Bioactive Phenolic Compounds from the Egyptian Red Sea
Seagrass *Thalassodendron ciliatum*

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Five flavonoids (rutin, asebotin, 3-hydroxyasebotin, quercetin-3-O-\(\beta\)-D-xylopyranoside,
and a racemic mixture of catechin) and caffeic acid were isolated and identified for the first
time from seagrass, *Thalassodendron ciliatum*, collected from the Hurghada region in Egypt.
The crude extract and the isolated pure compounds were evaluated for their cytotoxic activi-
ties against HCT-116, HEPG, MCF-7, and HeLa human cancer cell lines, for their antiviral
activity against Herpes Simplex and hepatitis A viruses, and for their antioxidant activity.

**Key words:** Flavonoids, Red Sea, *Thalassodendron ciliatum*

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**Introduction**

Seagrass is part of a critical, albeit fragile, eco-
system, widely distributed along temperate and
tropical coastlines of the world. It is the only
marine flowering plant which often lives entirely
submerged and completes its life cycle in sea-
water (Short *et al.*, 2007). It has ecological im-
portance (Hemminga and Duarte, 2000) and is
used as a traditional medicine (Torre-Castro and
Rönnbäck, 2004).

Interest in marine organisms as sources of
new substances is growing. With marine species
comprising approximately half of the total global
biodiversity, the sea offers an enormous resource of
novel compounds (De Vries and Beart, 1995),
and it has been classified as the largest remaining
reservoir of natural molecules to be evaluated for
drug activity (Gerwick and Bernart, 1993). Most
studies have focused on the importance of coral
reefs and mangroves, limited studies are found
on seagrass although it has been reported that
this plant is a rich source of secondary metabo-
lites, particularly phenolic compounds (McMillan
*et al.*, 1980) which are interesting for their bio-
logical activities.

Few reports exist on the biological activity of
either crude extracts or isolated compounds from
seagrass (Achamlale *et al.*, 2009; Kontiza *et al*.,
2005, 2008; Rowley *et al*., 2002; Carbone *et al*.,
2008). *Thalassodendron ciliatum* (Forssk.) Hartog
is one of the most common and longest seagrasses
along the Egyptian Red Sea. It is characterized by
many ‘tannin cells’ in its leaves, more than in any
other seagrass (Lipkin, 1988), which means a high
phenolic content. There are no previous studies in
the literature on the chemical constituents and bi-
ological activities of this species. Thus, for the best
of our knowledge, this is the first report on the
chemistry and biological activities of *T. ciliatum*.

**Results and Discussion**

As mentioned above, *T. ciliatum* is considered a
rich source of phenolic compounds. In this study,
five flavonoids and a phenolic acid have been iso-
lated for the first time from the methanolic ex-
tract of *T. ciliatum* collected from the shores of
the Red Sea: rutin (quercetin-3-O-rutinoside, 1) (Chaurasia and Wichl, 1987), asebotin (2',4,6'-tri-hydroxy-4'-methoxydihydrochalcone 2'-O-β-D-glucopyranoside, 2; Fig. 1) (Fuendjiep et al., 2002), 3-hydroxyasebotin (3; Fig. 1) (Yao et al., 2005), quercetin-3-O-β-D-xylopyranoside (4) (Debella et al., 2000), and trans-cafeic acid (6), in addition to a racemic mixture of (+)-catechin and (−)-catechin (5) (Davis et al., 1996).

Asebotin (2) and 3-hydroxyasebotin (3) are the first dihydrochalcones isolated from a marine organism. In addition, this is the second report for 3 as a naturally occurring compound, because it has previously been found only in the leaves of Pieris japonica by Yao et al. (2005).

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of the T. ciliatum total methanolic extract was found to be (71 ± 1.09)% at 1 mg/mL. In comparison, rutin (1) showed higher activity with an EC_{50} value of 0.99 mM than quercetin-3-O-β-D-xylopyranoside (4) (1.63 mM), 3-hydroxyasebotin (3) (1.62 mM), caffeic acid (6) (3.50 mM), and the racemic mixture of catechins (5) (3.82 mM), while asebotin (2) was inactive. The difference in antioxidant activity is related to the number and positions of hydroxy groups and the nature of any other substitutions on the aromatic rings (Nagendran et al., 2006; Heijnen et al., 2001); the hydroxy groups of the sugar moiety do not contribute to the antioxidant activity (Rezk et al., 2002). Concerning the dihydrochalcones Kozlowski and co-workers (2007) have shown the importance of the double bond between C8 and C9 for the redox capacity. Its absence decreases the antioxidant activity, and it was found that antioxidant activity depends on the presence of a hydroxy substitution at the C2, C6, and C4' positions (Bentes et al., 2011). The active dihydrochalcone has a conformation in which the aromatic A ring is orthogonal to the carbonyl group, while the inactive dihydrochalcone has a strongly hydrogen-bound phenolic hydroxy group, suggestive of a coplanar conformation. A hydroxy group at the 2' position of the dihydrochalcone A ring is an essential pharmacophore for radical scavenging potential (Nakamura et al., 2003).

Cytotoxic activity

The crude extract exhibited cytotoxic activity against HCT-116, HEPG, MCF-7, and HeLa human cancer cell lines with IC_{50} values of (4.2 ± 0.51), (8.12 ± 0.34), (4.12 ± 0.77), and (9.8 ± 0.25) μg/mL, respectively. Quercetin-3-O-β-D-xylopyranoside (4) showed higher activities against HEPG cells [(7.25 ± 0.39) μM] and HCT-116 cells [(11.17 ± 0.39) μM], 3-hydroxyasebotin (3) was found to be active against HCT-116 cells [(9.77 ± 0.52) μM], while asebotin (2) was active against HEPG cells [(8.55 ± 0.42) μM] (Table I). 3-Hydroxyasebotin and the racemic mixture of catechin exhibited cytotoxic activity only against the HCT-116 cell line. None of the isolated compounds showed activities against HeLa and MCF-7 cancer cell lines.

In vitro cytotoxic activities of plant polyphenols may be related to their prooxidant activity. Bhat et al. (2007) have proposed that such prooxidant action could be a common mechanism for anti-

Table I. Cytotoxic activity of compounds isolated from T. ciliatum against HCT-116 and HEPG human cancer cell lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HCT-116</th>
<th>HEPG</th>
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<tbody>
<tr>
<td>Rutin (1)</td>
<td>20.0 ± 0.81</td>
<td>32.76 ± 1.6</td>
</tr>
<tr>
<td>Asebotin (2)</td>
<td>14.32 ± 0.88</td>
<td>8.55 ± 0.42</td>
</tr>
<tr>
<td>3-Hydroxyasebotin (3)</td>
<td>9.77 ± 0.52</td>
<td>Inactive</td>
</tr>
<tr>
<td>Quercetin-3-O-β-D-xylopyranoside (4)</td>
<td>11.17 ± 0.39</td>
<td>7.25 ± 0.39</td>
</tr>
<tr>
<td>Racemic mixture of (±)-catechins (5)</td>
<td>49.95 ± 0.96</td>
<td>Inactive</td>
</tr>
<tr>
<td>trans-Cafeic acid (6)</td>
<td>23.03 ± 0.72</td>
<td>17.48 ± 0.38</td>
</tr>
<tr>
<td>Doxorubicin (positive control)</td>
<td>2.33 ± 0.14</td>
<td>2.02 ± 0.07</td>
</tr>
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Fig. 1. Chemical structures of asebotin and 3-hydroxyasebotin isolated from T. ciliatum.
cancer and chemopreventive properties of plant polyphenols. Indeed, a common mechanism would better explain the anticancer effects of polyphenols with diverse chemical structures as well as the preferential cytotoxicity towards cancer cells. The authors hypothesized that the anticancer mechanism of plant polyphenols involves mobilization of endogenous copper, possibly chromatin-bound copper, and the consequent prooxidant action. It is well established that plant polyphenols of various classes behave both as prooxidants and antioxidants.

Catechin, epicatechin, and rutin were reported to exhibit potent anticancer activity (Chen et al., 2009). Individual catechin and epicatechin shows free radical scavenging activities against DPPH radicals (Thiraviam et al., 2004). Epicatechin has low cytotoxicity to human breast cancer cells (MCF-7) with an IC50 value of 102 μM (Le Baila et al., 1998), and it is inactive against lung PC-6 and HEPG2 human cancer cell lines (Takashi et al., 2008). While epicatechin shows activity against HCT-116 cells (Uesato et al., 2001), catechin was found to be effective against pancreatic cancer (McMillan et al., 2007). There is no previous report on the activity of a mixture of catechin and epicatechin.

Caffeic acid exhibits antioxidant activity in vitro and has been shown to have multiple pharmacological properties including inhibition of HIV replication (Kashiwada et al., 1995) and induction of apoptosis in cancer cell lines, and causes tumour growth inhibition and regression in animals (Chung et al., 2004; Orsolic et al., 2004). Caffeic acid shows anticancer activity against HeLa cells at a concentration of 6.25 μg/mL and against subcutaneous tumour growth either given before tumour cell inoculation or after stabilization of tumour growth because of higher intracellular copper levels in cancer cells (Bhat et al., 2007).

**Antiviral activity**

The crude extract showed 100% inhibition of hepatitis A (HAV) and Herpes Simplex (HSV-1) viruses at lowest concentration tested (20 μg/mL). The antiviral activity of the crude extract against HAV was lost by fractionation, which could be explained by the synergistic action of several compounds in the crude extract.

Asebotin (2), quercetin-3-O-β-D-xylopyranoside (4), and trans-caffeic acid (6) showed viral percentage reduction [(96.6 ± 0.91)%, (70 ± 0.98)%, (53 ± 0.77)%, respectively] of plaque formation of HSV-1 at 1 mg/mL and were not cytotoxic.

It has been reported that rutin has no antiviral activity, while sulfated rutin demonstrated some anti-HSV-1 activity with an IC50 value of (88.3 ± 0.1) μM (Orhan et al., 2010; Tao et al., 2007). Caffeic acid exhibits strong activity against HSV-1, HSV-2, and ADV-3, catechin also strongly inhibits replication of acyclovir-resistant HSV-1-ACVr. Compounds with anti-HSV activity have a broad range of structures: polyphenols, polysaccharides, tannins, flavonoids, etc. (Thompson, 2006). The presence of ortho-trihydroxy groups in the B-ring is important for compounds exhibiting anti-HSV, complement classical pathway inhibition, and radical scavenging effects (De Bruyne et al., 1999).

Seagrasses are no link between marine algae and land-living higher plants but are derived from land plants which have secondarily reorganized marine habitats. Therefore, seagrasses share most features of their primary and secondary metabolism with their relatives from the Alismatales order which live in land and freshwater habitats (Hegelmeyer and Zidon, 2010). A previous study showed that the sterol compositions of some seagrasses are typical of higher plants (Gilan et al., 1984).

This is the first report on the chemical composition and biological activity of *T. ciliatum*. The isolation of dihydrochalcones from a marine organism is quite interesting from the chemotaxonomical point of view because the chemical composition of the seagrasses is not fully covered and the characterization of this type of marine aquatic plants is highly recommended based on the chemical profile. We also report the biological activity of the two dihydrochalcones as well as of the other pure compounds. Further studies are required on the toxicity of *T. ciliatum* to humans when consumed as food supplement and/or on its formulation as a therapeutic drug against HSV-1 and HAV and as an anticancer agent.

**Material and Methods**

**General experimental procedures**

Optical rotations were measured on a Perkin-Elmer model 343 Plus polarimeter (Überlingen, Germany) using a sodium lamp at 25 °C. NMR spectra were recorded on a Bruker AMX 500
instrument (Karlsruhe, Germany) operating at 500 MHz for $^1$H NMR and at 125 MHz for $^{13}$C NMR. Two-dimensional NMR spectra were obtained with the standard Bruker software. Sephadex LH-20 (Pharmacia, Uppsala, Sweden) was used for column chromatography. For thin layer chromatography (TLC) aluminum sheet silica gel 60 F$_{254}$ pre-coated plates (Merck, Darmstadt, Germany) were used.

**Plant material**

Seagrass samples of *Thalassodendron ciliatum* (Forssk.) Hartog were collected from Magawish city near Hurghada, Egypt in October 2008, and were identified by Prof. Dr. Monir Abd-El Ghaney, Botany Department, Herbarium, Faculty of Science, Cairo University, Cairo, Egypt.

**Extraction and isolation**

Fresh *T. ciliatum* (800 g) was blended in an electric blender with methanol; the process was repeated until exhaustion. The combined extracts were filtered, and the solvent was evaporated under reduced pressure at 45 °C. The crude extract was partitioned between ethyl acetate (EtOAc) and H$_2$O several times.

The ethyl acetate fraction (10.61 g) was chromatographed on a Sephadex LH-20 column (600 mm) with step gradient elution starting from 30% ethanol in H$_2$O to 100% ethanol. Fractions of 250 mL each were collected and those exhibiting similar TLC profiles were combined. Subfractions 20 columns with different elution systems to allow the purification and identification of six phenolic compounds; rutin (1), asebotin (2), 3-hydroxyasebotin (3), quercetin-3-O-β-D-xylopyranoside (4), a racemic mixture of (+)-catechin and (−)-catechin (5), and trans-cafeic acid (6).

**Asebotin (2)**: Fine colourless needles. – UV: $\lambda_{\max } = 282$ nm (MeOH). – M.p. 140 – 143 °C. – $[\alpha]_{D}^{22} = -44.4^\circ$ (c 0.275, MeOH). – $^1$H NMR: $\delta = 7.06$, 6.68 (2H each, d, 8.6 Hz, H-2/H-6 and H-3/H-5), 6.30 and 6.11 (1H each, $J = 2.4$ Hz, H-3′, 5′), 5.07 (d, $J = 7.3$ Hz, H-1′), 3.47 (2H, t, $J = 7.5$ Hz, α-H) and 2.88 (2H, t, $J = 7.5$ Hz, β-H) of C-α, β (Fuendjiep et al. 2002). – $^{13}$C NMR: $\delta = 206.9$ (qC, C=O), 167.4 (qC, C-6′), 167.2 (qC, C-4′), 161.9 (qC, C-2′), 156.4 (qC, C-4), 133.8 (qC, C-1), 130.4 (2CH, C-2 and C-6), 116.1 (2CH, C-3 and C-5), 107.5 (qC, C-1′), 102.2 (CH, C-1′′), 96.5 (CH, C-5′), 94.8 (CH, C-3′), 78.5 (2CH, C-3′′ and C-5′′), 74.7 (CH, C-2′′), 71.2 (CH, C-4′′), 62.5 (CH$_2$, C-6′′), 56.1 (CH$_3$, OCH$_3$-4′), 47.1 (CH$_3$, C-α), 30.7 (CH$_2$, C-β).

**3-Hydroxyasebotin (3)**: Feathery fine needles. – UV: $\lambda_{\max } = 279$ nm (MeOH). – M.p. 155 – 157 °C. – $[\alpha]_{D}^{22} = -36.0^\circ$ (c 0.519, MeOH). – $^1$H NMR: $\delta = 6.69$ (d, $J = 2.0$ Hz, H-2), 6.56 (dd, $J = 8.1$ and 2.0 Hz, H-6), 6.65 (d, $J = 8.1$ Hz, H-5), 6.30 and 6.11 (1H each, $J = 2.4$ Hz, H-3′, 5′), 5.07 (d, $J = 7.6$ Hz, H-1′), 3.46 (2H, t, $J = 7.6$ Hz, α-H) and 2.83 (2H, t, $J = 7.6$ Hz, β-H) of C-α, β (Yao et al., 2005). – $^{13}$C NMR: $\delta = 206.9$ (qC, C=O), 167.5 (qC, C-6′), 167.2 (qC, C-4′), 161.9 (qC, C-2′), 146.0 (qC, C-3′), 144.3 (qC, C-4), 134.6 (qC, C-1), 120.7 (CH, C-6), 116.6 (CH, C-2), 116.2 (CH, C-5), 107.5 (qC, C-1′), 102.1 (CH, C-1′′), 96.5 (CH, C-5′), 94.7 (CH, C-3′), 78.5 (2CH, C-3′′ and C-5′′), 74.7 (CH, C-2′′), 71.2 (CH, C-4′′), 62.5 (CH$_2$, C-6′′), 56.1 (CH$_3$, OCH$_3$-4′), 47.1 (CH$_2$, C-α), 31.0 (CH$_3$, C-β).

**Cytotoxic activity**

Cytotoxicity was determined using the sulforhodamine B colorimetric assay (SRB) and scored as percentage reduction of cell viability of treated culture versus untreated control culture. Triplicate wells were prepared for each individual concentration (5, 10, 20, and 30 μg/well). The IC$_{50}$ values of the cell growth were obtained from the drug dose-response curves using the software Origin 6.1 as previously described by De Vries and Beart (1995).

**Antioxidant activity**

Antioxidant activity was determined by measurement of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging percentage according to the method described by Chung et al. (2002).

**Antiviral activity**

The total extract as well as the isolated pure compounds were tested against HSV-1 and HAV-MBB. The cytotoxicity of samples was determined by a method previously described by Van den Berge et al. (1978). Tenfold serial dilutions of each sample were made starting from $10^0$ (l mg/mL) to a $10^2$ dilution. Each concentration was tested in triplicate. The antiviral activity of the samples was determined on non-cytotoxic concentration by the plaque assay method (Kaul et al., 1938).
Chaurasia N. and Wichtl M. (1987), Flavonol glycosides


