PLANTS have been used as a source of traditional medicine to treat many diseases and conditions for many years. They considered as excellent source of phytochemicals which showed antioxidant and anticancer activities.

The aim of the present study is to investigate the chemical composition and to determine the anticancer activity of *Salicornia fruticosa* (Chenopodiaceae) methanolic extract. *S. fruticosa* proved to be a source of isorhamnetin and its glycosides and showed anticancer activities. Seven major flavonoids were isolated and identified from the cytotoxic methanolic extract. The isolated compounds were identified, as quercetin 3',4'-dimethyl ether (1), isorhamnetin (2), isorhamnetin 3-O-rhamnoside (3), isorhamnetin 3-O-glucoside (4), isorhamnetin 3-O-rutinoside (5), isorhamnetin 3-O-neohesperidoside (6), isorhamnetin 3-O-rhamnosyl(1-2)arabinoside (7), by chromatographic analysis, chemical and spectroscopic tools (acid hydrolysis, UV, $^1$H and $^{13}$C NMR). Compounds 1 and 3-7 were isolated for the first time from the plant under investigation. The evaluation of cytotoxic activity of the methanolic extract against HCT-116, HepG2, A549 and MCF-7 human cancer cells, by MTT assay, revealed the higher potency of *S. Fruticosa* extract with IC$_{50}$ [2.6, 10.9, 37.9, 5.4 (mg/ml)] respectively, comparable to that of doxorubicin. The obtained results suggested that the investigated plant could be used for future development of naturally occurring anticancer agent. Subclinical and clinical trials on polar fractions of *S. fruticosa* are mandatory to pave the way for its use in treatment of cancer diseases (HCT-116, HepG2, A549 and MCF-7).

**Keywords:** *Salicornia fruticosa*, Chenopodiaceae, Flavonoids. isorhamnetin derivatives, Anticancer activity.

**Introduction**

*Salicornia* species display application on folk medicine for treatment of bronchitis, and diarrhea and showed important biological properties such as antioxidant, anti-inflammatory, antimicrobial, hypoglycemic and cytotoxic activities [1,2,3]. Oral administration of *S. europaea* is used in treatment of goiter, hypertension, cephalalgia and scurvy [4,5]. The phythochemical studies on this genus reported the isolation of the alkaloids, saliherbins and salicomine from *S. herbacea* [6] and chlorogenic acid derivatives were also isolated and tested for their DPPH radical scavenging activity [7]. From the red stems of *S. europaea*, two chromones derivatives were isolated [8]. Flavonoid derivatives (methylenedioxy flavone and isoflavones) and triterpenoid saponin were also reported from *S. europaea* [9,10]. Rare oleanane type 30-nortriterpenoids were reported from *S. bigelovii* [5]. Ferulic, acetic and galacturonic acids, arabinose and glucose were isolated from the cell walls of *S. ramosissima* [11]. The antioxidant activity, total phenolics and flavonoids of *S. brachiate* were also reported [12].

*Salicornia fruticosa* L. (also known as glasswort) is annual succulent herb of Chenopodiaceae family and one of the most salt tolerant plants. It is apparently leafless herb that have articulated and succulent stems [13]. It is growing on salt marshes and muddy seashores [14] and this family is represented in Egypt by 25 genera and about 300 species. Several species possess...
antibacterial and antihypertensive properties, also mentioned in folk medicine for relief of toothache and chronic rheumatic. Investigation of certain species of this family revealed that they contain large amounts of minerals, essential amino acids, essential fatty acids, coumarins, phenolic compounds and alkaloids [15].

Little has been reported on the isolation of phenolic constituents from *S. Fruticosa*. The available reports are those dealing with the isolation of acacetin and apigenin glucoside [15]. They also reported that n-butanol and ethyl acetate extract of *S. Fruticosa* display high DPPH radical scavenging effect, which is an evidence for its antioxidant activity. Also, the utilization of *S. Fruticosa* herb for producing antioxidants was studied [16]. The antioxidant and antimicrobial activities of the methanolic extract in minced beef was previously investigated [17].

The present study deals with the isolation and structure elucidation of seven flavonoids, six of them were isolated for the first time from *S. fruticosa*. Also, evaluation of cytotoxic activity of the methanol extract against HCT-116, HepG2, A549 and MCF-7 human cancer cell lines is reported as the anticancer activity has not been reported before.

![Flavonoid structures](image)

4. R= glucosyl; 5. R= rutinosyl
6. R= neohesperidosyl;
7. R= rhamnosyl(1-2)arabinosyl

**Materials and Methods**

**Plant material**

*S. fruticosa* L., family Chenopodiaceae, was collected in May 2016 from Serapium Road, El-Ismailia city and identified by Prof. S.A. Kawashty. A voucher specimen (no. 211) was deposited in the herbarium of the National Research Centre (CAIRC) [18]. The plant was left to dry in the shade at room temperature till reach a constant weight.

**Extraction and isolation**

Fine air-dried powder of *S. fruticosa* plant (500 g) was extracted with 70% methanol twice for two days, filtered and concentrated under reduced pressure. The obtained extract (60 g) was subjected to Silica gel column using chloroform-methanol system as an eluent in ascending sequence of polarity. Fractions eluted by 20% MeOH-CHCl₃ afforded the aglycones 1 and 2, while fractions eluted by 50% MeOH-CHCl₃ gave the monoglycosides 3 and 4. The diglycoside compounds 5, 6, and 7 were eluted from the column using 80% MeOH-CHCl₃. The collected fractions were further purified on Whatman 3 MM paper chromatography using different solvent systems. Final purification was achieved by Sephadex LH-20 column.

**Structure elucidation of the isolated compounds**

NMR spectra were recorded on a Jeol EX-500 spectrometer: 500 MHz (¹H NMR), 125 MHz (¹³C NMR). UV spectrophotometric analyses with Shimadzu UV-240. CC using Silica gel 60 (Merck, 0.063-0.2 mm) using CHCl₃/MeOH (9:1-9:9). For elution PC (descending) using Whatman No. 1 and 3 MM papers, and solvent systems: 1) H₂O, 2) 15% HOAc (H₂O–HOAc 85:15), 3) 50% H₂OAc (H₂O–HOAc 50:50), 4) BAW (n-BuOH–HOAc–H₂O 4:1:5, upper phase), 5) BBPW (C₆H₆–n-BuOH–pyridine–H₂O, 1:5:3:3, upper phase). Solvent 5 was used for sugar detection. Sephadex LH-20 (Pharmacia) eluted with methanol. Acid hydrolysis for O-glycosides (2N HCl, 2 hrs, 100 °C) were carried out and followed by paper co-chromatography with authentic samples to
identify the aglycones and sugar moieties.

**In-vitro cytotoxic activity**

Cell culture of HCT-116 (human colorectal carcinoma), HepG2 (human hepatocellular carcinoma), A549 (human lung carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO₂ (Regulatory Toxicology Lab, Centre of Excellence, NRC).

**MTT cytotoxicity assay**

The cytotoxicity activities on HCT-116, HepG2, A549 and MCF-7 human cancer cell lines were estimated using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the reduction of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [19,20,21]. Cells were dispensed in a 96 well sterile microplate (5 x 10⁴ cells/well), and incubated at 37°C with series of different concentrations, in DMSO, of the extract or doxorubicin (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm using a Spectra Max Paradigm Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in triplicate and repeated on three different days. All the values were represented as mean ±SD. IC₅₀'s were determined by probit analysis by SPSS Inc probit analysis (IBM Corp., Armonk, NY, USA).

**Compounds characterizations**

**Quercetin 3’,4’-dimethyl ether (1)**

R₂: 0.33 (BAW), 0.70 (50% AcOH), 0.50 (Phenol). UV/Vis λₑₓₘᵦₜ (MeOH): 255, 302, 371. ¹H-NMR in DMSO-d₆: δ 7.76 (1H, d, J = 2 Hz, H-2’), δ 7.77 (1H, d, J = 8 Hz, H-6’), δ 6.94 (1H, d, J = 8 Hz, H-5’), δ 6.48 (1H, d, J = 2 Hz, H-8), δ 6.20 (1H, d, J = 2 Hz, H-6), δ 3.85 (6H, s, OCH₃).

**Isorhamnetin 3-O-β-D-glucopyranoside (4)**

R₂: 0.41 (BAW), 0.15 (H₂O), 0.28 (15% AcOH). UV/Vis λₑₓₘᵦₜ (MeOH): 255, 268sh, 298sh, 356. ¹H-NMR in DMSO-d₆: δ 7.83 (1H, d, J = 2 Hz, H-2’′), δ 7.70 (1H, d, J = 8 Hz, H-6’), δ 6.90 (1H, d, J = 8 Hz, H-5’), δ 6.44 (1H, d, J = 2 Hz, H-8), δ 6.21 (1H, d, J = 2 Hz, H-6), δ 5.32 (1H, d, J = 7.5 Hz, H-1″), δ 3.85 (3H, s, OCH₃).

**Isorhamnetin 3-O-α-L-rhamnopyranoside (5)**

R₂: 0.25 (BAW), 0.45 (H₂O), 0.52 (15% AcOH). UV/Vis λₑₓₘᵦₜ (MeOH): 255, 268sh, 296sh, 356. ¹H-NMR in DMSO-d₆: δ 7.89 (1H, d, J = 2 Hz, H-2’), δ 7.49 (1H, d, J = 9 Hz, H-6’), δ 6.92 (1H, d, J = 8 Hz, H-5’), δ 6.37 (1H, d, J = 2 Hz, H-8), δ 6.15 (1H, d, J = 2 Hz, H-6), δ 5.42 (1H, d, J = 7.5 Hz, H-1″), δ 4.44 (1H, br.s, H-1‴), δ 3.85 (3H, s, OCH₃), δ 1.05 (3H, d, J = 5.5 Hz, H-6‴), 13C-NMR in DMSO-d₆: δ 177.32 (C-4), 165 (C-7), 161.24 (C-5), 156.56 (C-2), 156.47 (C-9), 149.5 (C-3′), 147 (C-4′), 133 (C-3), 122.31 (C-6′), 121.17 (C-1′), 115.32 (C-5′), 113.41 (C-2′), 103.8 (C-10), 101.9 (C-1″), 100.1 (C-1‴), 99 (C-6), 94 (C-8), 76.12 (C-3″), 76.43 (C-5″), 71.94 (C-4″), 71.28 (C-2″), 70.77 (C-3″′), 68.43 (C-2″′), 68.47 (C-5″′), 68.18 (C-4″), 66.91 (C-6″), 17.82 (C-6″′).

**Isorhamnetin 3-O-neohesperidoside (6)**

R₂: 0.30 (BAW), 0.42 (H₂O), 0.52 (15% AcOH). UV/Vis λₑₓₘᵦₜ (MeOH): 255, 269sh, 295sh, 356. ¹H-NMR in DMSO-d₆: δ 7.89 (1H, d, J = 2 Hz, H-2’), δ 7.50 (1H, d, J = 7.6 Hz, H-6’), δ 6.93 (1H, d, J = 7.6 Hz, H-5’), δ 6.41 (1H, d, J = 2 Hz, H-8), δ 6.19 (1H, d, J = 2 Hz, H-6), δ 5.40 (1H, d, J = 7.3 Hz, H-1″), δ 5.21 (1H, d, J = 2.2 Hz, H-1‴), δ 3.85 (3H, s, OCH₃), δ 1.05 (3H, d, J = 6.0 Hz, H-6‴).
showed downfield (≈6 ppm) for C-6 and C-2 of the sugars were identified as rhamnose for 3 and 3′ [27]. Acid hydrolysis of these compounds indicated a flavonol with occupations at positions 3 and 3′ [27]. The signals at d 4.44 and 5.21 were assigned to the terminal rhamnose H-1 for compounds 5 and 6. The coupling constants 2.5 and 2.2 Hz indicated α-configuration [23,33]. The $^{13}$C-NMR spectra of 5 and 6 corresponded well to the shift of isorhamnetin. The only difference being upfield shift by approximately 2.5 ppm. This shift is reported when the hydroxyl group at C-3 of the aglycone is glucosylated [34]. Also the spectra showed downfield (≥6 ppm) for C-6 and C-2 of the glucose moieties in 5 and 6 and upfield shift of the signal assigned to C-1 glucose in compound 6 (=2 ppm) confirming the rhamnosylation. Thus 5 and 6 were identified as isorhamnetin 3-O-rutinoside [33] and isorhamnetin 3-O-neohesperidoside [35].

Compound 7 showed $^1$H-NMR signal at d 5.41 assigned for H-1 arabinose and this chemical shift confirmed that arabinose is attached to the isorhamnetin directly. The coupling constant J=7.5 Hz indicated the β-configuration of glucose. While the signals at d 4.44 and 5.21 were assigned to the terminal rhamnose H-1 for compounds 5 and 6. The coupling constants 2.5 and 2.2 Hz indicated α-configuration [23,33]. The $^{13}$C-NMR spectra of 5 and 6 corresponded well to the shift of isorhamnetin. The only difference being upfield shift by approximately 2.5 ppm. This shift is reported when the hydroxyl group at C-3 of the aglycone is glucosylated [34]. Also the spectra showed downfield (≥6 ppm) for C-6 and C-2 of the glucose moieties in 5 and 6 and upfield shift of the signal assigned to C-1 glucose in compound 6 (=2 ppm) confirming the rhamnosylation. Thus 5 and 6 were identified as isorhamnetin 3-O-rutinoside [33] and isorhamnetin 3-O-neohesperidoside [35].

$^{1}$H-NMR spectra of 5 and 6 indicated that both are diglycosides of isorhamnetin on the basis of H-1 of glucose and rhamnose. The signals at d 5.42 for 5 and 5.40 for 6 were assigned to the H-1 of glucose. This chemical shift confirmed that glucose is directly attached to the aglycone and the coupling constant (J=7.5 Hz) indicated the β-configuration of glucose. While the signals at d 4.44 and 5.21 were assigned to the terminal rhamnose H-1 for compounds 5 and 6. The coupling constants 2.5 and 2.2 Hz indicated α-configuration [23,33]. The $^{13}$C-NMR spectra of 5 and 6 corresponded well to the shift of isorhamnetin. The only difference being upfield shift by approximately 2.5 ppm. This shift is reported when the hydroxyl group at C-3 of the aglycone is glucosylated [34]. Also the spectra showed downfield (≥6 ppm) for C-6 and C-2 of the glucose moieties in 5 and 6 and upfield shift of the signal assigned to C-1 glucose in compound 6 (=2 ppm) confirming the rhamnosylation. Thus 5 and 6 were identified as isorhamnetin 3-O-rutinoside [33] and isorhamnetin 3-O-neohesperidoside [35].

## Results and Discussion

The cytotoxic methanolic extract of *S. fruticosa* afforded seven flavonoids, six of them (1, 3-7) were isolated for the first time from the plant. The isolated glycosides are mainly isorhamnetin derivatives. The isorhamnetin and its glycoside derivatives are biologically active compounds [22-26]. Thus, the activity of the extract may be related to the existence of the isorhamnetin derivatives.

### Compounds identification

Compounds 1 and 2 were isolated in free form and showed chromatographic properties, UV and $^1$H-NMR data similar to those reported for 3′,4′-dimethoxyquercetin and isorhamnetin [27-30].

The UV spectral data, of the rest of the compounds, with diagnostic shift reagents indicated a flavonol with occupations at positions 3 and 3′ [27]. Acid hydrolysis of these compounds gave isorhamnetin as the main aglycone, while the sugars were identified as rhamnose for 3, glucose for 4, rhamnose and glucose for 5 and 6 and rhamnose and arabinose for 7 (Co-chromatography with authentic samples). Compounds 3 and 4 showed $^1$H-NMR spectra characterized to isorhamnetin glycosides. Thus compounds 3 and 4 are monoglycoside derivatives and identified as isorhamnetin 3-O-rhamnoside and isorhamnetin 3-O-glucose [27,31].

The $^1$H-NMR spectra of 5 and 6 indicated that both are diglycosides of isorhamnetin on the basis of H-1 of glucose and rhamnose. The signals at d 5.42 for 5 and 5.40 for 6 were assigned to the H-1 of glucose. This chemical shift confirmed that glucose is directly attached to the aglycone and the coupling constant (J=7.5 Hz) indicated the β-configuration of glucose. While the signals at d 4.44 and 5.21 were assigned to the terminal rhamnose H-1 for compounds 5 and 6. The coupling constants 2.5 and 2.2 Hz indicated α-configuration [23,33]. The $^{13}$C-NMR spectra of 5 and 6 corresponded well to the shift of isorhamnetin. The only difference being upfield shift by approximately 2.5 ppm. This shift is reported when the hydroxyl group at C-3 of the aglycone is glucosylated [34]. Also the spectra showed downfield (≥6 ppm) for C-6 and C-2 of the glucose moieties in 5 and 6 and upfield shift of the signal assigned to C-1 glucose in compound 6 (=2 ppm) confirming the rhamnosylation. Thus 5 and 6 were identified as isorhamnetin 3-O-rutinoside [33] and isorhamnetin 3-O-neohesperidoside [35].

Compound 7 showed $^1$H-NMR signal at d 5.41 assigned for H-1 arabinose and this chemical shift confirmed that arabinose is attached to the isorhamnetin directly. The coupling constant J=6.8 Hz indicated b-configuration. The $^{13}$C-NMR spectra of the sugar moieties of 7 showed downfield shift of the signal assigned to C-1 arabinose in compound 6 (=2 ppm) confirming the rhamnosylation. Thus 5 and 6 were identified as isorhamnetin 3-O-rutinoside [33] and isorhamnetin 3-O-neohesperidoside [35].

## Cytotoxicity screening

The methanolic extract of *S. fruticosa* is rich in isorhamnetin and its glycosides which reported to have cytotoxic activity against lung cancer [22], esophageal cancer cells [23] and liver carcinoma cells [24].

The methanolic extract was examined in vitro for its activity against HCT-116, HepG2, A549 and MCF-7 human cancer cells using the MTT assay. The percentages of intact cells were calculated and compared to those of the control. Activities...
of the extract against the four carcinoma cell lines were compared to the activity of doxorubicin as well. The extract suppressed the four cancer cells in a dose-dependent manner (Figs. 1-4). In case of HCT-116 human colorectal carcinoma cells, both Figure 1 and Table 1 showed that the extract was more potent cytotoxic as it showed 3.6 folds higher activity compared to that of doxorubicin. In case of MCF-7 human breast cancer cells, the extract was more potent cytotoxic as it showed 1.2 folds higher activity compared to that of doxorubicin (Figure 2 & Table 1). In case of HepG2 human liver carcinoma cells, the extract was equipotent to the reference drug doxorubicin (Figure 3 & Table 1). In case of A549 human lung carcinoma cells, the extract was more potent cytotoxic as it showed 1.5 folds higher activity compared to that of doxorubicin (Figure 4 & Table 1). From the above results, one can conclude that this extract is potent anticancer drug candidate for human colon, breast, lung and liver cancers. The obtained results are in accordance with a previous report that the extract of *S. europaea* L. showed significant cytotoxic activity against *Artemia salina* L. and *Daphnia magna* S and moderate antineoplastic activity [37]. Also, the results are in accordance with that reported for *S. herbacea* seeds extract which showed potent antioxidant and cytotoxic activities against HCT 116 and HT-29 colon cancer cells [38]. Also, it was reported for the treatment of intestinal aliment, nephropathy, cancer and tumours [39,40].

The isorhamnetin and its glycoside derivatives are interesting phytochemicals and are biologically active compounds [22,26]. Thus the cytotoxic activity of *S. fruticosa* methanol extract may be related to the existence of the isorhamnetin glycoside derivatives. These findings are in agreements with other reported data on the use of *S. herbacea* flavonoids, isorhamnetin 3-O-glucopyranoside and quercetin 3-O-glucopyranoside as natural chemopreventive agents for cancer, they successfully suppressed radical mediated DNA damage and had inhibitory effect on matrix metalloproteinase-9 (MMP-9) [41].

**Conclusion**

In the present investigation, we reported the isolation of six flavonoid compounds for the first time from *S. fruticosa*. The methanolic extract showed potent anticancer activity against human colon, breast, lung and liver cell lines. The methanolic extract is rich in isorhamnetin derivatives. The methanolic extract is rich in isorhamnetin derivatives. The isorhamnetin and its glycosides are known to have anticancer activity. The obtained results suggested that the herb could be used for future development of naturally occurring anticancer agent and may add a new property to the extract to act as potent drug. The anticancer mechanism of the extract and its valuable content need a greater research effort to confirm its medicinal industry.

**Acknowledgement**

The authors are grateful to NRC (National Research Centre) for supporting the present work through internal project 11010328.

TABLE 1: IC$_{50}$ of the extract against the four cancer cell lines according to the MTT assay

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC$_{50}$ ($\mu$g/ml) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extract</td>
</tr>
<tr>
<td>HCT-116</td>
<td>2.6 ± 1.3</td>
</tr>
<tr>
<td>MCF-7</td>
<td>5.4 ± 1.9</td>
</tr>
<tr>
<td>HepG2</td>
<td>10.9 ± 2.9</td>
</tr>
<tr>
<td>A549</td>
<td>37.9 ± 4.1</td>
</tr>
</tbody>
</table>
Figure 1. Dose dependent cytotoxic activities of the extract against HCT-116 cancer cells according to the MTT assay.

Figure 2. Dose dependent cytotoxic activities of the extract against MCF-7 cancer cells according to the MTT assay.
Figure 3. Dose dependent cytotoxic activities of the extract against HepG2 cancer cells according to the MTT assay.

Figure 4. Dose dependent cytotoxic activities of the extract against A549 cancer cells according to the MTT assay.
References


