Phytochemical screening and anticancer activities of some terrestrial and aquatic plants growing in saline habitat

F. Sh. Abd El-Gwaid [1,*], A. S. El Saied [2], Z. A. El-Swaify [1], R. A. Salah El Din [1]

[1] *Botany and Microbiology Department, Faculty of Science (Girls Branch), Al –Azhar University, Cairo, Egypt.*
[2] *Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.*

* Corresponding author E-mail:fatmaahmed2387.el@azhar.edu.eg (F. Abd El-Gwaid)

ABSTRACT

Halophytes are plants with significant economic potential for their nutritional and medicinal properties. High-performance liquid chromatography (HPLC) was used to identify the phenolics and flavonoids compounds of six halophyte plants (*Spergularia marina, Suaeda aegyptiaca, Arthrocnemum fruticosum, Halophila stipulacea, Halodule uninervis,* and *Thalassodendron ciliatum*) ethanol extracts, additionally, physiochemical parameters such as total ash, water-soluble ash acid in soluble ash, total carbohydrates, soluble carbohydrates, total nitrogen, and total protein were estimated. The results showed that nineteen phenolic and eleven flavonoid compounds were confirmed by HPLC qualitatively and quantitatively in ethanol extracts of all plants. The major compounds were chlorogenic, gallic, vanillic, naringin, and naringenin. Also, evaluation of their anticancer activities against cell lines such as pancreatic carcinoma (PA1), lung cancer (A549), prostate cancer (PC3), and colon cancer (Caco2) was carried out. The anticancer activities of *A. fruticosum* site (1), *S. marina*, and *A. fruticosum* site (2) extracts demonstrated the highest effective anticancer activities with IC50 values of 74.38 ± 3.04, 85 ± 2.11, and 88.922 ± 0.735 μg/mL, respectively, against human cancer cell lines (caco2) than other plants that were understudied. As a result of this research, halophyte plants can be viewed as a potential medicinal ingredient.

Keywords: Halophyte; Phenolic; Flavonoids compounds; Anticancer activities.

INTRODUCTION

Halophytes are plants that survive and complete their life cycles in extreme salinity in water or soil, besides various harsh environmental conditions (El-Din, 2012; Kumari *et al*., 2015; Joshi *et al*., 2015). Halophytes are found all over the planet, from deserts, which make up 8% of the total land surface, to coastal regions on the sea (O'Leary *et al*., 1994). Halophytes can be produced and collected as raw materials for food, animal feed, and pharmaceuticals, despite the high salt content in their tissues. Additionally, they stand out because of the existence of biologically active substances that could be helpful for treating diseases as well as be used as additives in the food industry (Menzel and Lieth, 2003; Ksouri *et al*., 2009). Plants from the aquatic ecosystem haven't been fully explored because of the complexity of the marine ecosystem with interconnected food chains, constant changes in the environment, and variation in marine species. One such group of under discovered marine plants are the sea grasses (Rengasamy *et al*., 2019). Sea grasses are the only flowering halophyte plants that grow when completely submerged in marine or shallow estuarine and coastal waters under extreme saline conditions. Additionally, they are a rich source of secondary metabolites such as phenolics, alkaloids, flavonoids, terpenes and each of these classes is comprised of thousands of different individual compounds and possesses a variety of biological activities such as anticancer, antibacterial, antibacterial anti-inflammatory, antifungal, and antioxidant, as well as their nutritional values. (Kannan *et al*., 2012; Baehaki *et al*., 2016; Hamdy *et al*., 2020; Parthasarathi *et al*., 2021; Kim *et al*., 2021)

Several study papers on different aspects of the physiology of halophytes have been published (Shabala 2013, Yin *et al*., 2019, and Yuan *et al*., 2019), including ecology and evolution (Lastiri-Hernández *et al*., 2020), biochemistry, and molecular biology (Rozentsvet *et al*., 2017). They are fascinating plants because they live and develop in harsh environments with high salt content. To survive in these environments, they have developed a variety of morphological, anatomical, physiological, and biochemical strategies (Ghanem *et al*., 2021; Grigore and Toma 2021; de la Fuente *et al*., 2021).

Phenolic compounds are substances with a wide range of biological functions, such as anti-cancer, anti-inflammatory, antioxidant, and anti-atherogenic properties. (Mori *et al*.,1999; Middleton *et al*., 2000; Medina *et al*., 2007; Badhani *et al*., 2015; Kumar and Goel, 2019). The phenolic compounds can be divided into two main groups: phenolic compounds

(phenols, phenolic acids, and phenol condensation) and flavonoids, which play important roles in plant defence against salt-induced stress Reginato *et al.* (2014). Derivatives of hydroxybenzoic acid, such as p-hydroxybenzoic, catechol, and vanillic acid, as well as hydroxycinnamic acid forms, such as p-coumaric, gallic, and caffeic acid have significant anticancer action (Rocha *et al.,* 2012; Tanaka *et al.,* 2011). Natural phenolic compounds have gained more attention as a result of their strong antioxidant activities and their notable contributions to the prevention of a number of oxidative stress-related illnesses, particularly cancer (Dai *et al*., 2010)

Cancer is a complex condition marked by excessive cell growth brought on by deficiencies in cellular regulation as well as impediments to cell cycle progression. It assaults, obliterates healthy cells, and destabilizes the body, with the potential to spread metastatically. Chemotherapy, radiation, and chemically generated medications that have a variety of negative effects on healthy cells are among the current cancer treatments. (Mansoori *et al*., 2017) Therefore, the discovery of new anticancer agents from natural products, particularly plants, is under investigation. Medicinal plants remain a good source for the discovery and improvement of anticancer agents. (Greenwell and Rahman 2015). (Custodio *et al* ., 2022) studied the anticancer activity of halophytes from different families, including, whenever possible, the bioactive molecules involved in such therapeutically properties along with possible mechanisms of action.

The current study aims to assess the proximate composition and secondary bioactive constituents of some dominant halophytes naturally occurring in Hurghada, the Red Sea, and Baharyia Oases, Egypt (*Halophila stipulacea, Halodule uninervis, Thalassodendron ciliatum sea grasses*, *Spergularia marina, Suaeda aegyptiaca,* and *Arthrocnemum fruticosum* terrestrial plants) with relation to soil variables, and additionally the anticancer activities of ethanolic extracts of plants that were under investigation against four cell lines.

MATERIAL AND METHODS:

Collection of Samples

Three fresh sea grass *species* (order *Alismatales*) were hand-collected in Hurghada, Red Sea, Egypt, at a depth of 0.5 to 1 m. Two of the *species*, *Thalassodendron ciliatum* (Forsskl) den Hartog and *Halodule uninervis* (Forsskl) *Ascherson*, are members of the family *Cymodoceaceae*, and *Halophila stipulacea* (Forsk.), One *species* of *Caryophyllaceae* (*Spergularia marina*), one *species* of *Amaranthaceae* (*Suaeda aegyptiaca*), and one *species* of *Chenopodiaceae* (*Arthrocnemum fruticosum*) were collected from four naturally growing populations of Bahariya oases, Egypt, between April 2020 and April 2021 and then identified by Ass. Prof. Al-Baraa Salah El-Din. The plants were cleaned, dried in the shade, and then ground.

Sediment analysis

A soil sample was collected in sealed plastic bags at a depth of 5–25 cm during the spring. The soil sample was air-dried, sieved through a 2mm mesh, and stored at room temperature for further physical and chemical analysis. Soil pH and electrical conductivity (EC) were measured in water suspension (1:2.5), as described by Jackson (1973), as were total soluble salts. Sulphates, carbonates, bicarbonates, and chlorides are determined by the titration method according to Jackson (1967). Calcium and Magnesium were determined by the titration method, according to Page (1982). Sodium and Potassium were determined using a flame photometer according to Allen et al. (1974) and Rowell (1994).

Physicochemical parameters

Total carbohydrates, soluble carbohydrates, total ash, water-soluble ash and acid-insoluble ash were detected by using British Pharmacopoeia (1980) methods. The total nitrogen was determined by the Kjeldahl method (Pirie *et al*., 1955), and the protein contents of the plant species were determined by multiplying the N contents by the factor 6.25.

Preparation of plant extract

The air-dried root and shoot parts of each plant (200 g) were extracted with slight modifications (Moustafa *et al.,* 2014). The extract was prepared by percolation of the material with 500 mL of ethanol (70%), and then fully extracted by percolation at ambient temperature on a shaker for 72 hours. The extracts were filtered using Whatman No. 1 paper and concentrated under reduced pressure at 40°C. They were dried by high vacuum, and the crude extracts were kept in the refrigerator at 4°C until used for the experiment. Yields for *Halodule uninervis, Halophila stipulacea, Thalassodendron ciliatum,*

Spergularia marina, Suaeda aegyptiaca, Arthrochnemum fruticosum site (1), and *Arthrochnemum fruticosum* site (2) were 9.1, ,8.5,9.25,.16.67,19.83,18.01 and 17.65 g, respectively.

Determination of phenolic compounds

Chemical reagents for HPLC analysis of phenolic compounds, ethanol, and acetonitrile (HPLC grade) were purchased from SDS (Peypin, France), phosphoric acid from Probus (Badalona, Spain), and acetic acid from Merck (Darmstadt, Germany). Pure water was obtained from Milli-Q (Millipore, MA, USA). Phenolic standards: protocatechuic acid, coumaric acid, quercetin, β- hydroxyl benzoic acid, alloevodionol, 5-hydroxyverotric acid, chlorogenic acid, neptein,3,4,'-dimethoxychrysin, 3,4'dimethoxychrysin, epicatechin, catechin, gallic acid, ferulic acid, caffeic acid, chlorogenic acid, trimethoxy quercetin, apigenin hyperoside, kaempferol, kaempferol-3-O-glucoside, keampferol-3rutinoside, kaempferol-7-O-neohesperidoside, quercitrin, isoquercitrin, iso-rhamnetin, luteolin, luteolin-6-C-glucoside, luteolin-8-C-glucoside, luteolin-7-O-glucoside, apigenin, apigenin-7-O-glucoside, apigenin-6-C-glucoside, apigenin-8-C-glucoside, amentoflavone, naringin, naringenin, naringenin-7-O-glucoside, isorhoifolin and rutin were purchased from Sigma Co. (St. Louis, MO, USA). The purity of the standards was 98%.

HPLC apparatus Reverse-phase HPLC apparatus, Agilent Series 1200 apparatus (Agilent, USA), equipped with an auto sampling injector, solvent degasser, quaternary HP pump (Series 1200), ultraviolet (UV) detector, and a Zobrax ODS C18 column (particle size 5 μm, 250 mm × 4.6 mm Ø) was used for phenolic compounds analysis. The experiment was carried out at the Soil, Water, and Environment Research Institute.

Quantitative determination of phenolic compounds by HPLC

The previously prepared ethanolic extract of seven plants (100 mg) was dissolved in HPLC-grade acetonitrile (2 mL). Separation of phenolic acids was carried out with the mobile phase consisted of three solvent A (CH3COOH 2.5%) in water, B (CH3COOH 8%) in water and C (acetonitrile). The gradient profile was as follows: at 0 min, A:B:C, 95:5:0; at 20 min, A:B:C, 90:10:0; at 50 min, A:B:C, 70:30:0; at 55 min, A:B:C, 50:50:0; at 60 min, A:B:C, 0:100:0; at 100 min, A:B:C, 50:50:50; at 110 min, A:B:C, 0:0:100 until 120 min (Pascale et al., 1999). Chromatography was performed at 35°C with a flow rate of 1 mL/min. The injection volumes were 10 L. UV traces were measured at 280 nm (Pascale et al., 1999). Flavonoid separation was done using a mobile phase consisting of 50 mM H3PO4, pH 2.5 (solution A), acetonitrile (solution B), and acetic acid (40:60, v/v) in the following gradient: isocratic elution 95% A:5% B, 0–5 min; linear gradient from 95% A:5% B to 50% A:50% B, 5–55 min; isocratic elution 50% A:50% B, 55–65 min; linear gradient from 50% A:50% B to 95% A:5% B, 65–67 min (Mattila *et al*., 2000). The temperature of the column was maintained at 35°C. The flow rate of the mobile phase was 0.7 mL/min. The volume injected was 5 L. UV traces were measured at 330 nm (Mattila *et al*., 2000). UV spectra were recorded between 200 and 600 nm for peak characterization. Standard flavonoids and phenolic acids were prepared as 10 mg/50 mL solutions in methanol, diluted to make concentrations (20–40 μg/mL) and injected into HPLC. The peak area of the external standards was used to quantify the phenolic compounds of the plant extracts, and the concentrations of the identified compounds were expressed.

Anticancer evaluation

Cell lines for pancreatic carcinoma (PA1), lung cancer (A549), prostate cancer (PC3), and colon cancer (Caco 2) were kindly provided by Dr. Mohammed Abd El-Baseir, Laboratory of Virology, Science Way for scientific researches and consultations, Faculty of Medicine, Al-Azhar University, Egypt.

Culturing of cell lines

A laminar airflow cabinet was used to maintain the sterility of the procedure. The cell culture was maintained in a Roswell Park Memorial Institute medium (RPMI 1640). It contained a 1% antibiotic antimycotic mixture (10,000 μg/mL streptomycin sulphate, 25 μg/mL amphotericin B and 10,000 U/mL potassium penicillin), 1% L-glutamine. Fetal bovine serum (10% heat-inactivated) was used to supplement the medium, and culturing and subculturing were performed (Thabrew *et al*., 1997).

MTT assay

The cytotoxicity was evaluated by the mitochondrial-dependent reduction of yellow MTT (3(4,5-dimethylthiazol-2-yl)–2,5-diphenyl tetrazolium bromide). The yellow MTT undergoes a mitochondrial reduction to form a purple formazan (Mosmann, 1983). The 96-well tissue culture microplate was inoculated at a cell concentration of 1 × 105 cells per well

in 100 µL of growth medium. The microplate was incubated at 37°C in 5% CO2 for 24 h to develop a complete monolayer sheet. The growth medium was decanted from 96 well microplates after the formation of the confluent layer of cells. The extract of the seeds was dissolved in dimethyl sulfoxide (DMSO). Serial dilutions of the dissolved extract were prepared by RPMI–1640 medium to give a final concentration of 156.25; 312.5; 625; 1250; 2500; 5000; 10,000 and µg/ml. Each concentration of the extract (0.1 mL) was added to confluent cell monolayers dispensed into 96-well microplate using a multichannel pipette. The treated cells were incubated at 37°C in 5% CO2 for 24 h. Three wells were used for each concentration of the extract. Control cells were incubated without the seeds extract. MTT powder was dissolved in PBS (Bio Basic Canada Inc.) to give a solution at the concentration of 5µg/ml. After the end of the incubation period, 20 µL of the MTT solution was added to each well. The mixing was allowed at 150 rpm for 5 min using the shaker (MPS-1, Biosan, London, UK). Then, the 96-well microplate was incubated at 37°C in 5% CO2 for 4 h to allow the MTT metabolism. Formazan (MTT metabolic product) was resuspended in 200 µL of DMSO and placed on a shaker at 150 rpm for 5 min for a thorough mixing (Mosmann, 1983). The optical density was recorded using a microplate reader (Mindray-96A, Mindray, Nanshan, Shenzhen, China) at 560 nm. The results were corrected using a reference wavelength of 620 nm as a background (Pang *et al*., 2010). All experiments were carried out in triplicate.

Microscope

An inverted microscope (Nikon, 118811) with an objective of 8x was used to observe the morphological structures of cell lines at varied concentrations of 70% ethanol halophyte plant extracts.

Statistical analysis

The parameters of the experiments were conducted, and results are presented as the mean ± standard deviation. The statistical significance of the data obtained from *in vitro* studies was evaluated by the one-way analysis of variance (ANOVA), followed by the general linear model approach in the Minitab 19 system. The least significant difference approach was used for mean comparisons.

RESULTS AND DISCUSSION

Sediment analysis

Results in Table (1) indicate that the highest values of, Cl^{-},Na^{+} , Ca^{+2} and Mg^{+2} in the case Of *A. fruticosum* site(2) soil sample were (549.238 , 208.704 , 64.870,420.240 Meq/l) respectively , while the lowest values recorded (2.599,50.370,39.132,0.255,6.228 and 13.536 Meq/l) respectively in case of *S. marina* soil sample also, Cl^{-} and Na^{+} the most dominant major cations (549.238 and 208.704Meq/l) respectively, in case of *A. fruticosum* site (2) soil sample of Bahryia oasis ,on the other hand K^{+} and SO_4 recorded the lowest values were 0.255 Meq/l and 5.431 Meq/l respectively in case of S. marina soil while total water soluble salt (TSS) recoded 3326 mg/l .The results indicated that the soil type is slightly alkaline. Elnaggar. (2017) found that in the Bahariya Oasis, soils are rich in alkali cations, calcium carbonates, and gypsum, and as a result, their pH values tend to rise towards alkalinity (pH greater than 7). Kudo et al. (2010) stated that the salt environment causes ion-harmful effects on the halophytes in addition to reducing their ability to absorb nutrients. The capacity of plants to remove Na and maintain the balance of nutrients in the body determines how much salt halophytes can tolerate.

Physicochemical parameters

Results in Table (2) showed that the total ash content reached its maximum value (33.50 and 30.70 gm/100 gm), respectively, for *T. ciliatum* and *S. aegyptiaca*, while reaching its minimum value (18.55 gm/100 gm) for *S. marina*. The ash content is used to determine the impact of saline soil on combustion. (Stavridou *et al*., 2016). The plant's ash content is essential because it affects the amount of various types of minerals in the food. (Mohanty *et al.,* 2019). High ash content significantly reduces the energy output derived from a specific biomass source (James *et al*., 2012). The composition difference might be explained by variations in origin, plant species, age, climatic conditions, and ecology; alternatively, it could be related to the physiological condition of the plant and nutrients present in the soil. (Abd-El Gawad *et al*., 2021; Elshamy *et al*., 2019; Hassan *et al*., 2021; Assaeed *et al*., 2020; Singha *et al*., 2017). On the other side, the total carbohydrate reached its maximum value (16.75 gm/100 gm and 12.95) for *S. aegyptiaca* and *A. fruticosum* site 1, respectively, while the minimum values are (0.019 gm/100 gm and 0.039 gm/100 gm) for *T. ciliatum* and *H. stipulacea*, respectively. The

accumulation of total carbohydrates was closely correlated with the stress of both drought and salinity. Therefore, it may be considered one of the most effective physiological characteristics and safeguards or adaptive mechanisms against response stress conditions. Teimouri *et al*., (2009) reported that increasing salinity increases the concentration rate of plant soluble carbohydrates, which shows that salinity has a negative influence on these materials. Parida and Das (2005) showed that under salt stress, carbohydrates, including sugar (glucose, sucrose, fructose), and starch accumulate.

Total nitrogen and total protein

It is observed from Table (3) that the maximum percentage of nitrogen was recorded in *H uninervise* (2.07%), while the minimum amount was attained by *A. fruticosum* site (1) (0.39%). It is evident from this study that the sea grasses contain a higher content of nitrogen and protein than other plants under investigation. Proteins are compounds of fundamental importance for all functions in the cell (Dose, 1980). Osuagwu and Edeoga (2012) reported that the increment in nitrogen content under stress conditions could be due to the metabolism of nitrogen ions in the leaves for the synthesis of special proteins and amino acids in the plants as a defense mechanism against the effects of water and salinity stresses. On the other hand, Jalal *et al*., (2012) mentioned that many studies showed a decrease in protein concentration in many plant species grown under drought stress due to decreased protein biosynthesis and enhanced degradation. A common effect of drought stress is to cause oxidative damage. The production of reactive oxygen species (ROS) under water stress conditions increases the breakdown of proteins. The accumulation of ROS during drought stress, along with increasing H_2O_2, often enhances protein oxidation in plant species, while other reporters investigated whether reducing protein content was a result of the negative effect of drought on nitrate reducate activity and nitrogen metabolism.

Identification of the active Phenolic compounds using HPLC

The phytocompounds are the most essential non-nutritive components secreted by plants and are also known as secondary metabolites that aren't released by the plants but are used as a natural defense system for plants against microbes, environmental stresses, or disease treatment purposes for humans (Das and Gezici, 2018). These secondary metabolites are relatively easily produced in small quantities in plants and have important economic and medicinal values (Dang, 2018).

It is clear from the results using high-performance liquid chromatography (HPLC) analysis for *H. uninervis, H. stipulacea, T. ciliatum, S. marina, S. aegyptiaca, A. fruticosum* site (1), and *A. fruticosum* site (2) ethanol extract, tabulated in Table (4) and Figs. (1–10) contains a high variety of phenolic compounds. It showed that seventeen different chemical classes' of phenolic compounds were present. The main components were chlorogenic acid (3695.17 ppm) for *S. marina*, followed by gallic acid (3605.81 ppm) for *A. fruticosum* site (1). Meanwhile, 3-OH Tyrosol (5.66 ppm) for *H. stipulacea* and Cinnamic acid (3.19 ppm) for *A. fruticosum* site (1) are considered the minor components.

This variation may be caused by a variety of factors, including salt stress, environmental changes, and UV radiation. Halophytes produce secondary metabolites such as phenolic compounds in a range of quantities. (Al Jitan *et al*., 2018). It has been detected that the total phenolic compounds of *Sarcocornia* and *Arthrocnemum* have antioxidant capacity, and it has been shown that *Sarcocornia* and *Arthrocnemum* may be used as food due to their high contents of nutrients (Castañeda-Loaiza *et al*., 2020; Oliveira-Alves *et al*.,2021). Sea grasses share more characteristics of their primary and secondary metabolism with terrestrial plants due to the fact that they are created from land plants that have secondarily recolonized marine environments.

The exposure of halophytes to saline conditions in their natural environment has an effect on the contents of the bioactive phytochemical compounds that are present in them, particularly as a protection mechanism against the oxidizing agents produced in these harsh environmental conditions. (Nikalje et al., 2019)

Our results agree with Zengin *et al*. (2018), who identified several phenolic acids in *Arthrocnunm macrostachyum* extracts: chlorogenic acid, gallic acid, protocatechuic acid, p-coumaric acid, rosmarinic acid, and caffeic acid. also identified several flavonoids as quercetin, naringenin, hesperidin, and rhamnetin for A. macrostachyum. Elsharabasy *et al*. (2019) identified compounds using HPLC and found nine phenolic compounds were present in the ethanolic extract of *Sueada*

pruinosa, while eight compounds were found in the ethanolic extract of *S. monoica*. The extracts of all plants contained gallic acid, catechin, caffeic acid, syringic acid, rutin, coumaric acid, vanillin, and quercetin, but cinnamic acid was found only in the ethanolic extract of *S. pruinosa*. Also, Hegazi *et al.* (2021) studied the metabolic profiles of *Halophila stipulacea* and *Thalassia hemprichii species* by using ultra-high-performance liquid chromatography and high-resolution mass spectrometry analysis. Also investigated were 144 metabolites, including phenolic acids, flavonoids, terpenoids, and lipids. Three new phenolic acids, methoxy benzoic acid-O-sulphate, O-caffeoyl-O-hydroxyl dimethoxy benzoyl tartaric acid, dimethoxy benzoic acid-O-sulphate, a new flavanone glycoside, hexahydroxy-monomethoxy flavanone-O-glucoside, and a new steviol glycoside, rebaudioside-O-acetate, have been prescribed. As well as estimated the anti-diabetic activities of *H. stipulacea* and *T. hemprichii* species.

Identification of the active flavonoid compounds from Halophyte (terrestrial and aquatic) plants ethanolic extracts using HPLC.

The high-performance liquid chromatography analysis for *H. uninervis, H. stipulacea, T. ciliatum, S. marina, S. aegyptiaca, A. fruticosum* site (1) and *A. fruticosum* site (2) ethanol extracts results tabulated in Table (5) showed that twelve different chemical classes of flavonoids are present. The major components are Naringin (1906.1 ppm) for *H. uninervis* and Naringenin (432.7 ppm) for T. ciliatum. Meanwhile, the minor components are Apigenin, quercetin, and rosmarinic acid for *S. aegyptiaca*, *A. fruticosum1,* and *H. stipulacea* (6.96, 10.74, and 18.2 ppm, respectively).

Flavonoids are a large class of naturally occurring substances that are commonly dispersed in the plant kingdom. Many of these substances have been reported to have a wide range of strong biological activities, such as anti-oxidative tissue protection and tumour inhibition. (Krant *et al*., 2005; Kim *et al*.,2007; Matanjun *et al*., 2008). (Jaganathan and Mandal 2009) indicated that quercetin and kaempferol have advanced as promising pharmaceuticals for treating cancer. It is widely accepted that increasing the intake of flavonoids in the diet may lower the prevalence of some human diseases. The health advantages of flavonoids may result from their interactions with numerous biological systems, as they have antioxidant capacity, free radical scavenging activities, anticancer activities, cardiovascular disease preventive potential, and anti-HIV properties. Numerous papers proved that flavonoids have antioxidant effects (DeGroot, 1994; Halliwe, 1995; Korkina and Afanas, 1997; and Mozaffari *et al*., 2011). Therefore, they should increase their antioxidant requirements as a defense against harsh environmental conditions. Also, they have anti-atherosclerotic properties, anti-inflammatory, anti-tumor, anti-thrombogenic, anti-osteoporotic properties, and anti-viral activities (Arai et al., 2000; Vinson *et al*., 2006; Damas *et al*., 1985; Xing *et al*., 2013; Caltagirone *et al*., 2000; He *et al*., 2012; Bojić *et al*., 2011; Uchida *et al*., 2010; Lagari *et al*., 2012; Harris *et al*., 2003; Andres *et al*., 2009) It is common knowledge that different plant species and the various parts of the same plant exhibit a vast range of chemical compositions. Furthermore, diverse geographic regions, ages, climates, and soil conditions resulted in varying chemical compositions in different plants. These substances' ability to survive in environments of high salinity and drought, as well as their added value for consumption, are both supported by their existence in various halophytes. In halophytes, salt tolerance and nutritional interest are correlated, and the wide range of chemicals found supports these Sánchez-Gavilán *et al*., (2021).

Anticancer evaluation

According to the World Health Organization, deaths from cancer are more common in advanced nations than in developing countries. Cancer is the second-most prevalent cause of mortality and is characterized by the unchecked proliferation of human cells**.** (Mathers 2020; Akindele *et al*. 2015). Currently, 60% of the drugs used to treat cancer come from natural sources Newman and Cragg. (2016).

Six halophyte plants' ethanol extracts were tested for their ability to inhibit the growth of several human cancer cells, including PA1, A549, PC3, and Caco 2, using the MTT assay. Product cell viability is assessed using the MTT (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide) test. The MTT assay is based on the reduction of MTT by mitochondrial dehydrogenase, which results in the formation of the purple-coloured formazan product from the colorless tetrazolium. The present study that is tabulated in Table (6) and Figs. [11 (b), 11 (c), 11 (d),11 (e)] reveals that the anticancer activity of ethanol extracts of *A. fruticosum* site(1), *S. marina* and *A. fruticosum* site (2) have higher activities against human

cancer cell lines (caco2) with IC50 (74.38 ± 3.04, 85 ± 2.11, and 88.922 ± 0.735), respectively, than other plants that understudied extracts, while *H. stipulacea* has the lowest activities against human cancer cell lines Pc3, A549,caco2 with IC50 (776.4 ± 24.1 and 494.9 ± 41.8), respectively. Phenolic compounds might interfere in several of the steps that prevent the development of tumors, including protecting DNA from oxidative damage, preventing carcinogen activation, and stimulating carcinogen-detoxifying systems (Hodek *et al*., 2002; Byrd *et al*., 1998; Nakagawa *et al*., 1996; Rodrguez-Garca *et al*., 2019; Abotaleb *et al*., 2018; Yahfoufi *et al*., 2018).

CONCLUSION

Halophytes are plants that produce numerous metabolites with promising nutritional values and bioactive effects such as antioxidants, antimicrobials, and anticancer agents. The material from *Halodule uninervis, Halophila stipulacea , Thalassodendron ciliatum , Spergularia marina, Suaeda aegyptiaca* and *Arthrocnemum fruticosum* contained high contents of macromolecular antioxidants phenolic constituents , i.e. Chlorogenic acid, Gallic acid, Naringin, Rosmarinic, Apignin -7-glucoside, Quercetin, Caffeic and Kaempferol 3-2-p-coumaroylglucose, rise their antioxidant demands as a defense against the harsh environmental conditions and their remarkable contributions to the prevention of a number of oxidative stress-related illnesses, particularly cancer so, natural phenolic compounds have attracted greater attention. *A. fruticosum* and *S. marina* showed promising potential activities against human cancer cell lines (caco2). So, halophyte plants can be considered to have promising nutritional, pharmaceutical, and medicinal properties.

REFERENCES

Abd-ElGawad, A.M., El Gendy, A.E.N.G., Assaeed, A.M., Al-Rowaily, S.L., Alharthi, A.S., Mohamed, T.A., Elshamy, A.I. 2020: Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. Plants, 10(1), 36. https://doi.org/10.3390/plants10010036

Abotaleb, M., Samuel, S.M., Varghese, E., Varghese, S., Kubatka, P., Liskova, A., Büsselberg, D. 2018: Flavonoids in cancer and apoptosis. Cancers, 11(1), 28. https://doi.org/10.3390/cancers11010028

Akindele, A.J., Wani, Z.A., Sharma, S., Mahajan, G., Satti, N.K., Adeyemi, O.O., Mondhe, D.M., Saxena, A.K. 2015: In vitro and in vivo anticancer activity of root extracts of Sansevieria liberica Gerome and Labroy (Agavaceae*). Evidence-Based Complementary and Alternative Medicine. 2015*. *https://doi.org/10.1155/2015/560404*

Al Jitan, S., Alkhoori, S.A., Yousef, L.F. 2018: Phenolic acids from plants: Extraction and application to human health. *Stud. Nat. Prod. Chem.*, 58, 389–417. https://doi.org/10.1016/B978-0-444-64056-7.00013-1

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C., Roberts, J.D. 1974: Chemical Analysis of Ecological Materials. *Blackwell Scientific Publications; London*.

Andres, A., Donovan, M.S., Kuhlenschmidt, M.S. 2009: Soy isoflavones and virus infection.*J. Nut. Bio*., 20980:563-569. https://doi.org/10.1016/j.jnutbio.2009.04.004

Arai, Y., Watanab, S., Kimira, M., Shimoi, K., Mochizuki, R., Kinae, N. 2000: Ditary intakes of flavonols, flavones and isovlavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration.*J.Nutr*., 130(9):2243-2250.

Assaeed, A., Elshamy, A., El Gendy, A.E.N., Dar, B., Al-Rowaily, S., Abd-ElGawad, A. 2020: Sesquiterpenes-rich essential oil from above ground parts of Pulicaria somalensis exhibited antioxidant activity and allelopathic effect on weeds. *Agronomy.* 10(3), 399.

Baehaki, A., Agus, S., Pratama, M.C. 2016: Antioxidant Activity of Methanol Extract of Halodule uninervis Seagrass from the Coastal of Lampung, Indonesia. Research Journal of Pharmaceutical, Biological and Chemical Sciences.7 (3): 1173-1177.

Baghdady, A., Awad, S., Gad, A. 2018: Assessment of metal contamination and natural radiation hazards in different soil types near iron ore mines, Bahariya Oasis, Egypt. *Arabian Journal of Geosciences. 11, 1-12.*

Bojić, M., Debeljak, Z., Tomićić, M., Medić-Śarić, M., Tomić, S. 2011: Evaluation of anti-aggregatory activity of flavonoid aglycone series. *Nut.J*. 10:73.

British, pharmacopoeia. 1980: Published on recommendation of the Medicines commission.Printed in England for her majesty's stationary office at the university press, *Cambridge, U. K., vol. 11, pp.561.*

Byrd, J.C., Shinn, C., Waselenko, J.K., Fuchs, E.J., Lehman, T.A., Nguyen, P.L., Flinn, I.W., Diehl, L.F., Sausville, E., Grever, M.R. 1998: Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase-3 without evidence of bcl-2

modulation or dependence on functional p53. Blood, *The Journal of the American Society of Hematology, 92(10), pp.3804-3816.*

Caltagirone, S., Rossi, C., Poggi, A., Ranelletti, F.O., Natali, P.G., Brunetti, M., Aiello, F.B., Piantelli, M. 2000: Flavonoids apigenin and quercetin inhibit melanoma growth and metastic potential. *Int.J. Cancer*., 87:595-600

Castañeda-Loaiza, V., Oliveira, M., Santos, T., Schüler, L., Lima, A.R., Gama, F., Salazar, M., Neng, N.R., Varela, J., Barreira, L. 2020: Wild vs cultivated halophytes: Nutritional and functional differences. *Food Chem.* 333,127536. https://doi.org/10.1016/j.foodchem.2020.127536

Custodio, L., Garcia-Caparros, P., Pereira, C.G., Castelo-Branco, P. 2022: Halophyte Plants as Potential Sources of Anticancer Agents: A Comprehensive Review. Pharmaceutics, 14(11), 2406. https://doi.org/10.3390/pharmaceutics14112406

Damas, J., Bourdon, V., Remacle-Volon, G., Lecomte, J. 1985: Pro-inflammatory flavonoids which are inhibitors of prostaglandin biosynthesis.*Prostaglandins Leukot.Med.,* 19:11-24.

Dai, J., Mumper, R.J. 2010: Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules . 15, 7313-7352

Das, K., Gezici, S. 2018: Secondary plant metabolites, their separation and identification, and role in human disease prevention. *Ann. Phytomedicine*, 7, 13-24.

Dang, R. 2018: Role of antinutrient metabolites of plant on production of secondary metabolites and human health. Ann. Phytomed., 7(1):1-4

de la Fuente, V., Sánchez-Gavilán, I., Ramírez, E., Rufo, L., Sánchez-Mata, D. 2021: Morphological variability of halophytes: salicornioideae on Iberian Peninsula. Handbook of Halophytes: *From Molecules to Ecosystems towards Biosaline Agriculture, 1223-1258*.

DeGroot, H. 1994: Reactive oxygen species in tissue injury. *Hepato-gastroenterology*, 41:328-332.

Dose, K. 1980: Biochemie. Springer Berlin,Heidelberg, New York.

El -Naggar, A.A. 2017: Development of land capability and suitability maps for Bahariya Oasis, Egypt. *Egyptian Journal of Soil Science, 57(4*), p.489-503.

El-Amier, Y.A., Abd El-Gawad, A.M. 2017: Plant communities along the international coastal highway of Nile delta, Egypt. *J. Sci. Agric*, 1, 117-131.

El-Din, A.T. 2012: Halophytes as feedstuffs in feeding farm animals, a review. Journal of Agriculture & Environmental Science, Damanhour University, Egypt, 11(2), 1-33.

Elshamy, A.I., AbdElGawad, A.M., El-Amier, Y.A., El Gendy, A.E.N.G., Al-Rowaily, S.L. 2019: Interspecific variation, antioxidant and allelopathic activity of the essential oil from three Launaea species growing naturally in heterogeneous habitats in Egypt. *Flavour and fragrance journal, 34(5), 316-328.56.*

Elsharabasy, F.S., Metwally, N.S., Mahmoud, A.H., Soliman, M.S., Youness, E.R., Farrag, A.H. 2019: Phytoconstituents and hepatoprotective effect of Suaeda monoica Forssk and Suaeda pruinosa Lange. *Biomedical and Pharmacology Journal*, 12(1): 117-129.

Ghanem, A.M.F., Mohamed, E., Kasem, A.M., El-Ghamery, A.A. 2021: Differential salt tolerance strategies in three halophytes from the same ecological habitat: Augmentation of antioxidant enzymes and compounds. *Plants*, 10(6), 1100.

Greenwell, M., Rahman, P.K.S.M. 2015: Medicinal Plants: Their Use in Anticancer Treatment. *Int. J. Pharm. Sci. Res*., 6, 4103–4112.

Grigore, M.N., Toma, C. 2021: Morphological and anatomical adaptations of halophytes: A review. Handbook of Halophytes: *From Molecules to Ecosystems towards Biosaline Agriculture, 1079-1221.*

Halliwell, B. 1995: How to characterize an anti-oxidant:an update.*Biochem.Soc.Symmp.,61:73-101.*

Harris, S., Bishop, K.N., Sheehy, A.M., Craig, H.M., Petersen-Mahrt, S., Watt, I.N., Neiberger, M., Malim, M.H. 2003: .DNA deamination mediates innate immunity to retroviral infection. *Arch.Intern.Med.,*113(6) :803-809

Hassan, E.M., El Gendy, A.E.N.G., Abd-ElGawad, A.M., Elshamy, A.I., Farag, M.A., Alamery, S.F., Omer, E.A. 2020: Comparative chemical profiles of the essential oils from different varieties of Psidium guajava L. *Molecules, 26(1), 119.*

He, J., Xu, Q., Wang, M., Li, C., Qian, X., Shi, Z., Liu, L.Z., Jiang, B.H. 2012: Oral administration of apigenin inhibits metastasis through AKT/P70S6K1/MMP-9 pathway in orthotopic ovarian tumor.Mod. *Int.J. Mol. Sci*.,13(6):7271-7282.

Hegazi, N.M., Saad, H.H., Marzouk, M.M., Abdel Rahman, M.F., El Bishbishy, M.H., Zayed, A., Ezzat, S.M. 2021: Molecular networking leveraging the secondary metabolomes space of Halophila stipulaceae (Forsk.) Aschers. and Thalassia hemprichii (Ehrenb. ex Solms) Asch. in tandem with their chemosystematics and antidiabetic potentials. *Marine drugs,* 19(5), 279

Hodek, P., Trefil, P., Stiborová, M. 2002: Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. *Chemico-biological interaction*s, 139(1), 1-21.

Jackson, M.L. 1973: Soil Chemical Analysis. Prentice Hall of India Private limited, New Delhi.pp.498.

Jackson, M.L. 1967: Soil chemical analysis prentice-Hall of India private, *New Delhi, India.*

Jaganathan, S.K., Mandal, M. 2009: Antiproliferative effects of honey and of its polyphenols: A review. *J. Biomed. Biotechnol*., 830616, 1– 13.

Jalal, M., Mansouri, E., Sharifipour, M., Pouladkhan, A.R. 2012: Mechanical, rheological, durability and microstructural properties of high performance self-compacting concrete containing SiO2 micro and nanoparticles. *Materials & Design*, 34, 389-400.

James, A.K., Thring, R.W., Helle, S., Ghuman, H.S. 2012: Ash management review—applications of biomass bottom ash. *Energies*, 5(10), 3856-3873.

Joshi, R., Mangu, V.R., Bedre, R., Sanchez, L., Pilcher, W., Zandkarimi, H., Baisakh, N. 2015: Salt adaptation mechanisms of halophytes: Improvement of salt tolerance in crop plants. In Elucidation of Abiotic Stress Signaling in Plants; *Springer: New York, NY, USA,* pp. 243–279]

Kim, I.H., Lee, D.G., Lee, S.H., Ha, J.M., Ha, B.J., Kim, B.J., Lee, J.H. 2007: Antibacterial activity of Ulva lactuca against Methicillin- resistant Staphylococcus aureus (MRSH).Biotechnol. Bioprocess Eng., 12: 579–582.

Kim, D.H., Mahomoodally, M.F., Sadeer, N.B., Seok, P.G., Zengin, G., Palaniveloo, K., Rengasamy, K.R. 2021: Nutritional and bioactive potential of seagrasses: A review. South African Journal of Botany, 137, 216-227.

Korkina, L.G., Afanas, I.B. 1997: Antioxidant and chelating properties of flavonoids.*Adv.Pharacol*.,138:151-163.

Kudo, N., Sugino, T., Oka, M., Fujiyama, H. 2010: Sodium tolerance of plants in relation to ionic balance and the absorption ability of microelements. Soil Sci. Plant Nutr. 56, 225–233.

Krant, K., Schlesier, K., Bitsch, R., Hermann, H., Rohe, M., Bohm, V. 2005: Comparing antioxidative food additives and secondary plant products use of different assays.*Food Chem.,* 93:171–175.

Ksouri, R., Falleh, H., Megdiche, W., Trabelsi, N., Mhamdi, B., Chaieb, K., Bakrouf, A., Magné, C., Abdelly, C. 2009: Antioxidant and antimicrobial activities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. *Food Chem. Toxicol.*;47: 2083–2091.

Kumari, A., Das, P., Parida, A.K., Agarwal, P.K. 2015: Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Front. Plant Sci*.;6: 537.

Kumar, N., Goel, N. 2019: Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports, 24, e00370.

Lagari, V.S., Marin, O.G., Levis, S. 2012: Differences in Vitamin D3 Dosing Regimens in Geriatric Community-Dwelling Population. *J. Endocrine Practice*.18 (6):847-854.

Lastiri-Hernández, M., Cruz-Cárdenas, G., Álvarez-Bernal, D., Vázquez-Sánchez, M., Bermúdez-Torres, K. 2020: Ecological Niche Modeling for Halophyte Species with Possible Anthropogenic Use in Agricultural Saline Soils. *Environ. Model. Assess*.;25: 429–440.

Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S., Baradaran, B. 2017: The Different Mechanisms of Cancer Drug Resistance: A Brief Review. Adv. Pharm. Bull. 7, 339–348.

Matanjun, P., Mohamed, S., Mustapha, N., Muhammad, K., Ming, C. 2008: Antioxidant activities and phenolics content of eight species of Seaweeds from North Borneo. *J. Appl. Phycol. 20: 367– 373.*

Mathers, C.D. 2020: History of global burden of disease assessment at the World Health Organization. *Archives of Public Health*, 78(1), 1-13.

Mattila, P., Astola, J., Kumpulainen, J. 2000: Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *Journal of Agricultural and Food Chemistry, 48(12), pp.5834-5841.*

Menzel, U., Lieth, H. 2003: Halophyte Database,Halophyte Uses in Different Climates II. Halophyte Crop Development for Different Climates. *Progress in Biometeorology., The Netherlands: 2003. pp. 127–133.*

Mori, H., Kawabata, K., Yoshimi, N., Tanaka, T., Murakami, T., Okada, T., Murai, H. 1999: Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. Anticancer Res. 19 : 3775–3778. [PubMed] [Google Scholar]

Middleton, E., Kandaswami, C., Theoharides, T.C. 2000: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52: 673–751. [PubMed] [Google Scholar]

Medina, I., Gallardo, J.M., Gonzalez, M.J., Lois, S. 2012: Hedges N. Effect of molecular structure of phenolic families as hydroxycinnamic acids

and catechins on their antioxidant effectiveness in minced fish muscle. J. Agric. Food Chem.55: 3889–3895. [PubMed] [Google Scholar]

Badhani, B. 2015: Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. RSC Adv.5:27 540–27557.

Mohanty, B.P., Mahanty, A., Ganguly, S., Mitra, T., Karunakaran, D., Anandan, R. 2019: Nutritional composition of food fishes and their importance in providing food and nutritional security. *Food Chem*.;293 :561–570.

Mosmann, T. 1983: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods, 65(1-2), 55-63.*

Moustafa, S.A., Menshawi, B.M., Wassel, G.M., Mahmoud, K., Mounier, M.M. 2014: Screening of some plants in Egypt for their cytotoxicity against four human cancer cell lines. *Int J PharmTech Res.; 6(3): 1074-1084.*

Mozaffari, S., Esmaily, H., Rahimi, R., Baeeri, M., Sanei, A., Shahmirzadi, Y.A., Hossein, M., Abdollahi, M. 2011: Efects of Hypericum perforatum extract on rat irritable bowel syndrome. *Pharmacog. Mag.*, 7(27):213-223.

Nakagawa, Y., Moldéus, P., Moore, G.A. 1996: Relationship between mitochondrial dysfunction and toxicity of propyl gallate in isolated rat hepatocytes. Toxicology, 114(2), 135-145.

Newman, D.J., Cragg, G.M. 2016: Natural products as sources of new drugs from 1981 to 2014. *Journal of natural products*, 79(3), 629-661.

Nikalje, G.C., Bhaskar, S.D., Yadav, K., Penna, S. 2019: Halophytes: Prospective Plants for Future. *In Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes*; 221-234.

O'leary, J.W., Glenn, E.P., Squires, V., Ayoub, A.T. 1994: Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands.

Oliveira-Alves, S.C., Andrade, F., Prazeres, I., Silva, A.B., Capelo, J., Duarte, B., Caçador, I., Coelho, J., Serra, A.T., Bronze, M.R. 2021: Impact of Drying Processes on the Nutritional Composition, Volatile Profile, Phytochemical Content and Bioactivity of Salicornia ramosissima. J. Woods. *Antioxidants*. 10(8), 1312.

Osuagwu, G.G.E., Edeoga, H.O. 2012: The influence of water stress (drought) on the mineral and vitamin content of the leaves of Gongronema latifolium (Benth). *International Journal of Medician and Aromatic Plants, 2(2), 301-309.*

Page, A.L. 1982: Methods of Soil Analysis, Plants I and II.Agronomy No.9.American Society of Agronomy, Madison.

Pang, R., Tao, J.Y., Zhang, S.L., Zhao, L., Yue, X., Wang, Y.F., Ye, P., Dong, J.H., Zhu, Y., Wu, J.G. 2010: In vitro antiviral activity of lutein against hepatitis B virus. Phytother Res. 24: 1627–1630

Kannan, R.R., Arumugam, R., Anantharaman, P. 2012: Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. Food chemistry. 135(4):2470-2473.

Hamdy, A.H., El-Fiky, N.M., El-Beih, A.A., Mohammed, M.M., Mettwally, W.S. 2020: Egyptian red sea seagrass as a source of biologically active secondary metabolites. Egyptian Pharmaceutical Journal. 19(3):224.

Parthasarathi, P., Umamaheswari, A., Banupriya, R., Elumalai, S. 2021: Phytochemical screening and in-vitro anticancer activity of ethyl acetate fraction of Seagrass Halodule uninervis from Mandapam Coastal Region Rameswaram Gulf of Mannar India. International Journal of Pharmaceutical Sciences and Drug Research. 13(6):677-684.

Parida, A.K., Das, A.B. 2005: Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and environmental safety*, 60(3), 324-349.

Pirie, N.W., Proteins. K., Tracey, M.V. 1955: Modern Methods of Plant Analysis. *Springer; Berlin, Germany*: pp. 23–68.

Rocha, L.D., Monteiro, M.C., Anderson, J.T. 2012: Anticancer properties of hydroxycinnamic acids-A Review. Cancer and clinical oncology 1: 1927–4866.

Tanaka, T., Tanaka, T., Tanaka, M. 2011: Potential Cancer Chemopreventive Activity of Protocatechuic Acid. J Exp Clin Med 3: 27–33.

Rengasamy, K.R.R., Sadeer, N.B., Zengin, G., Mahomoodally, M.F., Czi´aky, Z., Jek, O.J., Diuzheva, A., Abdallah, H.H., Kim, D.H. 2019: Biopharmaceutical potential, chemi- cal profile and in silico study of the seagrass Syringodium isoetifolium (Asch.) Dandy. *S. Afr. J. Bot.* 127, 167–175.

Reginato, M.A., Castagna, A., Furlán, A., Castro, S., Ranieri, A., Luna, V. 2014: Physiological responses of a halophytic shrub to salt stress by Na2SO4 and NaCl: oxidative damage and the role of polyphenols in antioxidant protection. AoB Plants, 6.

Rodríguez-García, C., Sánchez-Quesada, C., Gaforio, J.J.2019: Dietary flavonoids as cancer chemopreventive agents: An updated review of human studies. *Antioxidants*, 8(5), 137.

Rowell, D.L. 1994: Soil Science: Methods and Applications. *Longman Publishers, Singapor*, PP. 350.

Rozentsvet, O., Nesterov, V., Bogdanova, E. 2017: Structural, physiological, and biochemical

aspects of salinity tolerance of halophytes. *Russ. J. Plant Physiol*.;64: 464–477.

Shabala, S. 2013: Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.* 2013;112 :1209–1221.

Singha, B.L., Hassan, Y. 2017: Analysis of carbohydrate, moisture content and specific gravity of bambusa tulda with special reference to its harvesting season. *Int. J. Sci. Res*, 6(1), 279-283.]

Slama, I., Ghnaya, T., Messedi, D., Hessini, K., Labidi, N., Savoure, A., Abdelly, C. 2007: Effect of sodium chloride on the response of the halophyte species Sesuvium portulacastrum grown in manniol-induced water stress. *J. Plant Res*.120:291-299

Stavridou, E., Hastings, A., Webster, R.J., Robson, P.R. 2016: The impact of soil salinity on the yield, composition and physiology of the bioenergy grass.

Teimouri, A., Jafari, M., Azarnivand, H. 2009: Effect of proline, soluble carbohydrates and water potential on resistance to salinity of three Salsola species (S. rigida, S. dendroides, S. richteri). *Desert*, 14(1), 15-20.

Thabrew, M.I, Hughes, R.D., McFarlane, I.G. 1997: Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *Journal of pharmacy and pharmacology, 49(11), pp.1132-1135.*

Uchida, H., Yamazaki, K., Fukuma, M., Yamada, T., Hayashida, T., Hasegawa, H., Kitagawa, Y., Sakamoto, M. 2010: Overexpression of leucine-rich repeat-containing G protein coupled receptor in colorectal cancer issue. *Cancer Sci*., 101(7):1731-1737.

Vinson, J.A., Proch, J., Bose, P., Muchler, S., Taffera, P., Shuta, D., Samman, N., Agbor, G.A.2006: Chocolate is a powerful ex-vivo and in vivo anti-oxidant, an anti-atherosclerotic agent in an animal model, and a significant contribution to anti-oxidant in the European and American Diets. *J.Agric.Food.Chem*., 54(21):8071-8076.

Xing, J., Peng, K., Cao, W., Lian, X., Wang, Q., Wang, X. 2013: Effects of total flavonoids from Dracocephalum moldavica on the proliferation ,migration and adhesion molecule expreesion of rat vascular smooth muscle cells induced by TNF-α. *Pharm. Biol*., 51(1):74-83.

Yahfoufi, N., Alsadi, N., Jambi, M., Matar, C. 2018: The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients*, 10(11), 1618.

Yin, Z., Zhang, H., Zhao, Q., Yoo, M.J., Zhu, N., Yu, J., Yu, J., Guo, S., Miao, Y., Chen, S. 2019: Physiological and comparative proteomic analyses of saline-alkali NaHCO3- responses in leaves of halophyte Puccinellia tenuiflora. *Plant Soil.* 437:137–158.

Yuan, F., Guo, J., Shabala, S., Wang, B. 2019: Reproductive physiology of halophytes: Current standing. *Front. Plant Sci*.;9:1954.

Zengin, G., Aumeeruddy-Elalfi, Z., Mollica, A., Yilmaz, M.A., Mahomoodally, M.F. 2018: In vitro and in silico perspectives on biological and phytochemical profile of three halophyte species—A source of innovative phytopharmaceuticals from nature. *Phytomedicine*, 38, 35-44.

Table 1: Chemical analysis of the sediment profiles associated with Halophyte (terrestrial and aquatic) plants (terrestrial and aquatic) plants:

Plants	pH	EC ds/m	TSS mg/l	SO4 Meq/l	CO3- -	HCO3 Meq/l	Cl Meq/l	Na Meq/l	K Meq/l	Ca Meq/l	Mg Meq/l
A. fruticosum site *(1)*	8.15	29.41	17611	24.960	-	1.560	289.799	186.964	1.658	37.365	80.173
A. fruticosum site *(2)*	8.46	68.03	37365	131.040	-	11.698	549.238	208.704	0.816	64.870	420.240
S. marina	8.28	5.84	3326	5.431	-	2.599	50.370	39.132	0.255	6.228	13.536
S. aegyptiaca	8.04	15.3	8373	13.520	-	1.690	138.000	56.524	1.785	31.138	64.555
Sediment of Sea grasses	8.0	9.3	5520	13.5	0.779	0.910	178.960	76.090	1.301	3.321	12.470

Table 2: Total ash, Water soluble ash, Acid insoluble ash and total carbohydrates gm/100gm contents of Halophyte (terrestrial and aquatic) plants.

parameters	Total ash gm/100gm	Water soluble ash gm/100gm	Acid insoluble ash gm/100gm	total carbohydrates gm/100gm	In soluble carbohydrates gm/100gm	soluble carbohydrates gm/100gm
S.marina	18.55	21.70	26.935	11.90	0.285	11.615
A. fruticosum site (1)	23.14	22.78	14.789	12.95	0.425	12.525
A. fruticosum site (2)	21.70	20.84	21.128	10.65	0.260	10.39
S. aegyptiaca	30.70	24.17	9.349	16.75	0.388	16.362
H .uninervise	20.70	28.68	17.97	0.125	0.003	0.122
H . stipulaceae	25.60	33.6	23.18	0.039	0.001	0.038
T. ciliatum	33.50	21.27	19.39	0.019	0.002	0.017

Table 3: Total nitrogen and total protein of Halophyte (terrestrial and aquatic) plants:

Plants	Total N %	Total protein%
S.marina	0.89	5.54
A. fruticosum site (1)	0.39	2.42
A. fruticosum site(2)	0.84	5.27
S. aegyptiaca	0.88	5.49
H .uninervise	2.07	12.93
H. stipulaceae	1.31	8.21
T. ciliatum	1.62	10.09

Table 4: Identification of the active Phenolic compounds from Halophyte (terrestrial and aquatic) plants ethanolic extract using HPLC**:**

No	Phenolic compounds	*H. uninervis*		*T. ciliatum*		*H. stipulacea*		*A. fruticosum site (1)*		*A. fruticosum site (2)*		*S. marina*		*S. aegyptiaca*	
		Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm
1	Pyrogallol	4.23	391.94	4.192	342.26	4.061	107.01	-	-	-	-	-	-	-	-
2	Gallic	4.729	25.44	4.767	33.59	4.730	33.82	3.369	1453.34	3.369	3605.81	3.369	565.14	3.368	246.77
3	3-OH Tyrosol	5.823	17.15	5.935	29.84	5.803	5.66	-	-	-	-	-	-	-	-
4	Catechol	6.109	558.75	6.193	355.48	6.040	180.84	6.875	125.35	6.782	0	6.858	812.77	6.782	0
5	4-Aminobenzoic	6.518	21.53	6.481	41.53	6.470	10.24	-	-	-	-	-	-	-	-
6	Catechein	-	-	-	-	6.623	387.77	4.651	144.48	4.652	524.21	4.603	0.00	4.638	36.71
7	Chlorogenic	-	-	7.350	214.77	7.466	220.48	-	0	4.219	42.99	4.328	3695.17	4.218	377.44
8	Benzoic	7.637	246.22	7.512	228.40	-	-	-	-	-	-	-	-	-	-
9	p-OH-benzoic	-	-	-	-	7.513	13.86	-	-	-	-	-	-	-	-
10	Caffeic	7.957	238.46	7.887	374.10	7.830	238.74	-	0	5.882	79.29	6.104	22.40	6.059	0
11	Vanillic	-	-	8.129	87.94	8.057	121.96	9.842	170.39	9.848	296.51	9.701	18.94	9.667	2651.86
12	Caffeine	-	-	8.702	142.57	8.643	109.16	-	-	-	-	-	-	-	-
13	Ferulic	9.592	90.63	9.765	518.54	9.720	336.43	14.168	34.03	10.216	193.27	10.378	65.78	10.269	57.33
14	Ellagic	10.499	562.42	10.483	1074.08	10.433	648.68	8.882	208.35	8.890	520.08	8.826	1386.61	8.973	0
15	Coumarin	11.478	141.69	11.391	45.49		77.20	-	-	9.180	0	9.383	479.41	-	0
16	Methyl gallate	-	-	-	-	-	-	5.415	88.01	5.398	336.97	5.551	259.94	5.582	62.69
17	Syringic acid	-	-	-	-	-	-	6.390	578.57	6.389	1342.67	6.442	120.57	6.661	12.77
18	Cinnamic acid	-	-	-	-	-	-	14.168	3.19	14.162	10.44	13.733	46.80	13.955	8.50

Table 5: Identification of the active flavonoid compounds from Halophyte (terrestrial and aquatic) plants ethanolic extracts using HPLC

No	Flavonoids	*H. uninervis*		*T. ciliatum*		*H. stipulacea*		*A. fruticosum1*		*A. fruticosum2*		*S. marina*		*S. aegyptiaca*	
		Rt.	Ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm	Rt.	ppm
1.	Rutin	9.903	39.6	-	-	9.910	42.4	7.903	65.96	7.865	175.24	7.985	378.56	8.176	31.47
2.	Naringin	10.108	145.6	10.150	1906.1	10.057	235.2	-	-	-	-	-	-	-	-
3.	Rosmarinic	10.341	30.9	10.293	85.7	10.343	18.2	-	-	-	-	-	-	-	-
4.	Quercetrin	10.951	56.0	11.017	63.2	10.927	73.7	-	-	-	-	-	-	-	-
5.	Apignin 7-glugose	11.856	82.3	11.843	391.6	11.814	61.2	-	-	-	-	-	-	-	-
6.	Quercetin	12.153	138.5	12.019	224.8	11.991	140.7	13.074	10.74	12.582	40.72	12.787	35.39	12.819	255.37
7.	Naringenin	12.547	69.4	12.602	432.7	12.556	419.	10.486	37.22	10.482	180.39	10.719	96.09	10.480	6.06
8.	Kampferol 3-2-p-coumaroyl glucose	12.776	726.5	-	-	12.829	342.3	-	-	-	-	-		-	-
9.	Kampferol	13.032	57.8	12.986	176.6	13.030	61.7	15.168	0	15.168	0	15.140	105.33	15.408	12.61
10	Apigenin	14.143		13.990	30.4	14.086	34.9	14.655	0	14.655	0	14.671	7.95	14.692	6.96
11	Daidzein	-	-	-	-	-	-	12.303	15.39	12.303	33.88	12.035	28.10	12.312	150.35

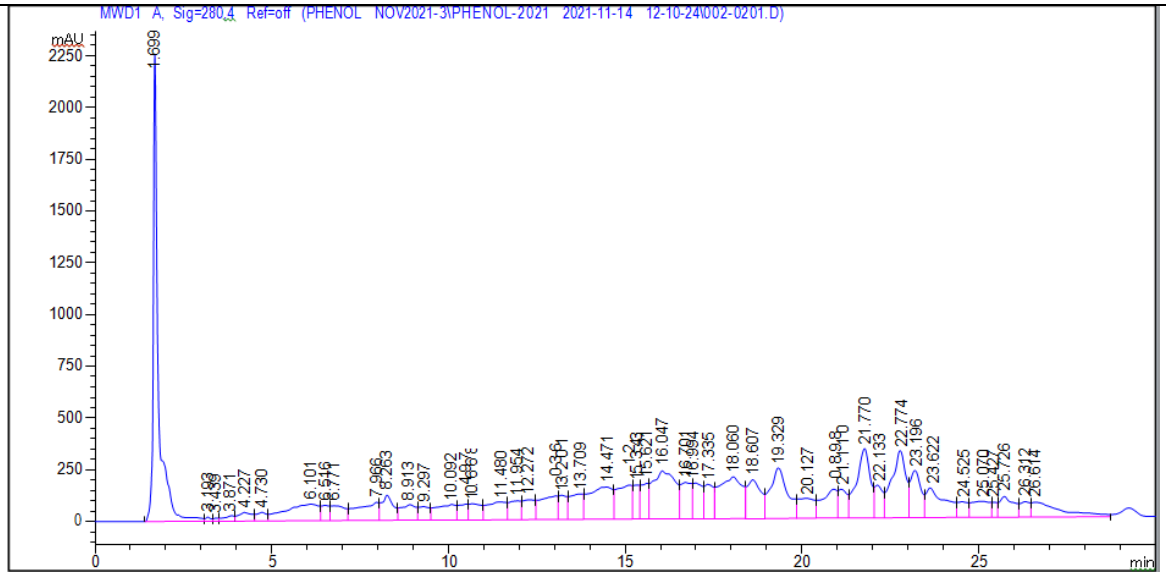


Figure 1: HPLC of phenolic compounds of *H. uninervis* ethanol extract.

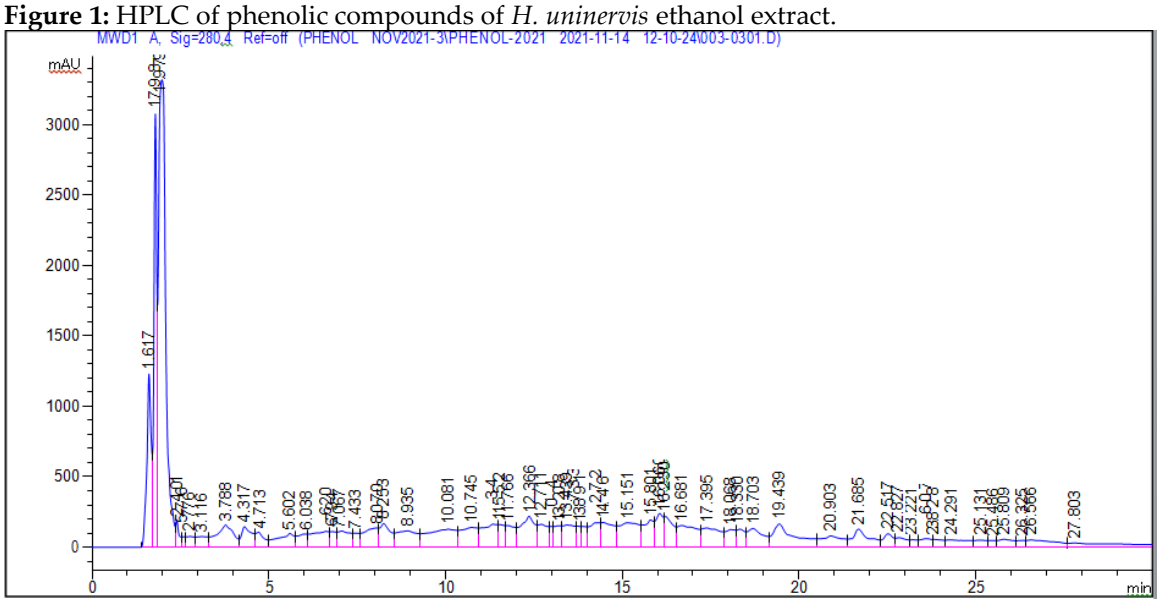


Figure 2: HPLC of phenolic compounds of *H. stipulacea* ethanol extract.

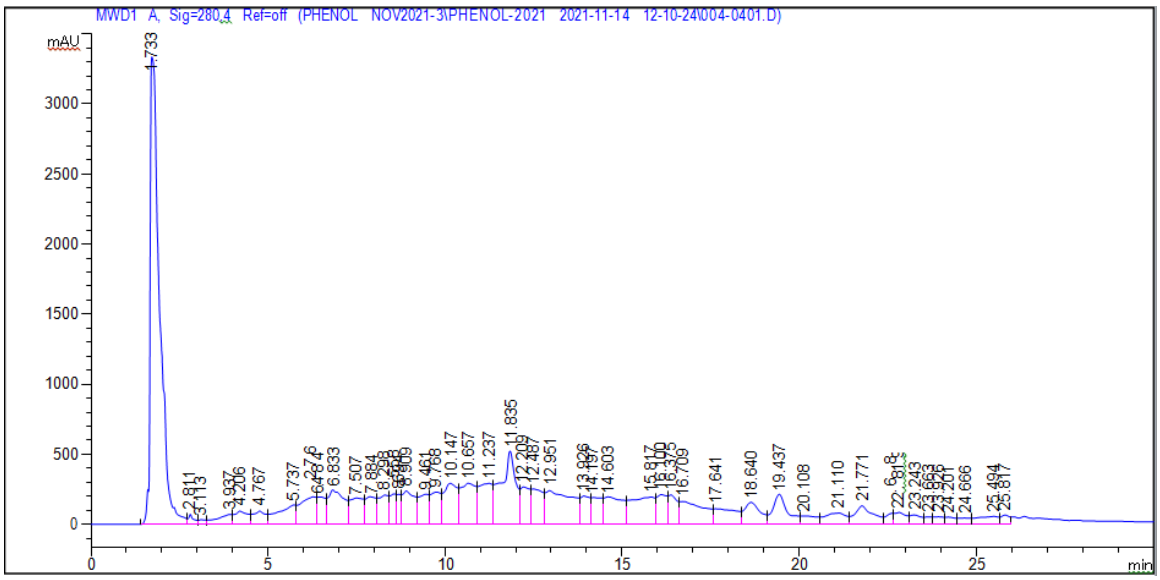


Figure 3: HPLC of phenolic compounds of *T. ciliatum* ethanol extract.

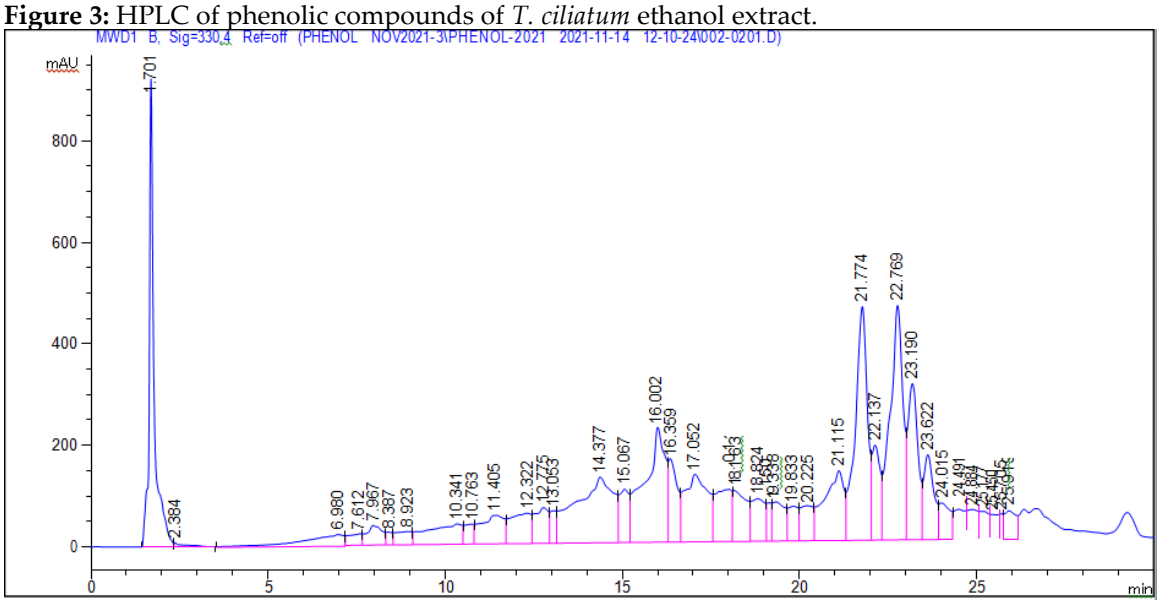


Figure 4: HPLC of flavonoid of *Halodule uninervis* ethanol extract.

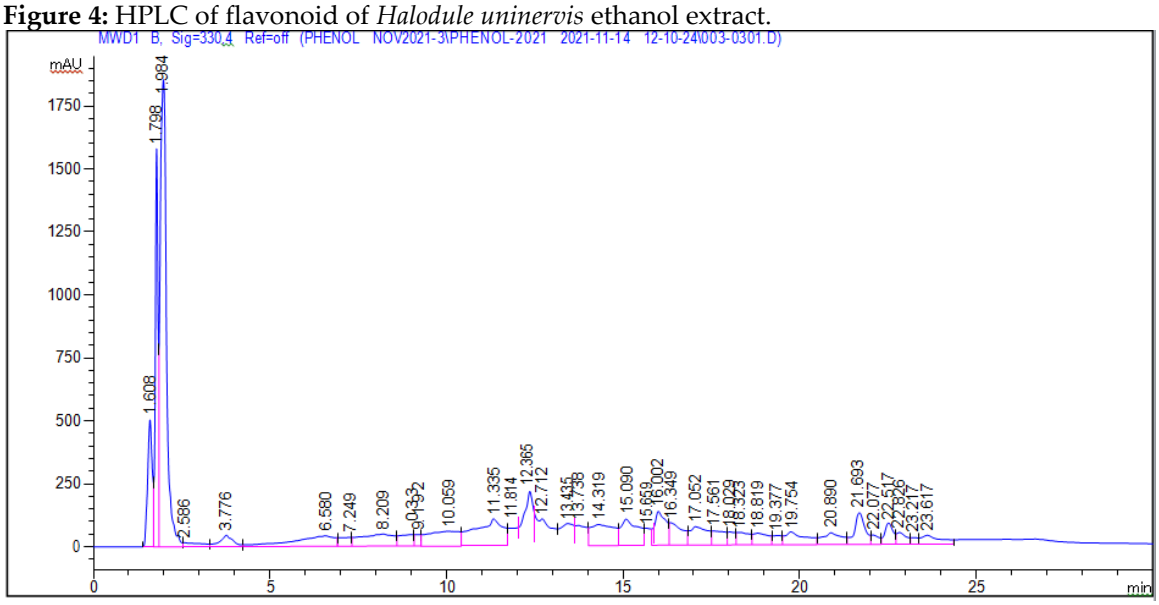


Figure 5: HPLC of flavonoids compounds of *Halophila stipulacea* ethanol extract.

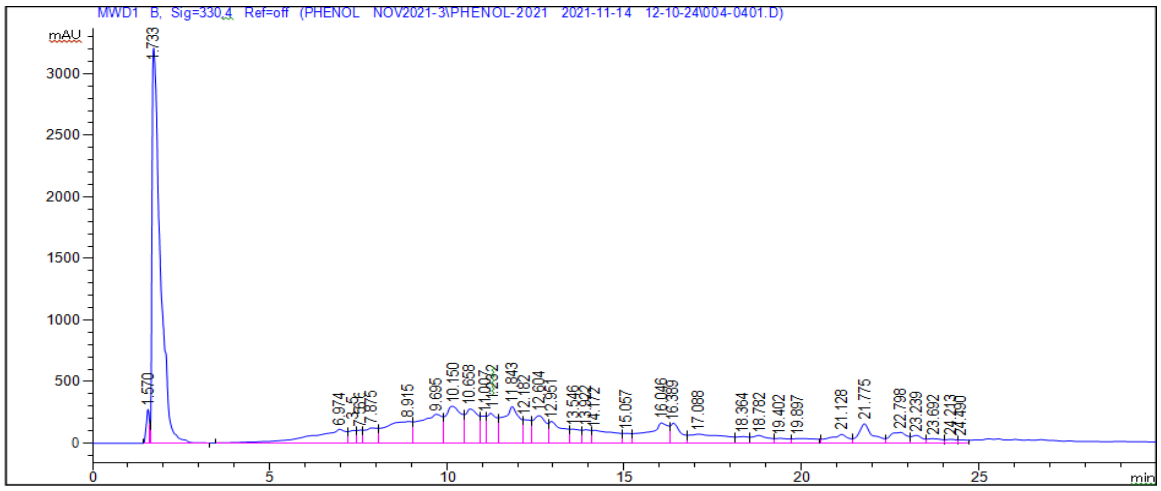


Figure 6: HPLC of flavonoid compounds of *T. ciliatum* ethanol extract.

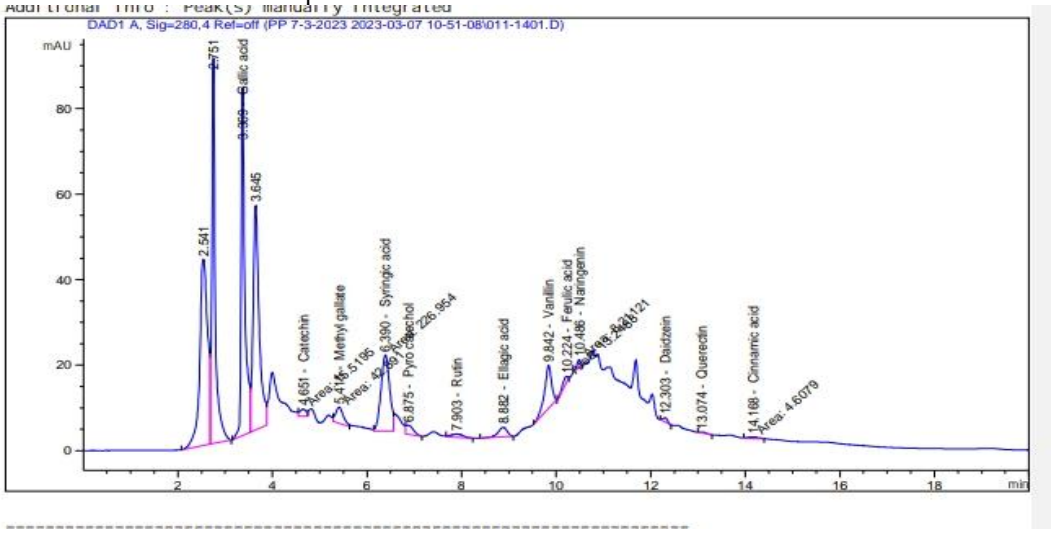


Figure 7: HPLC of phenolic and flavonoid compounds of *A. fruticosum site (1)* ethanol extract.

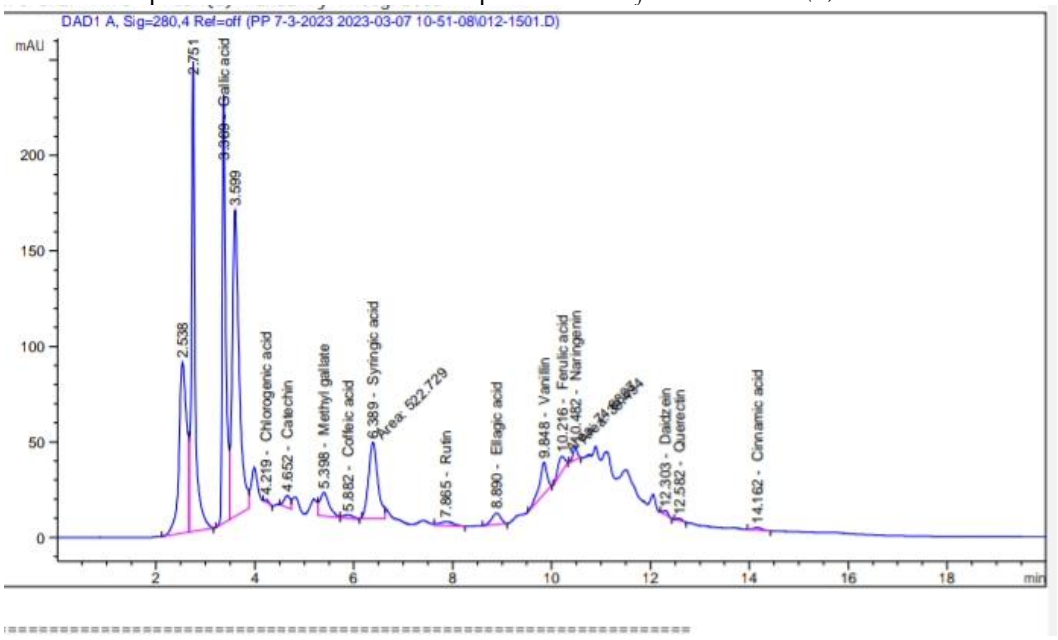


Figure 8: HPLC of phenolic and flavonoid compounds of *A. fruticosum* site (2) ethanol extract.

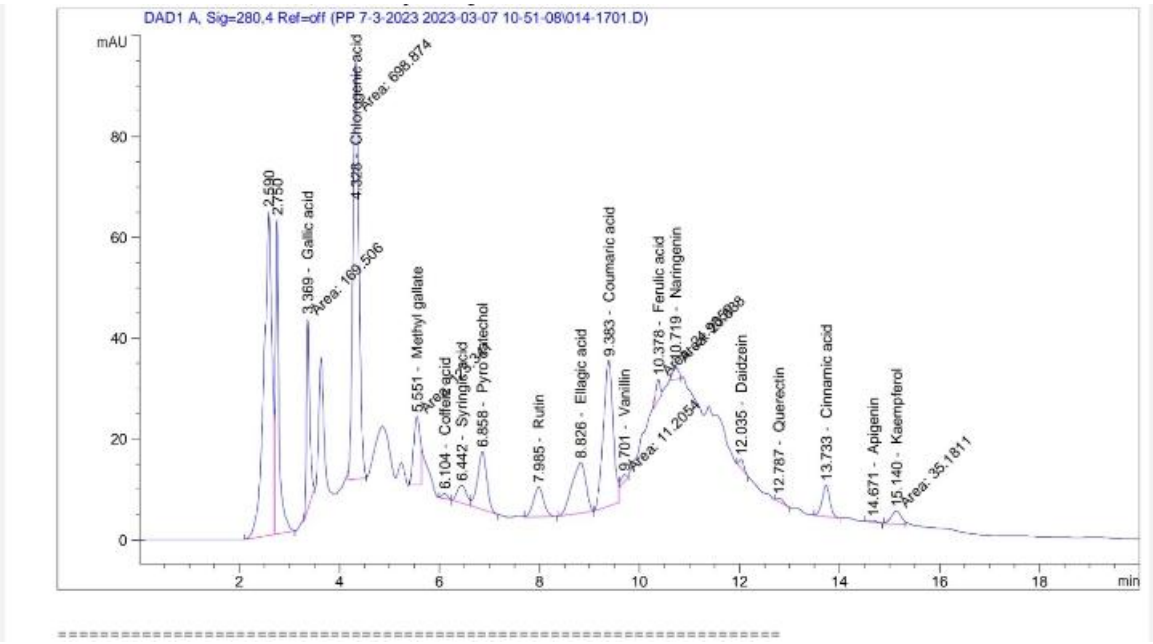


Figure 9: HPLC of phenolic and flavonoid of *S. marina* ethanol extract

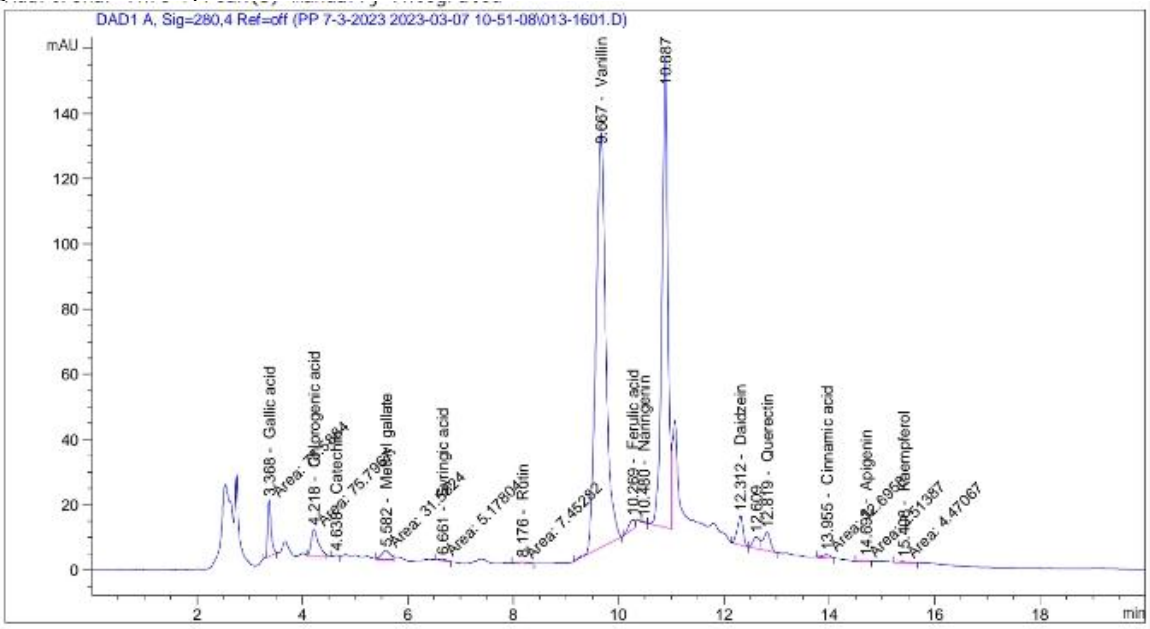


Figure 10: HPLC of phenolic and flavonoid compounds of *S. aegyptiaca* ethanol extract.

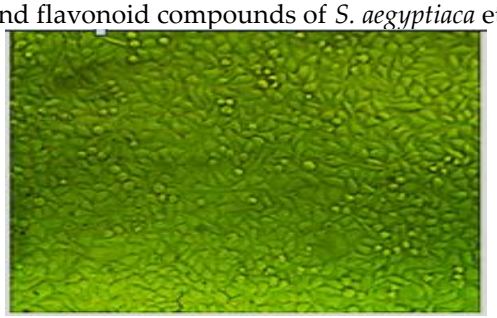


Figure 11 a: Control Caco2 cells

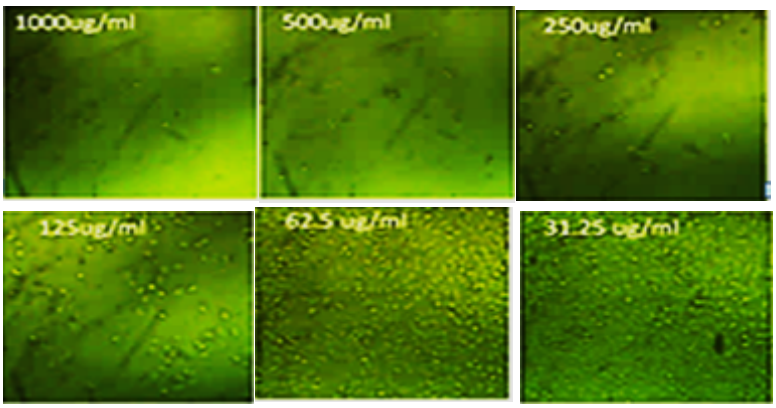


Figure 11 (b): Morphological features by optical microscope after 24 hours for Caco2 cells treated with ethanol extract of *A. fruticosum* site (2) concentrations (1000 ,500 ,250 ,125 µg/mL).

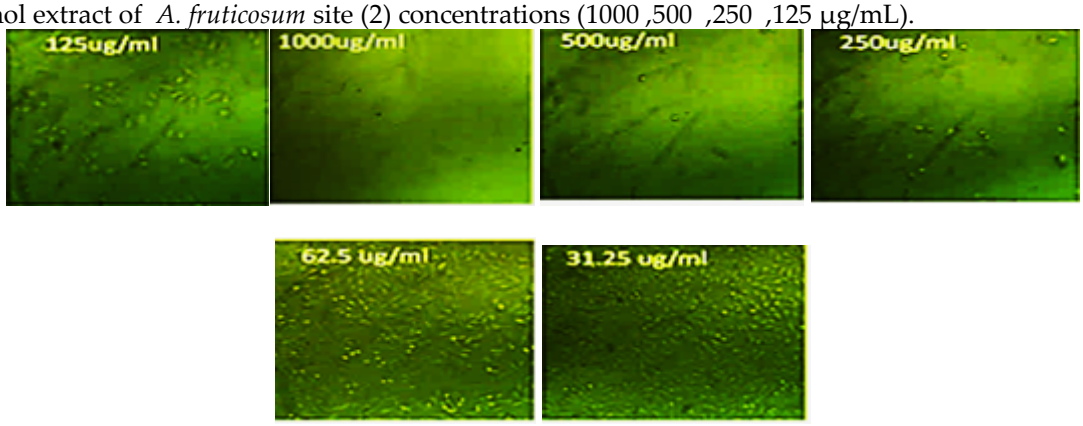


Figure 11 (c): Morphological features by optical microscope after 24 hours for Caco2 cells treated with ethanol extract of *Suaeda* concentrations (1000 ,500 ,250 ,125 µg/mL).

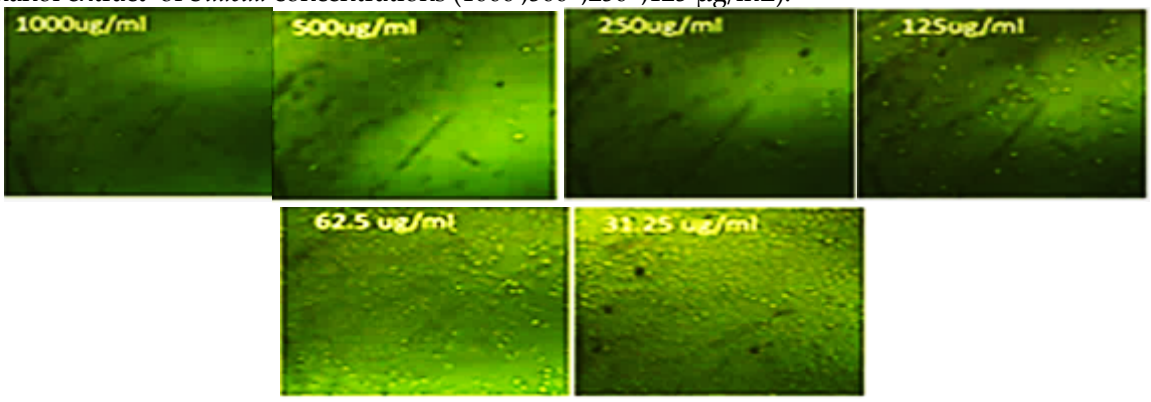
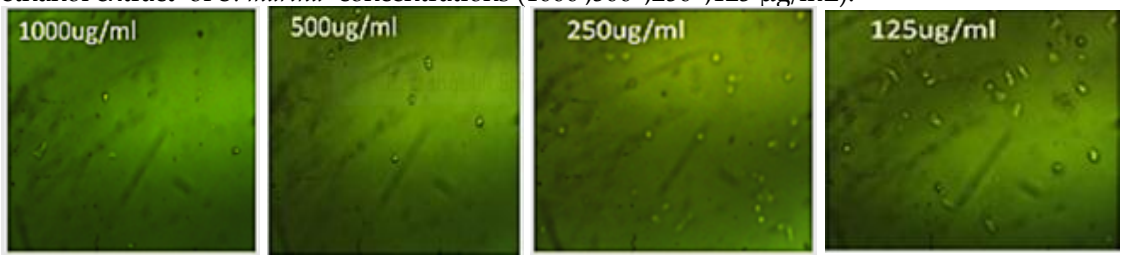


Figure 11 (d): Morphological features by optical microscope after 24 hours for Caco2 cells treated with ethanol extract of *S. marina* concentrations (1000 ,500 ,250 ,125 µg/mL).



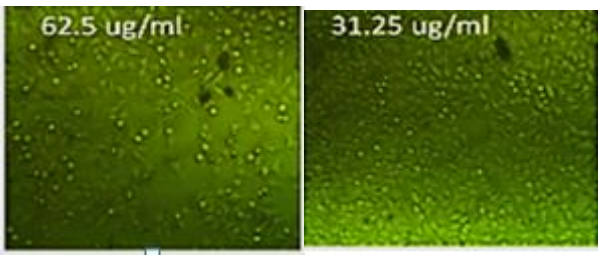


Figure 11 (e): Morphological features by optical microscope after 24 hours for Caco2 cells treated with ethanol extract of *A. fruticosum* site (1) concentrations (1000 ,500 ,250 ,125 µg/mL).

دراسة الفحص الكيميائي النباتي والأنشطة المضادة للسرطان لبعض النباتات الارضية والمائية الناميه في بيئات مالحة

فاطمة شعبان عبدالجواد أحمد [1,*]، البراء صلاح الدين محمود السعيد [2]، زينب أحمد السويفي [1]، روحية عبداللطيف صلاح الدين [1]

[1] *قسم النبات والميكروبيولوجى, كلية العلوم (بنات), جامعة الأزهر, القاهرة, مصر.*
[2] *قسم النبات والميكروبيولوجى, كلية العلوم (بنين), جامعة الأزهر, القاهرة, مصر.*

* البريد الإلكتروني للباحث الرئيسي: fatmaahmed2387.el@azhar.edu.eg

الملخص العربى

النباتات الملحية هي نباتات ذات إمكانات اقتصادية كبيرة لخصائصها الغذائية والطبية. تم استخدام جهاز الكروماتوغرافيا السائل عالي الاداء لتحديد مركبات الفينولات والفلافونويد للمستخلص الايثانولي لستة نباتات ملحية (*Spergularia marina, Suaeda aegyptiaca , Arthrocnemum fruticosum , Halophila stipulacea, Halodule uninervis and Thalassodendron ciliatum*) ، بالإضافة إلى المعلمات الفيزيوكيميائية مثل الرماد الكلي ، الرماد القابل للذوبان في الماء ، الرماد الغير قابل للذوبان ، الكربوهيدرات الكلية ، الكربوهيدرات القابلة للذوبان ، النيتروجين الكلي والبروتين الكلي. أظهرت النتائج أنه تم تأكيد تسعة عشر مركبا من الفينول وأحد عشر مركبا من مركبات الفلافونويد بواسطة HPLC نوعيا وكميا في مستخلصات الإيثانول لجميع النباتات. وكانت المركبات الرئيسية هي الكلوروجينيك ، الغال ، أحماض الفانيليك ، نارينجين ونارينجينين و ايضا تم إجراء تقييم لأنشطتهم المضادة للسرطان ضد سرطان البنكرياس (PA1) ، وسرطان الرئة (A549) ، وسرطان البروستاتا (PC3) ، وسرطان القولون (Caco2) وقد أظهرت الأنشطة المضادة للسرطان للمستخلصات ان النباتات *A. fruticosum* site1 و *S. marina* و *A. fruticosum* site 2 أعلى فعالية لفعالية الأنشطة المضادة للسرطان بقيمة3.04 ± 74.38) IC50 ، 2.11 ± 85و 88.922 ± 0.735 ميكروجرام / مل) على التوالي ، ضد الخلايا القولون السرطانية (caco2) من النباتات الأخرى قيد الدراسة. من هذه الدراسة يمكن اعتبار النباتات الملحية كعامل طبي وصيدلاني واعد.

الكلمات الاسترشادية: مركبات الفينوليك، فلافونويد، الأنشطة المضادة للسرطان.