ELSEVIER

Food Chemistry 76 (2002) 187–195

Food Chemistry

www.elsevier.com/locate/foodchem

Stress metabolites from *Corchorus olitorius* L. leaves in response to certain stress agents

Aisha Hussein Saleh Abou Zeid

Department of Pharmacognosy and Chemistry of Medicinal Plants, National Research Centre, Dokki, Cairo, Egypt

Received 27 February 2001; received in revised form 7 June 2001; accepted 7 June 2001

Abstract

Five coumarins were found to be produced as phytoalexins (stress metabolites) from the fresh young leaves of *Corchorus olitorius* L. plant, in response to inoculation with biotic stress agent, such as the spore suspension of the fungus *Helminthosporium turcicum* and with chemical stress agents, such as aqueous solutions of mercuric chloride and cupric chloride. The five compounds were isolated, purified and subjected to melting point and spectroscopic determinations. They were identified as scopoletin, fraxinol, isopimpinellin, xanthotoxol and peucedanol. They were tested for their antimicrobial activities. The volatile components of the natural fresh leaves and the cupric chloride-treated leaves were prepared and subjected to GC/MS analysis. Fifty five and 49 components were identified in the control and treated leaves, respectively. *cis*-3-Hexen-1-ol, *cis*-4-hexen-1-ol, terpinolene, sabinene and phytol were the major compounds in the control leaves, while those of the treated leaves were *cis*-4-hexen-1-ol, *cis*-3-hexen-1-ol, tetradecanal and phytol. The percentage of the total oxygenated compounds were found to be increased in the cupric chloride-treated leaves much more than the control leaves (77.3 and 47.4%, respectively).

Keywords: *Corcorus olitorins*; Stress metabolites; Coumarins; Volatile constituents

1. Introduction

The genus *Corchorus* comprises certain herbs and shrubs. It contains about 100 species which are distributed in the tropics and subtropics, chiefly South-East Asia and South America (Ahmad, Ali, Ali, Baqai, & Zafar, 1998). One of these species is *Corchorus olitorius* Linn. plant (Fam. Tiliacea) which is cultivated to provide bark for the production of fibres (Jute) and mucilagenous leaves for using in food as a vegetable. The seeds are used as a purgative and the leaves as a tonic and diuretic (Chopra, Nayar, & Chopra, 1956; Oliver, 1960). The aqueous solution of the leaves was found to decrease hyperglycaemea in mice, guinea-pigs and rabbits. It did not act in absence of pancrease; it promoted the degradation of glucose (Goldner, 1958). The leaf powder and its water-soluble viscous solution were found to decrease total serum and liver cholesterol concentrations and increase the fecal excretion of bile acids, total neutral sterols and cholesterol (Inammi, Nakamura, Tabata, Wada, & Takita, 1995). New cardenolide oligoglycosides, corchorusosides A, B, C, D and E, were isolated from *C. olitorius* seeds (Yoshikawa et al., 1998).Two digitoxigenin glycosides (coroloside and glucoeva tromonoside), as well as, four strophanthidine glycosides (erysimoside, olitoriside, corchoroside A and helveticoside) were identified as main cardiac glycosides in the methanol extract of *C. olitorius* leaves (Goda, Sakai, Nakamura, Akiyama, & Toyodo, 1998). Three cardenolides were isolated from *C. olitorius* seed-leaves and their cytotoxic activities were evaluated against six cancer cell lines (Abdel Wahab, Islam, & El Tanbouly,1999). Four flavonoid glycosides, astragaline, tolifolin, isoquercetin and juglanin, as well as oleanolic acid glyceryl monopalmitate, β- sitosterol and β- sitosterol-3- glucoside, were isolated from the leaves of *C. olitorius* (Kohoda, Tanaks, Yamaoka, Morinaga, & Ohhara, 1994). A new coumarin (4-,7-dihydroxy coumarin) was isolated from the chloroform extract of the defatted seeds of *C. olitorius* (Mukherjee, Mitra, & Ganguly, 1998). Six phenolic antioxidative compounds were identified in the leaves of *C. olitorius*. Their antioxidant activities were measured and this showed that 5-caffeoylquinic acid was a predominant phenolic antioxidant in *C. olitorius* leaves (Azuma et al., 1999).

Phytoalexins are antimicrobial compounds synthesized by a plant in response to infection or stress. The chemistry of these compounds is usually uniform within a plant family but is diverse within the plant kingdom. Accumulation of these compounds is determined by

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00264-3

rates of synthesis in the plant and by rate of metabolism by the infecting organism and possibly also by the plant (Bailey & Mansfield, 1982).

A large proportion of Phytoalexin studies has involved plants belonging to the family Leguminosae, sub-family papilionoideae and, consequently, a rich assortment of phytoalexins has been discovered in these plants (Hargreaves, & Mansfield, 1976; Ingham, 1978; Martin & Dewick, 1979; Robenson, 1978; VanEtten, 1973; Woodward, 1980)

Phytoalexins were found to be elicited in plants by: 1-biotic agents (fungi, bacteria, viruses or animals); 2-abiotic agents (salts of heavy metals such as $HgCl_2$ and $CuCl_2$, antibiotics, such as actinomycin D, some respiratory inhibitors such as iodoacetate and plant growth regulators, such as ethylene); 3- microbial metabolites, such as, peptides, glycopeptides and polysaccharides; 4-physical stress such as by irradiation of parts of the tissues with short-wavelength ultra violet light, partial freezing of the tissues and wounding of the tissues by cutting, bruising or picking (Bailey & Mansfield, 1982). The identification of so many types of biological, chemical and physical stimuli as effective phytoalexin-inducers has allowed phytoalexins to be regarded as a sub-group of a class of substances termed stress compounds (Stoessl, Stothers, & Ward, 1976).

The aim of this study is to investigate the induction of antifungal compounds (phytoalexins or stress metabolites) by *C. olitorius* leaves, in response to application of certain stress agents and to test their anti-microbial activity, as well as, their volatile constituent composition comparing the natural untreated fresh leaves with treated leaves.

2. Materials and methods

2.1. Plant materials

C. olitorius L. seeds were obtained from the Agricultural Research Centre, Egypt. They were cultivated in the Experimental Farm of the Cultivation and Production of Medicinal Plants Department, National Research Centre, Egypt. The date of cultivation was September 1998. The date of collection of the young leaves was in November 1998.

2.2. Microbial materials

2.2.1. Micro-organisms

The bacterial strains *Bacillus subtilis*, *Escherichia coli* and the yeast strain *Saccharomyces cerevisae* were kindly donated from Microbial Genetics Department, National Research Centre, Egypt. The fungal strains, *Helminthosporium turcicum* and *Penicillium digitatum,* were kindly donated from Legume Disease Section, Plant Pathology Research Institute, Agricultural Research Centre, Egypt.

2.2.2. B-Media

Bacteria were grown on Lauri-Bertani medium (Moniatis, Fritssh, & Sambrook, 1982), yeasts were grown on yeast extract peptone medium (Dillon, Nasim, & Nestmann 1985); fungi were grown on potato dextrose agar medium (Subba Rao, 1977). Glucose mineral salts medium (Homans & Fuchs, 1970) is the liquid medium used for preparation of the fungal spore suspension used for spraying TLC plates of the different extracts for detecting the fungi-toxic compounds on TLC (Bioautography; Homans & Fuchs, 1970).

2.3. Apparatus

1. Koffler's heating stage microscope for determination of the melting points.
2. Ultra Violet Visible Recording Spectrophotometer model 240, Shimadzu, Kyoto, Japan, for determination of UV spectra.
3. Fourier Transform Infra Red Spectrophotometer FT/IR-820,1PC Shimadzu, for determination of IR spectra.
4. GC/MS Finnigan mat SSQ 7000, Digital DEC EL ev 70 for MS determinations and GC/MS analysis of the volatile constituents.
5. Nuclear Magnetic Resonance Spectrophotometer Jeol EX-270 NMR Spectrometer for determination of ^{1}HNMR.

2.4. Conditions of GC/MS analysis of the volatile constituents

A capillary column of DB-5 Fused silica (5% phenyl methyl polysiloxane), 30 m length, 0.25 mm id and 0.25 μm thickness was used; carrier gas, helium at 13 psi, temperature programming, 60 °C for 3 min, 60–100 °C at a rate of 2 °C/min, 100–250 °C at a rate of 5 °C/min, 250 °C for 5 min; splitless detector.

2.5. Production of stress metabolites (phytoalexins)

2.5.1. Treatment of the leaves by the drop diffusate method (Ingham, 1981)

Young expanded leaves, freshly detached from the *C. olitorius* plant, were separated and placed with the abaxial surface downwards onto a perforated diaphragm of polyethylene boxes. The bottoms of the boxes were lined with filter papers moistened with distilled water to keep a high humidity inside the boxes. The boxes were divided into four groups. The leaves in the first group were treated by dropping 0.05–0.1ml of 10^{-2} M aqueous solution of mercuric chloride onto their adaxial surface, 1–2 drops/leaf. The leaves in the second group received drops of 3×10^{-2} M aqueous solution of cupric chloride. The leaves in the third group were inoculated with drops of the spore suspension of

the fungus *Helminthosporium turcicum* (5×10^4 spores/ml in mineral salts–glucose liquid medium). In the fourth group, the leaves received drops of sterile distilled water and were kept as a control. All boxes were covered and incubated at 25 °C for 72 h. The concentrations of $CuCl_2$ and $HgCl_2$ were chosen, according to Cruickshank and Perrin (1963),who reported that the effective concentrations of cupric and mercuric chloride were observed not to be phytotoxic to the tissues of the plant.

2.5.2. Extraction of the leaves

At the end of the incubation period the drops were collected by suction, using a special dropper, and the tissues underlying it were cut by a cork borer. Both the drops and the tissues were extracted by homogenization with 95% ethanol in a mixer. The extract was filtered and the residue was washed with ethanol. The filtrate and washings were combined and concentrated under vacuum at 40 °C. Water was added and the aqueous solution was successively extracted with hexane, ether and ethyl acetate. The aqueous solution remaining was evaporated under vacuum and the residue was dissolved in methanol. All extracts of the treatments and control were separately filtered over anhydrous sodium sulphate and evaporated to dryness.

2.5.3. TLC of the different extracts

The hexane, ether, ethyl acetate and methanol extracts of the different treatments and the corresponding control were subjected to TLC examination on aluminium sheets pre-coated with silica gel 60 F 254 (Merck). The spots were applied in as equal amounts as possible. The plates were developed in the following developing solvent systems:

1. benzene–acetone (9:1) for the hexane extract.
2. benzene–ethyl acetate (4:1) for the ether and ethyl acetate extracts
3. chloroform–methanol (95:5) for the methanol extract.

After development, the plates were examined under UV light (365 nm) to locate any additional spots in the different extracts of the treatments in comparison with that of the corresponding control extracts.

2.5.4. Detection of the antifungal compounds by bioautography (Homans & Fuchs, 1970)

Further TLC plates were prepared the same manner as before. After development in the specified solvent system, they were sprayed with the spore suspension of the fungus *Penicillium digitatum* in glucose-mineral salts medium and incubated in a moist place at 28 °C for 72 h. At the end of the incubation period the antifungal compounds appeared as white inhibition zones in the dark green growth of the fungus.

2.5.5. Preparative TLC of the anti-fungal compounds

The R_f values of the antifungal compounds which appeared as white inhibition zones were determined and compared with that of the unsprayed plates, to detect their colours under UV light (365 nm). Several plates were prepared and the antifungal compounds were separated by preparative TLC and purified by repeated PTLC in different solvent systems. The pure compounds were subjected to mp, UV, IR, MS, and, if possible, ^{1}HNMR determinations, depending on the quantity of the isolated compounds.

2.6. Antimicrobial activity test of the isolated compounds

The isolated compounds were tested for their antimicrobial activity against the above-mentioned microorganisms by the paper-disc antibiotic assay method (Gnanamanickam & Mansfield, 1981). Ampicillin was used as a standard antibacterial agent and clotrimazol was used as a standard antifungal agent. The discs were loaded with 100 μg of the isolated compounds or standard. They were placed on the surface of the specified medium in Petri dishes seeded with the test organisms and incubated at 28, 30 or 37 °C for fungi, yeasts or bacteria, respectively. Diameters of inhibition zones were measured in millimeters, after incubation for 24–48 h or 48–72 h in the case of bacteria and yeasts or fungi, respectively.

2.7. Investigation of the volatile constituents

Five hundred grams of both the fresh untreated leaves of *C. olitorius* (control) and the leaves, treated with 3×10^{-2} M aqueous solution of $CuCl_2$ and incubated for 72 h, were subjected to steam-distillation in a modified Likens and Nickerson apparatus (Macleod & Cave, 1975), which allowed the simultaneous extraction of the volatile components in organic solvent (*n*-pentane). The solvent was removed carefully. The obtained volatile constituents were subjected to GC/MS analysis, following the above-mentioned conditions. The volatile components were identified by comparing their retention times and mass fragmentation pattern with those of the published data (Adams, 1989; Eight peaks index of mass spectra, 1980; Jennings & Shibamoto, 1981). Quantitative determinations were carried out, based on peak area measurements.

3. Results and discussion

3.1. General

The antifungal compounds produced in the leaves of *C. olitorius* in response to treatment with heavy metal salts ($HgCl_2$ and $CuCl_2$) and biotic agent (spore suspension of the fungus *H. turcicum*) were detected on the

TLC plates by bioautograpy as white inhibition zones in the dark green growth of the fungus *Penicillium digitatum*. Also, these compounds were detected as additional spots in the extracts of the different treatments.

Stress agent application on young leaves of *C. olitorius*, as indicated by the previous TLC examination, resulted in the production of five antifungal compounds, three in the ether extract and two in the ethyl acetate extract.

$CuCl_2$ solution was the most suitable stress agent for the production of phytoalexins, as shown by the relatively higher concentrations of stress metabolites compared with their concentrations in the other stress agents. This result was in agreement with the reported data about the production of phytoalexins from certain plants belonging to the family Leguminoseae (Abou Zeid, 1990). There were no detectable differences between the hexane and methanol extracts of the treatments and control.

The five antifungal compounds were separated from the ether and ethyl acetate extracts of the leaves treated with $CuCl_2$ by repeated preparative TLC, using the solvent system: benzene–ethyl acetate (4:1) as the developing system and further purification was performed using the solvent system ether–hexane (3:1).

The isolated compounds were identified by determination of the mp and spectroscopic measurements, as well as comparison with the available authentic samples and the published data. They were found to be coumarins in nature and were identified as compunds I–IV (Fig. 1)

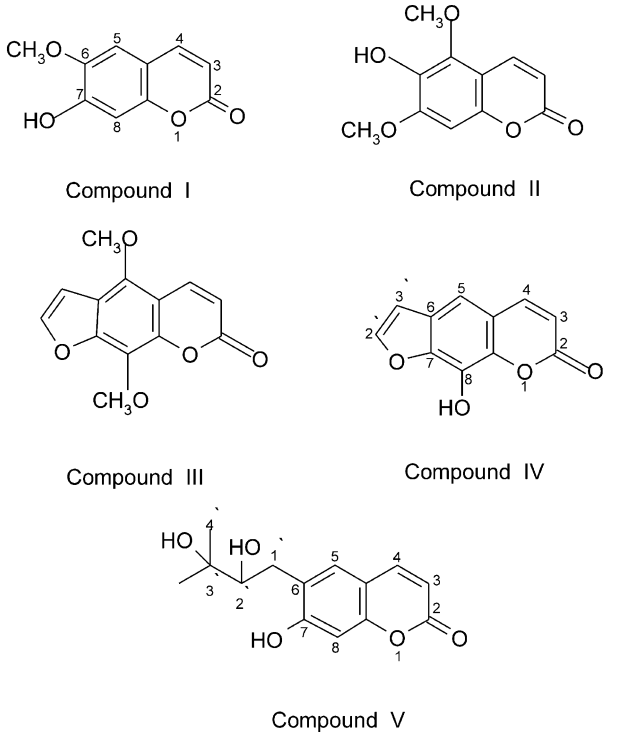

Fig. 1. Compounds isolated.

3.2. Compound I

Crystallized from methanol (42 mg), R_f : 0.30 in benzene–ethyl acetate (4:1), with blue florescent colour under UV light, 365 nm, mp, 204–206° (reported mp 203–204°, Shafizadeh & Melnikoff, 1970).

UV λ_{max} ($CHCl_3$): 244, 260, 296, 345 nm. IR ν_{max} (KBr disc) cm^{-1}: 3448 (OH), 1728 (C=O), 2939, 2850(CH), 1490, 1589 (aromatic). MS:192 [M^+], 177 [M^+–CH_3], 164[M^+–CO], 149[M^+–CH_3–2CO], 124, 115, 105, 91, 89, 87.

^{1}HNMR, ppm. ($CDCl_3$): 10.0 (1H, s, OH), 7.6(1H, d, J = 10 Hz, H-4), 7.05(1H, s, H-5), 6.75(1H, s, H-8), 6.27 (1H, d, J = 10 Hz, H-3), 3.9 (3H, s, OCH_3).

Compound I could be identified as scopoletin (7-hydroxy,6-methoxy coumarin $C_{10}H_8O_4$), as the above data are identical with the reported ones (Shafizadeh & Melnikoff, 1970). The identification was confirmed by comparison with an authentic sample (mp, mixed mp, co TLC). This compound was found to be present in the control extract in trace amounts, which increased in concentration after application of the different stress agents.

3.3. Compound II

This crystallized from methanol (15 mg), R_f : 0.34, faint yellowish-brown colour under UV light, mp, 170–172° (reported mp 171–173° Murray, Mendz, & Brown, 1982).

UV λ_{max} ($CHCl_3$): 240, 254, 295, 342 nm. IR ν_{max} (KBr disc) cm^{-1}: 3448 (OH), 2939, 2860 (CH), 1728 (C=O), 1520, 1590 (aromatic).

MS:222 [M^+], 221 [M^+-1], 191 [M^+–OCH_3], 163 [M^+–OCH_3–CO], 135 [M^+–OCH_3–2 CO], 204 [M^+–H_2O].

From these data it could be concluded that compound II is fraxinol (6-hydroxy, 5,7 dimethoxy coumarin, $C_{11}H_{10}O_5$).

Fraxoside was isolated from different species of *Tilia*, Fam. Tiliacea (Paris & Theallet, 1961; Plouvier, 1968) . It is the glucoside of fraxetol (7,8- dihydroxy, 6-methoxy coumarin).

3.4. Compound III

Crystallized from methanol (20 mg), R_f: 0.36, light brown under UV light, mp,147–150° (reported mp, 148–151°(Murray, Mendz, & Brown, 1982).

UV λ_{max} ($CHCl_3$): 250, 265, 302 nm. IR ν_{max} (KBr disc) cm^{-1}: 2923, 2520 (CH), 1728 (C=O), 1590, 1520 (aromatic) 1276, 1090 (Furane CO).

MS:246 [M^+], 231[M^+–CH_3], 216 [231–CH_3], 203[M^+–CH_3–CO], 175 [M^+–CH_3–2CO], 188 [203–CH_3], 160 [188–CO], 149,91.

From the above data, compound III was identified as isopimpinellin (5,8-dimethoxy psoralene, $C_{13}H_{10}O_5$), as

compared with an authentic sample (mp, mmp, co-TLC) and with the published data (Nielssen, 1971).

3.5. Compound IV

Crystallized from methanol (25 mg), R_f 0.15, light brown colour under UV, mp, 249–252° (reported mp 250–253° Murray, Mendz, & Brown, 1982).

UV λ_{max} ($CHCl_3$): 250, 260, 266, 305 nm.

IR ν_{max} cm^{-1}: 3425 (OH), 1730 (C=O) 2923, 2854 (CH), 1260, 1100 (furan CO), 1596,1480 (aromatic).

MS:202 [M^+], 201 [M^+–1],188, 173 [201–CO],145 [201–2CO], 117, 91, 89.

^{1}HNMR, ppm. ($CDCL_3$): 7.75(1H, d, J = 10 Hz, H-4), 7.65(1H, d, J = 2.5Hz, H-2′), 7.25 (1H, s, H-5), 6.75(1H, d, J = 2.5Hz, H-3′), 6.3 (1H, d, J = 10 Hz, H-3), 6.2(1H, s, OH).

Compound IV was identified as xanthotoxol (8-hydroxy psoralene, $C_{11}H_6O_4$) by comparing the above data with the published data (Chatterjee & Sen, 1969) and comparison with an authentic sample (mp, mmp, co-TLC).

3.6. Compound V

Crystallized from methanol (15 mg), R_f : 0.2, faint blue colour under UV, mp, 174–176° (reported mp, 174–175° Murray, Mendz, & Brown, 1982)

UVλ_{max} $CHCl_3$: 245, 290, 332 nm.

IR ν_{max} (KBr disc) cm^{-1}: 3433 (OH), 1728 (C=O), 2923, 2860 (CH), 1589, 1485 (aromatic).

MS: 264 [M^+], 246 [M^+–H_2O], 236 [M^+–CO], 208 [M^+–2CO], 228 [M^+–$2H_2O$], 213, 193, 176, 175, 163, 149, 147, 177, 165.

By comparing these data with published reports (Lemmich & Havelund, 1978) compound V could be identified as peucedanol [6-(2′,3′- dihydroxy-3′-methyl butyl), 7-hydroxy coumarin, $C_{14}H_{16}O_5$].

3.7. Antimicrobial activity test

The antimicrobial activity test (Table 1) proved that the isolated compounds had good activity against the microorganisms used in the test. These results are in agreement with the following reports that many coumarins are formed in plants in response to infection or stress as phytoalexins and their antimicrobial activity was proved.

The production of coumarin compounds, as phytoalexins, from plants belonging to different families in response to stress agents, has been reported by many authors. These include, scopolin from *Nicotiana tabacum* (Tangy & Martin, 1972), scopoletin from *N. tabacum* (Reuveni & Cohen, 1978) and *Gossypium hirsutum* (Zeringue, 1984), scopoletin and umbelliferone from *Platanus arcerifolia* (Elmodafar, Clerievet, Fleuriet, & Macheix, 1999), scopoletin and ayapin from *Helianthus annuus* (Tal & Robeson, 1986) 6,7-dimethoxy coumarin from *citrus* spp. (Afek, Sztejnberg, & Carmely, 1986), xanthotoxin from *Postinaca sativa* (Johnson, Brannon & Kuc, 1973), *Apium graveolens* (Lord, Epton, & Frost, 1988), coumestrol from *Glycine max* (Keen, Zaki, & Sims, 1972), *Phaseolus lunatus* (Rich, Keen, & Thomason, 1977), *Phaseolus vulgaris* (Woodward, 1980), *Vigna sinensis* (Partridge, 1973), sajagol from *Glycine max* (Keen et al., 1972) and psoralidin from *Phaseolus vulgaris* (Rich et al., 1977).

Table 1
Antimicrobial activity of the isolated compounds

Micro-organism	Diameter of inhibition zone (mm) Compound					
	I	II	III	IV	V	St.
Bacillus subtilis	14	12	13	12	10	13
Echerichia coli	12	13	11	10	12	18
S. cerevisiae	16	14	12	15	12	17
H. turcicum	15	13	15	14	15	22
P. digitatum	14	14	16	16	13	20

These reports, indicate the importance of coumarins in playing a role in the defence mechanisms of plants against stress and infection, so the above-mentioned reports support our results and are in agreement with it.

3.8. Chemical composition of the volatile constituents

The volatile constituents of both the control and $CuCl_2$ treated fresh leaves of *C. olitorius* are obtained as a faint yellow oil with a characteristic odour in yields of 0.05 and 0.03% v/w, respectively. The treated leaves show a marked decrease in the percentage of the volatile content. This is in agreement with what is reported about the decrease in the volatile oil contents of mentha and parsley herbs after infection (Hashem & Sahab, 1999). This was also confirmed by (Mahran, Saleh, Fathy, Motawe, & Hashem, 1995) who reported that when plants were exposed to certain damage, a decrease in volatile oil production resulted. Also, our results are in agreement with what is published about rose-scented geranium (*Pelargonium* sp.) plants, which when infected with little leaf disease, showed 91–67% reduction in essential oil yield of the diseased plants in comparison with healthy plants (Rajeswara Rao, Kau, Mallavarapu, & Ramesh, 2000)

Results of GC/MS analysis are indicated in Table 2. It includes the identified compounds, the retention time relative to *cis*-4-hexen-1-ol, the base peak, the molecular weight and the percentage of each compound in both the control and treated leaves.

Fifty five and 49 components, representing 86.9 and 82.6% of the total volatile constituents, are identified in the control and treated leaves, respectively.

Table 2
Results of GC/MS analysis of the volatile constituents of the control and $CuCl_2$ treated leaves of *Corchorus olitorius*

Compound	RR_t[a]	Base peak	Mol Wt.	Area%	
				Control leaves (%)	Treated leaves (%)
Methyl butane	0.79	43	72	0.14	0.25
Isobutyl aldehyde	0.83	43	72	0.27	0.35
cis-3-hexen-1- ol	0.89	41	100	18.17	13.01
cis-4-hexen-1- ol	1.00	67	100	10.75	44.91
n-heptane	1.01	43	100	1.13	0.22
n-hexanal	1.02	43	100	2.94	3.31
1- Nonene	1.05	43	126	0.14	0.35
3- Methyl-2-hexanone	1.08	43	114	0.24	0.18
n- Nonane	1.09	43	128	1.88	0.20
1-Hepten-3-ol	1.13	43	114	0.21	0.36
1-Octen-3-ol	1.26	43	128	0.38	0.51
α-Pinene	1.32	93	136	1.54	0.23
Camphene	1.35	93	136	2.40	0.17
4,5-Dimethyl octane	1.58	43	142	0.17	0.14
2-methyl nonane	1.61	43	142	0.15	0.14
Sabinene	1.77	93	136	7.28	0.14
n-Decane	1.79	43	142	0.64	0.61
2-Carene	1.78	93	136	0.76	-
3-Carene	2.03	93	136	2.08	-
1,4-Cineol	2.06	43	154	-	0.17
4-Hexen-1-yl acetate	2.09	43	142	0.82	0.13
α-Terpinene	2.26	121	136	3.85	-
P-Cymene	2.32	119	134	1.63	-
cis-ocimene	2.35	93	136	3.76	-
Phenyl acetaldehyde	2.54	91	120	-	0.48
Terpinolene	2.78	93	136	7.88	-
Allo-ocimene	3.09	121	136	0.88	-
Linalool	3.32	93	154	1.32	-
n-Undecane	3.47	57	156	0.22	0.15
4-Terpineol	4.58	71	154	0.77	0.17
p-Cymen-9- ol	4.83	43	150	0.41	0.15
2 -Hydroxy- 5-methoxy benzaldehyde	5.21	152	152	0.18	0.12
1-Tetradecene	7.27	43	196	0.16	0.14
n-Tetradecane	7.32	57	198	0.14	0.12
3-buten-1-one,4-[2,6,6-trimethyl-1-cyclohexen-1-yl]	7.45	177	192	0.29	0.13
β-Ionone	8.00	177	192	0.47	0.68
n-Pentadecane	8.18	57	212	0.25	0.25
n-Hexadecane	8.79	57	226	0.34	0.25
n-Heptadecane	9.45	57	240	0.36	0.25
Tetradecanal	9.53	57	212	0.23	4. 78
4-(1- Methyl-1-phenyl ethyl) phenol	9.55	197	212	0.24	0.20
1-Octadecene	9.97	43	252	0.16	0.25
n-Octadecane	9.99	57	254	0.20	0.22
Methyl-9,12,15- octadecatrienoate	10.01	79	292	-	1.06
Dibutyl phthalate	10.30	149	278	0.15	0.41
n-Nonadecane	10.51	57	268	0.20	-
Methyl -14-methyl pentadecanoate	10.69	74	270	0.19	-
Hexadecanoic acid	10.99	43	256	2.17	0.52
2,6-Bis (t-butyl)-4-(dimethyl benzene) phenol	11.28	309	324	0.53	0.26
n-Heneicosane	11.50	57	324	0.30	0.08
Phytol	11.55	71	296	6.26	4.55
n-Docosane	11.97	57	310	0.14	0.10
11-Tricosene	12.27	43	322	0.20	0.30
n-Tricosane	12.44	57	324	0.19	0.23
Dioctyl hexadecanoate	12.77	129	370	0.12	0.11
n-Tetracosane	12.80	57	338	0.24	0.10
2- Nonadecyl thiophene	13.15	97	350	–	0.35
2,4-Bis(dimethyl benzyl)-6-t-butyl phenol	13.27	371	386	0.24	0.71
n-Hexacosane	13.73	57	366	0.16	0.12
Total identified constituents	–	–	–	86.92	82.62
Unidentified constituents	–	–	–	13.08	17.38

[a] RR_t = Retention time relative to *cis*-4- hexen-1-ol

Table 3
Chemical composition of the volatile constituents of the control and $CuCl_2$ treated leaves of *Corchorus olitorius*

Chemical class	Area %	
	control leaves	treated leaves
I- Oxygenated compounds		
A- Alcohols		
cis-3-hexen-1- ol	18.17	13.01
cis-4-hexen-1- ol	10.75	44.91
1- Hepten -3- ol	0.21	0.36
1- Octen-3- ol	0.38	0.51
Linalool	1.32	–
4- Terpineol	0.77	0.17
p-Cymen-9- ol	0.41	0.15
Phytol	6.26	4.55
Total	38.27	63.66
B- Aldehydes		
Isobutyl aldehyde	0.27	0.35
n-Hexanal	2.94	3.31
Phenyl acetaldehyde	–	0.48
2-Hydroxy-5-methoxy benzaldehyde	0.18	0.12
Tetradecanal	0.23	4. 78
Total	3.62	9.04
C-Ketones		
3- Methyl - 2 - hexanone	0.24	0.18
3-buten-1-one,4-[2,6,6-trimethyl-1-cyclohexen-1-yl	0.29	0.13
β-Ionone	0.47	0.68
Total	1.00	0.99
D-Acids		
Hexadecanoic acid	2.17	0.52
Total	2.17	0.52
E- Esters		
4- Hexen-1-yl acetate	0.82	0.13
Dibutyl phthalate	0.15	0.41
Methyl-9,12,15- octadecatrienoate	–	1.06
Methyl-14-methyl pentadecanoate	0.19	–
Dioctyl hexadecanoate	0.12	0.11
Total	1.28	1.71
F- Phenols		
4-(1- Methyl-1-phenyl ethyl) phenol	0.24	0.20
2,6-Bis (t-butyl) -4- (dimethyl benzene) phenol	0.53	0.26
2,4-Bis(dimethyl benzyl)-6-t-butyl phenol	0.24	0.71
Total	1.01	1.17

Chemical class	Area %	
	control leaves	treated leaves
G- Oxides		
1,4 - Cineol	–	0.17
Total	–	0.17
II- Non-oxygenated compounds		
A-Non-terpenoid		
Methyl butane	0.14	0.25
n-Heptane	1.13	0.22
1-Nonene	0.14	0.35
n-Nonane	1.88	0.20
4,5-Dimethyl octane	0.17	0.14
2-Methyl nonane	0.15	0.14
n-Decane	0.64	0.61
n-Undecane	0.22	0.15
1-Tetradecene	0.16	0.14
n-Tetradecane	0.14	0.12
n-Pentadecane	0.25	0.25
n-Hexadecane	0.34	0.25
n-Heptadecane	0.36	0.25
1-Octadecene	0.16	0.25
n-Octadecane	0.20	0.22
n-Nonadecane	0.20	–
n-Heneicosane	0.30	0.08
n-Docosane	0.14	0.10
11-Tricosene	0.20	0.30
n-Tricosane	0.19	0.23
n-Tetracosane	0.24	0.10
n-Hexacosane	0.16	0.12
Total	7.51	4.47
B-Terpenoid		
α-Pinene	1.54	0.23
Camphene	2.40	0.17
Sabinene	7.28	0.14
2-Carene	0.76	–
3-Carene	2.08	–
α-Terpinene	3.85	–
P-Cymene	1.63	–
cis-ocimene	3.76	–
Terpinolene	7.88	–
Allo-ocimene	0.88	–
Total	32.06	0.54
Sulphur compounds		
2- Nonadecyl thiophene	–	0.35
Total	–	0.35

The major compounds in the control leaves are *cis*-3-hexen-1-ol (18.2%), *cis*-4-hexen-1-ol (10.75%), terpinolene (7.88%), sabinene (7.28%) and phytol (6.26%), while those of the treated leaves are *cis*-4-hexen-1-ol (44.9%), *cis*-3-hexen-1-ol (13.0%), tetradecanal (4.78%) and phytol (4.55%). Some compounds are increased in concentration after treatment; some others are decreased in concentration and some others are not detected.

Ten compounds are present in the control leaves and not detected in the treated leaves, including, 2-carene, 3-carene, α-terpinene, p.cymene, *cis*-ocimene, terpinolene, allo-ocimene, linalool, nonadecane and methyl-14-

methyl pentadecanoate. Four compounds were identified in the treated leaves but not detected in the control leaves, including, 1,4-cineol, phenyl acetaldehyde, methyl 9,12,15-octadecatrienoate and 2-nonadecyl thiophene.

Table 3 illustrates the identified compounds, in different chemical classes and the percentage of each compound and each chemical class in the control and treated leaves.

Table 4 represents a summary of Table 3. It includes the number of compounds in each chemical class and their percentages. It is obvious that the percentage of the total oxygenated compounds increased very much in the treated leaves, reaching 77.3%, corresponding to 43.3% in the control leaves.

The reverse occurs in the percentage of non-oxygenated compounds, which were found to be much decreased in the treated leaves (5.01%), as compared with that of the control leaves (39.6%). Also, the number of the non-oxygenated identified components is decreased from 32 in the control leaves to 24 in the treated leaves. The high percentage of the oxygenated compounds in the treated leaves is mainly attributed to alcohols, especially, *cis*-4-hexen-1-ol (44.9%). One sulphur-containing compound, 2-nonadecyl thiophene was detected in treated leaves only.

The compounds which were detected only in the treated leaves and which also are increased in concentrations after $CuCl_2$ treatment may play a role in the defence mechanism of *C. olitorius* leaves in response to infection or damage. Our results were in agreement with what is published about rose-scented geranium (*Pelargonium* sp.) plants which, when infected with little leaf disease, showed many morphological changes in the plant and chemical changes in the composition of the essential oil (Rajeswara et al., 2000).

Our results are also in agreement with what is reported about the release of preformed inhibitors or the increase in concentration of antifungal compounds or synthesis of fungitoxic compounds from immediate or remote precursors in response to infection or damage (Bailey & Mansfield, 1982).

Table 4
Percentage and number of compounds in the different classes of the volatile constituents of the control and $CuCl_2$ treated leaves of *Corchorus olitorius*

Chemical class	No. of compounds in		Area (%) in	
	control leaves	treated leaves	control leaves	treated leaves
Oxygenated compounds				
1- Alcohols	8	7	38.27	63.66
2- Aldehydes	4	5	3.62	9.04
3- Ketones	3	3	1.00	0.99
4- Acids	1	1	2.17	0.52
5- Esters	4	4	1.28	1.71
6- Phenols	3	3	1.01	1.17
7- Oxides	–	1	–	0.17
Total	23	24	47.35	77.26
Non-oxygenated compounds				
1- Non-terpenoids	22	21	7.51	4.47
2- Terpenpids	10	3	32.06	0.54
Total	32	24	39.57	5.01
Sulpher compound	–	1	–	0.35

References

Abdel Wahab, S. M., Islam, W. T., & El Tanbouly, N. D. (1999). Cardenolides from *Corchorus olitorius* L. Seed-leaves. *Bulletin Faculty of Pharmacy, Cairo University*, *37*, 149–153.

Abou Zeid, A.H. (1990). *Phytoalexins production by certain species of the family leguminosae in response to infection by certain fungi*, Ph. D. thesis, Faculty of Pharmacy, Cairo University.

Adams, R. B. (1989). *Identification of essential oils by ion trap mass spectroscopy*. New York: Academic Press.

Afek, U., Sztejnberg, A., & Carmely, S. (1986). 6,7-Dimethoxycoumarin, a citrus phytoalexin conferring risistance against *Phytophthora gummosis*. *Phytochemistry*, *25*, 1855–1856.

Ahmad, V. U., Ali, A., Ali, Z., Baqai, F. T., & Zafar, F. N. (1998). Cycloartane triterpene glucoside from *Corchorus depressus*. *Phytochemistry*, *49*, 829–834.

Azuma, K., Nakayama, M., Koshioka, M., Ippoushi, K., Yamaguchi, Y., Kohata, K., Yamauchi, Y., Ito, H., & Higashio, H. (1999). Phenolic antioxidants from the leaves of *Corchorus olitorius* L. *Journal of Agriculture and Food Chemistry*, *47*, 3963–3966.

Bailey, J. A., & Mansfield, J. W. (1982). *Phytoalexins*. Glasgow: Blackie.

Chatterjee, D. K., & Sen, K. (1969). New synthesis of xanthotoxol and xanthotoxin. *Tetrahedron Letters*, *59*, 5223–5224.

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1956). *Glossary of Indian medicinal plants*. New Delhi: Councel of Scientific and Industrial Research.

Cruickshank, I. A. M., & Perrin, D. R. (1963). Studies on phytoalexins VI. Pisatin: the effect of some factors on its formation in *pisum sativum* L. and the significance of pisatin in disease resistance. *Australian Journal of Biological Science*, *16*, 111–128.

Dillon, J. R., Nasim, A., & Nestmann, E. R. (1985). *Recombinant DNA methodology*. New York: John Wiley.

Eight Peaks Index of Mass Spectra (1980). 2nd ed. Published by Mass Spectrometry data Centre. AWRE Aldermaston, Reading, UK.

Elmodafar, C., Clerievet, A., Fleuriet, A., & Macheix, J. J. (1999). Inoculation of *platanus acerifolia* with *ceratocystis fimbriata* F Sp. Platani induces scopoletin and umbelliferone accumulation. *Phytochemistry*, *34*, 1271–1276.

Gnanamanickam, S. S., & Mansfield, J. W. (1981). Selective toxicity of wyerone and other phytoalexins to gram positive bacteria. *Phytochemistry*, *20*, 997–1000.

Goda, Y., Sakai, S., Nakamura, T., Akiyama, H., & Toyodo, M. (1998). Identification and analysis of main cardiac glycosides in *Corchorus olitorius* seeds and their acute oral toxicity to mice. *Shokuhim Eiseigaku Zasshi*, *39*, 256–265.

Goldner, M. G. (1958). Oral hypoglycaemic agents past and present. *Archives of International Medicine*, *102*, 830–840.

Hargreaves, J. A., Mansfield, J. W., Coxon, D. T., & Price, K. R. (1976). Wyerone epoxide as a phytoalexin in *vicia faba* and its metabolism by *botrytis cinerea* and *B. faba* in vitro. *Phytochemistry*, *15*, 1119–1121.

Hashem, F. A., & Sahab, A. F. (1999). Chemical response of parsley and mentha herbs to certain stress agents. *Food Chemistry*, *65*, 29–33.

Homans, A. L., & Fuchs, A. (1970). Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *Journal of Chromatography*, *51*, 325–327.

Inammi, S., Nakamura, K., Tabata, K., Wada, M., & Takita, T. (1995). Soluble viscous substances of Jew's mellow leaves lowers serum and liver cholesterol concentrations and increase fecal steroid excretion in rats fed on rich cholesterol diet. *Journal of Nutrition, Science and Vetaminology*, *41*, 465–475.

Ingham, J. I. (1978). Isoflavonoid and stilbene phytoalexins of the genus *Trifolium*. *Biochemical Systematic Ecology*, *6*, 123–217.

Ingham, J. L. (1981). Phytoalexine induction and its taxonomic significance in the leguminosae subfamily papilionoideae. *Proceedings of the International Legume Conference (Advanced Legume Systematics) 1981, 599–626*.

Jennings, W., & Shibamoto, T. (1981). *Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography*. New-York: Academic Press.

Johnson, C., Brannon, D. R., & Kuc, J. (1973). Xanthotoxin: a phytoalexin of *pastinaca sativa*. *Phytochemistry*, *12*, 2961–2962.

Keen, N. T., Zaki, A. I., & Sims, J. J. (1972). Biosynthesis of hydroxyphaseollin and related isoflavonoids in disease resistant soy bean hypocotyles. *Phytochemistry*, *11*, 1031–1039.

Kohda, H., Tanaks, S., Yamaoka, Y., Morinaga, S., & Ohhara, Y. (1994). Constituents of *Corchorus olitorius* L. *Natural Medicine*, *48*, 213–214.

Lemmich, J., & Havelund, S. (1978). Coumarin glycosides of *Seseli montanum*. *Phytochemistry*, *17*, 139–141.

Lord, K. M., Epton, H. A. S., & Frost, R. R. (1988). Virus infection and furanocoumarins in celery. *Plant Pathology*, *37*, 385–389.

Macleod, A. J., & Cave, S. J. (1975). Volatile flavour compounds of eggs. *Journal of Science and Food Agriculture*, *26*, 351–358.

Mahran, G. H., Saleh, M. M., Fathy, M. M., Motawe, H. M., & Hashem, F. A. (1995). Effect of pollutants on the volatile oil of *Mentha piperita* L. leaves and cardiac glycosides of *Digitalis lanata* Ehrh. leaves. *Bulletin Faculty of Pharmacy, Cairo University*, *33*, 3–10.

Martin, M., & Dewick, P. M. (1979). Biosynthesis of 2- arylbenzofuran phytoalexin vignafuran in *Vigna unguiculata*. *Phytochemistry*, *18*, 1309–1317.

Moniatis, T., Fritssh, E. F., & Sambrook, J. (1982). *Molecular cloning*. Cold Spring Harbor Laboratory.

Mukherjee, K. K., Mitra, S. K., & Ganguly, S. N. (1998). A new coumarine from the seeds of Jute (*Corchorus olitorius* L.). *Natural Product Science*, *4*, 51–52.

Murray, R. D. H., Mendz, J., & Brown, S. A. (1982). *The natural coumarins, occurrence, chemistry and biochemistry*. New York: John Wiley.

Nielesen, B. E. (1971). The biology and chemistry of umbelliferae. In V. H. Haywood (pp. 325). London: Academic Press.

Oliver, B. (1960). *Medicinal plants in Nigeria*. Nigerian College of Art, Sciences and Technology.

Paris, R. R., & Theallet, J. P. (1961). Biochemical studies on several lime tree species (*Tilia argenta,T. cordata, T. platyphylla*); the isolation of fraxoside by separation of the bark of the silver lime. *Annales Pharmaceutiques Francaises*, *19*, 580–586.

Partridge, J. (1973). *Fungus disease defense response in legumes*. Ph.D.Thesis, University of California, USA.

Plouvier, V. (1968). Fraxoside and coumarin heteroside occuring in various botanical groups. *C.R.Hebd. Saences, Acad.Sci.*, *267 D*, 1883–1885.

Rajeswara Rao, B. R., Kaul, P. N., Mallavarapu, G. R., & Ramesh, S. (2000). First observation of little leaf disease and its impact on the yield and composition of the essential oil of rose-scented geranium (*Pelargonium* sp.). *Flavour and Fragrence Journal*, *15*, 137–140.

Reuveni, M., & Cohen, Y. (1978). Growth retardation and changes in phenolic compounds with special reference to scopoletin in mildewed and ethylene-treated *tobacco* plant. *Physiology and Plant Pathology*, *12*, 179–189.

Rich, J. R., Keen, N. T., & Thomason, I. J. (1977). Assosiation of coumestans with hypersensitivity of Lima bean roots to *Pratylenchus scribneri*. *Physiology and Plant Pathology*, *10*, 105–116.

Robenson, D. J. (1978). Furanoacetylenes and isoflavonoid phytoalexins in *Lens culinaris*. *Phytochemistry*, *17*, 807–808.

Shafizadeh, F., & Melnikoff, A. B. (1970). Coumarins of *Artemisia tridentata* SSP. Vaseyana. *Phytochemistry*, *9*, 1311–1316.

Stoessl, A., Stothers, J. B., & Ward, E. W. B. (1976). Sesquiterpenoid stress compounds of the Solanaceae. *Phytochemistry*, *15*, 855–872.

Subba Rao, N. S. (1977). *Soil micro-organisms and plant growth*. Oxford: Mohan Primlani Publisher.

Tal, B., & Robeson, D. J. (1986). The induction by fungal inoculation of ayapin and scopoletin biosynthesis in*Helianthus annuus*. *Phytochemistry*, *25*, 77–79.

Tangy, J., & Martin, C. (1972). Phenolic compounds and the hypersensitivity reaction in *Nicotiana tabacum* infected with *tobacco mosaic* virus. *Phytochemistry*, *11*, 19–28.

VanEtten, H. D. (1973). Identification of a second anti-fungal isoflavan from diseased *Phaseolus vulgaris* tissues. *Phytochemistry*, *12*, 1791–1792.

Woodward, M. D. (1980). Phaseoliene formation and metabolism in Phaseolus vulgaris. *Phytochemistry*, *19*, 921–927.

Yoshikawa, M., Murakani, T., Shimada, H., Fukada, N., Matsuda, H., Sashid, Y., & Yamahara, J. (1998). Corchorusoside A,B,C,D and E, new cardiotonic oligoglycosides from the seeds of *Corchorus olitorius* L. (*Moroheiya*). *Heterocycles*, *48*, 869–873.

Zeringue Jr., H. T. (1984). The accumulation of five fluorescent compounds in the cotton leaf induced by cell-free extracts of *Aspergillus flavus*. *Phytochemistry*, *23*, 2501–2503.