CHAPTER 3

RESULTS

Inhibitory Effect from Methanolic Extracts of E. camaldulensis.

1. Germination Inhibition.

The first series of experiments was conducted to test the hypothesis that methanolic extracts from green leaves, fallen leaves and bark of E. camaldulensis contained germination inhibitory phytotoxin(s). The numerical results of these experiments are summarized in Table 7 - 10 which show inhibition of M. pigra seed germination in Table 7 - 9 and O. sativa seed germination in Table 10.

1.1 Methanolic Extract of Green Leaves.

As shown in Table 7, methanolic extract of green leaves began to manifest its germination inhibitory activity towards M. pigra seeds at dose of 1 g. At this dose, germination of M. pigra seeds was reduced to 61.17, 59.63 and 88.10% of control after 24, 48 and 72 hours, respectively. Complete inhibition of M. pigra seed germination occurred at dose of 5 g. Only dose of 5 g had effect on germination of O. sativa seeds. At this dose, O. sativa seed germination was reduced to 6.65, 51.56 and 71.65% of control after 48, 72 and 90 hours, respectively (Table 10).

1.2 Methanolic Extract of Fallen Leaves.

As shown in Table 8, methanolic extract of fallen leaves began to elicit its germination inhibitory activity towards M. pigra seeds at the lowest dose tested (.1 g). At this dose, M. pigra seed germination was reduced to 65.92, 93.00 and 98.27% of control after 24, 48 and 72 hours, respectively. At doses of 1 and 5 g, M. pigra seed germination was completely inhibited.

1.3 Methanolic Extract of Bark.

As shown in Table 9, M. pigra seed germination was reduced by methanolic extract of bark at all doses tested (.1, 1 and 5 g) after 24 hours. After that the extract had no inhibitory activity against germination of M. pigra even at the highest dose tested (5 g).

2. Seedling Growth Inhibition.

The second series of experiments was conducted to elucidate whether extracts from green leaves, fallen leaves and bark of E. camaldulensis contained substance(s) having growth inhibitory activity against M. pigra seedlings. The results of these experiments are shown in Table 11 - 17 which give the hypocotyl and radicle length of M. pigra seedlings.

2.1 Methanolic Extract of Green Leaves.

As shown in Table 11, methanolic extract of green leaves began to elicit its growth inhibitory effects against M. pigra seedlings at dose of 1 g. At this dose, the length of hypocotyl was reduced to 25.54% of control and the length of radicle to 5.20% of control. Almost completely inhibition occurred at dose of 5 g.

At this dose, the length of hypocotyl was reduced to 3.09% of control and the length of radicle to 1.35% of control.

2.2 Methanolic Extract of Fallen Leaves.

Inhibitory effects of methanolic extract of fallen leaves against M. pigra seedling growth are recorded in Table 12. As summarized in the Table, the extract began to manifest its growth inhibitory activity towards M. pigra seedlings at the lowest dose (.1 g) tested. At this dose, the length of hypocotyl was reduced to an average of 50.66% of control and the length of the longest root to an average of 21.90% of control. Both doses of 1 and 5 g completely suppressed growth of hypocotyl and radicle of M. pigra.

2.3 Methanolic Extract of Bark.

The growth suppressing effect of M. pigra treated with bark methanolic extract is presented in Table 15. As summarized in the Table, the extract began to elicit its growth inhibitory activity towards M. pigra seedlings at dose of 1 g. At this dose, the length of hypocotyl was reduced to an average of 44.33% of control and the length of radicle to an average of 8.02% of control. At dose of 5 g, hypocotyl and the longest root length were reduced to 25.85 and 3.07% of control, respectively.

Isolation of the Inhibitory Factor(s).

The third series of experiments was conducted to isolate the toxic principle in the methanolic extracts of green leaves and bark of E. camaldulensis. The numerical results of these experiments are summarized in Table 13 - 14 and 16 - 21.

1. Effect of Solvent Extracts of the Methanolic Extracts.

1.1 Effect of Solvent Extracts of the Green Leaf Methanolic Extract.

After fractionation of the green leaf methanolic extract into n-hexane, ethyl acetate and aqueous fractions, severe growth reduction resulted from the aqueous fraction with slight reduction in the ethyl acetate and n-hexane fractions. As shown in Table 13 and 14, the aqueous extract began to manifest its growth inhibitory activity towards M. pigra seedlings at dose of 1 g. At this dose, the length of hypocotyl was reduced to 73.99% of control and the length of radicle to 30.63% of control. Severe growth reduction occurred at dose of 5 g. At this dose, the length of hypocotyl was reduced to 7.45% of control and that of radicle to 2.50% of control.

1.2 Effect of Solvent Extracts of the Bark Methanolic Extract.

After fractionation of the bark methanolic extract into n-hexane, ethyl acetate and aqueous fractions, the activity resulted from the aqueous and ethyl acetate fractions, but the n-hexane fraction was inactive (Table 16 and 17). The inhibitory activity of aqueous extract was approximately two times of that of ethyl acetate extract. Both aqueous and ethyl acetate extracts began to manifest their growth inhibitory activity towards M. pigra seedlings at dose of 1 g. At this dose, the ethyl acetate extract reduced hypocotyl length of M. pigra to 62.38% of control, and the longest root length to 49.08% of control. At dose of 5 g, the ethyl acetate extract reduced hypocotyl length of M. pigra to 28.20% of control and the longest root length to 6.17% of control.

At dose of 1 g, the aqueous extract reduced hypocotyl length of M. pigra to 39.92% of control and the longest root to 51.19% of control. At dose of 5 g, the aqueous extract reduced hypocotyl length of M. pigra to 12.74% of control and the longest root length to 3.47% of control.

2. Charcoal-Celite Column Chromatography.

Charcoal-celite column chromatography of methanolic extract of green leaves gave six fractions of eluate. The numerical results of growth inhibitory effect of these eluates on M. pigra seedlings are summarized in Table 18 and 19. As shown in Table 19, most of the activity was concentrated in the 50, 60 and 70% acetone fractions. Among the six fractions eluted from the column, the 50% acetone fraction showing the strongest inhibitory activity had the effect equal to or even greater than that exerted by the original methanolic extract. Increasing percent of acetone in the eluate resulted in decreasing the inhibitory activity. The last fraction eluted, the 100% acetone fraction, was slightly toxic to hypocotyl but had no inhibitory activity towards the seedlings of M. pigra. The 50% acetone fraction which would be subjected to further purification began to manifest its growth inhibitory activity towards M. pigra seedlings at dose of .1 g. At this dose, hypocotyl and the longest root length were reduced to 76.71 and 77.60% of control, respectively. At dose of 1 g, hypocotyl length was reduced to 12.27% of control and the longest root length to 3.06% of control. At dose of 5 g both hypocotyl and the longest root growth were completely inhibited.

3. Thin-Layer Chromatography.

Since the fraction eluted with acetone/water (50/50, v/v) was the most inhibitory fraction, it was subjected to Kieselgel 60 F_{254} (0.25 mm thick) thin-layer chromatography with butanol/water/acetic acid (80/15/5, v/v/v). After developing for more than 10 cm, the chromatogram was observed under UV-light and divided into eight fractions (including the origin) as shown in Table 20 to test for M. pigra growth-inhibitory activity. As indicated in the table, fraction one and five showed growth inhibitory activity towards M. pigra seedlings. Both fractions inhibited both root and growth of the seedlings. Using the 50% acetone fraction taken from 1 g fresh weight of green leaves, fraction one depressed hypocotyl length to an average of 57.28% of control and the longest root length to an average of 27.69% of control. More inhibitory activity was shown by fraction five reducing hypocotyl length to an average of 43.89% of control and the longest root length to an average of 18.93% of control.

Fraction one and five also inhibited growth of O. sativa seedlings. Using the 50% acetone fraction taken from 1 g fresh weight of green leaves, fraction one severely retarded O. sativa root growth but had little effects on the second leaf sheath whereas fraction five halved the growth of the longest root but completely unaffected growth of the second leaf sheath (Table 21).

Of the five authentic compounds cochromatographed with the water/acetone (50/50, v/v) fraction, chlorogenic acid showed the same color, namely bright white, under short wave UV-light, and the same R_f value as fraction five (Fig. 1). Thus, fraction five was assumed to be chlorogenic acid.

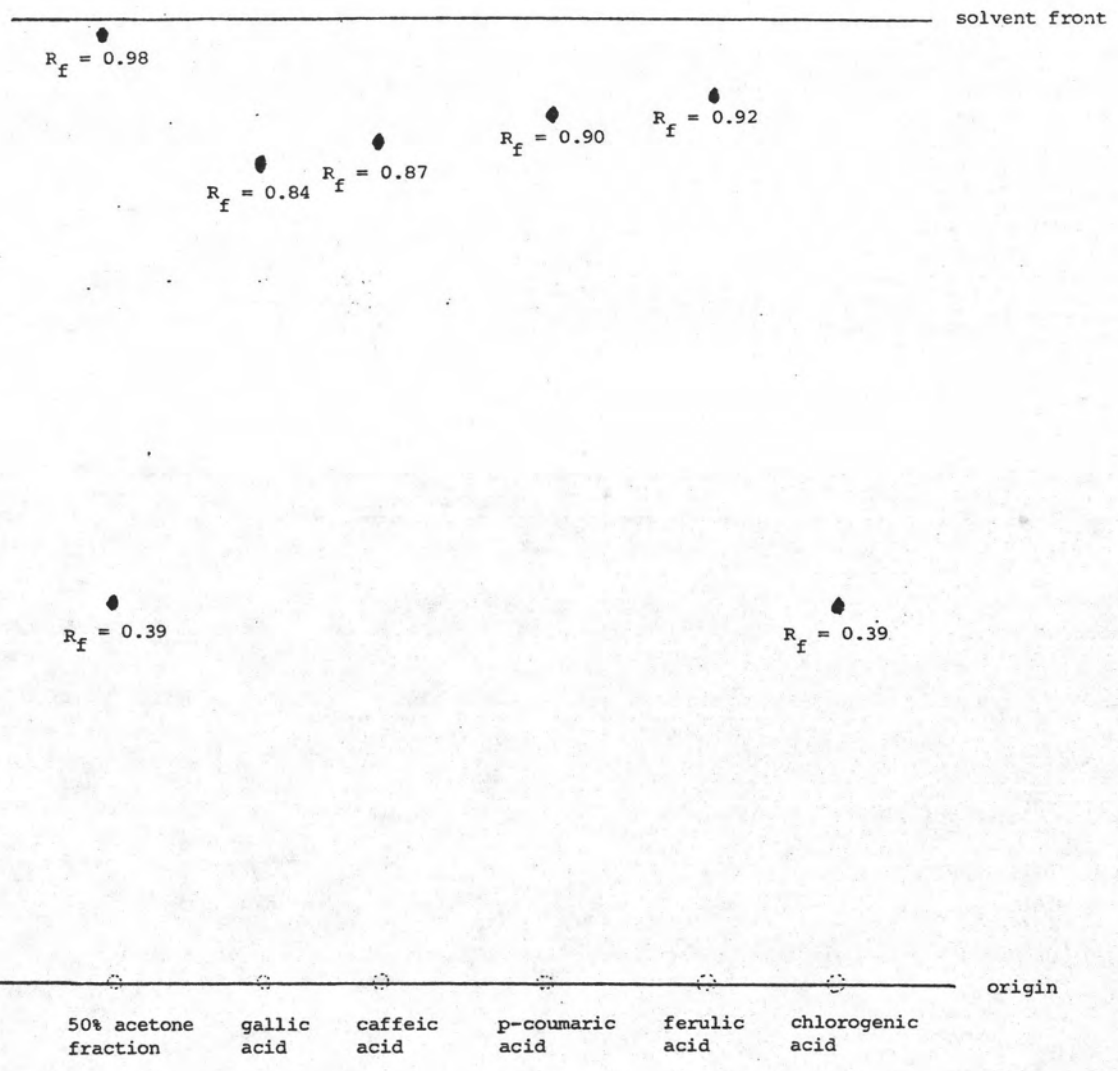

Fig. 1 Thin-layer chromatographic pattern of five phenolic acids and the 50% acetone fraction derived from charcoal-celite column chromatography of the green leaf methanolic extract of E. camaldulensis.

Fraction one was tentatively identified. This fraction being detected as a dark spotted area under UV-light and a yellow spotted area under white light had a little higher R_f value than those of the four remained authentic compounds cochromatographed with it. R_f values of fraction one, ferulic acid, p-coumaric acid, caffeic acid and gallic acid were 0.98, 0.92, 0.90, 0.87 and 0.84, respectively.

Effects of 13 Phenolic Compounds on Growth of M. pigra and O. sativa Seedlings.

For purpose of comparison, the test compounds were subdivided according to their chemical structure into cinnamic and benzoic acid derivatives. Two exceptional compounds, i.e. vanillin and thymol do not belong to either class albeit they are listed in benzoic acid derivative

Many phenolic compounds, notably benzoic and cinnamic acid derivatives are widely distributed in higher plant tissues and micro-organisms. Through the action of a variety of different mechanisms, these phenolic compounds are introduced into a soil system. The occurrence of these compounds in soil may be in the concentration range of 10^{-5} to 10^{-4} M (about 100 ppm) depending upon soil type, vegetation and seasonal variation (Whitehead, 1964). In this comparative experiments the phenolic compounds studied were tested at concentrations of .1, 1, 10, 100 and 1000 ppm. However, the concentrations other than 100 ppm were not used for comparing the effect upon test plant but were employed in order to determine the relative inhibitory capacities of the compounds concerned.

1. Effect on M. pigra.

With the exception of gallic and caffeic acids, all phenolic compounds tested showed severely inhibition on the growth (both hypocotyl and root) of M. pigra. The numerical results of these experiments are summarized in Table 22, 23, 24 and 25.

Among the benzoic acid derivative, at dose of 100 ppm, benzoic acid and salicylic acid were the most inhibitory, followed by gentisic, protocatechuic and gallic acids, respectively. However, at concentrations being lower than 100 ppm, gentisic acid was much more toxic than salicylic acid. At the lowest rate tested (.1 ppm), only benzoic and gentisic acids severely depressed growth (both hypocotyl and the longest root) of the seedlings. At this dose, other benzoic acid derivatives except gallic acid slightly reduced M. pigra seedling growth. Vanillin and thymol whose chemical structure do not belong to the benzoic acid derivative class are also toxic to M. pigra seedling growth. Collectively, the inhibitory activity they exerted on M. pigra seedlings was more than gallic acid but less than the other benzoic acid derivatives. Increasing concentration of benzoic acid, salicylic acid and vanillin in a range of .1 to 100 ppm, however, no increase in inhibitory effects was observed.

Among the cinnamic acid derivatives, at dose of 100 ppm, t-cinnamic acid was the most inhibitory, followed by ferulic acid, chlorogenic acid, methyl cinnamate, p-coumaric acid and caffeic acid, respectively. M. pigra seedling growth was completely inhibited at a concentration of 100 ppm t-cinnamic acid. At concentrations being lower than 100 ppm, however, ferulic and chlorogenic acids were much

more inhibitory than t-cinnamic acid. From Table 22, 23, 24 and 25 it can be seen that M. pigra seedlings were severely poisoned at as dilute a concentration as .1 ppm of ferulic and chlorogenic acids. Increasing concentration of chlorogenic acid, however, a slightly increase in inhibitory effect was observed, even but at 1000 ppm complete growth inhibition did not occur. Increasing concentration of methyl cinnamate in the range of .1 to 100 ppm constant inhibitory effect was also observed. Caffeic acid was the least inhibitory; it did not reduced growth of M. pigra seedlings to any extents. In a concentration lower than 1000 ppm, the substance showed no inhibitory effect on the growth of M. pigra seedlings.

All of the substances tested poisoning to hypocotyl also poisoned to the longest root to approximately the same degree.

2. Effect on O. sativa

Rice, on the other hand, almost all of the phenolic compounds tested seemed to have no effect on both root and the second leaf sheath length at concentrations as high as 100 ppm (Table 26, 27, 28 and 29). Here, however, growth of O. sativa was severely reduced by t-cinnamic acid, the only case in which this occurred. As shown in Table 27 and 29, length of the longest root was reduced to 9.46% of control and the second leaf sheath to 33.99% of control.

Among the benzoic acid derivatives, only protocatechuic acid and thymol were slightly inhibitory at dose of 100 ppm. However, these two substances did not elicit the same extent of activity : thymol was slightly toxic to both root and the second leaf sheath

whereas protocatechuic acid was toxic to only the longest root length which was reduced to 51.36% of control. Benzoic, salicylic and gentisic acids being severely inhibitory to M. pigra seedling growth at as dilute a concentration as .1 ppm had no inhibitory effect towards growth of O. sativa seedlings in the concentration range of .1 to 100 ppm.

Among the cinnamic acid derivatives, the most toxic of all to growth of both root and the second leaf sheath was t-cinnamic acid at a concentration of 100 ppm which severely impaired growth of the rice seedlings. The others being inhibitory to O. sativa seedlings were p-coumaric, caffeic and methyl cinnamate, however, all of these substances were inactive to the second leaf sheath and slightly toxic to the longest root. Chlorogenic acid was the least inhibitory. It was inactive to both root and the second leaf sheath at concentrations as high as 100 ppm and toxic to only the longest root at dose of 1000 ppm. Ferulic and chlorogenic acids being severely inhibitory to M. pigra seedling growth at as dilute a concentration as 0.1 ppm had no inhibitory effect towards growth of O. sativa seedlings at concentration as high as 100 ppm.

All of the six phenolic compounds (protocatechuic acid, thymol, t-cinnamic acid, p-coumaric acid, caffeic acid and methyl cinnamate) being inhibitory to O. sativa seedling growth were more poisonous to root than the second leaf sheath. Of these six compounds, thymol and t-cinnamic acid inhibited both root and the second leaf sheath. The others were poisonous to root but much less inhibitory to the second leaf sheath.

Table 7 *M. pigra* seed germination in Petri dishes with methanolic extracts from .1, 1 and 5 g of green leaves of *E. camaldulensis* (N = number of individuals measured = 20 for all means). Values in this and all subsequent tables are means ± S.D. and/or percent of germination or growth (compare to control).

Hours after	Control	Dose					
		1		1		5	
	Means ± S.D.	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
24	15.67 ± 1.53	15.00 ± 2.00	97.72	9.67 ± 4.16	61.17	0.00 ± 0.00	0.00
48	19.00 ± 0.00	18.83 ± 0.58	99.11	11.33 ± 4.73	59.63	0.00 ± 0.00	0.00
72	19.67 ± 0.58	19.67 ± 0.58	100.00	17.33 ± 0.58	88.10	0.00 ± 0.00	0.00

Table 8 *M. pigra* seed germination in Petri dishes with methanolic extracts from .1, 1 and 5 g. of fallen leaves of *E. camaldulensis* (N = 20 for all means).

Hours after	Control	Dose					
		.1		1		5	
	Means ± S.D.	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
24	13.57 ± 0.58	10.33 ± 3.79	65.92	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
28	19.00 ± 1.00	17.67 ± 0.58	93.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
72	19.67 ± 0.58	19.33 ± 1.15	98.27	0.00 ± 0.00	0.00	0,00 ± 0.00	0.00

Table 9 *M. pigra* seed germination in Petri dishes with methanolic extracts from .1, 1 and 5 g of bark of *E. camaldulensis* (N = 20 for all means).

Hours after	Dose					
	.1		1		5	
	Means ± S.D.	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
24	13.67 ± 0.58	6.67 ± 1.53	48.79	5.67 ± 0.58	41.48	1.67 ± 0.58	2.22
48	19.00 ± 0.00	20.00 ± 0.00	105.26	19.33 ± 0.38	101.74	17.33 ± 1.53	91.21
72	20.00 ± 0.00	20.00 ± 0.00	100.00	19.67 ± 0.35	98.35	18.33 ± 1.53	91.65

Table 10 O. sativa cv. RD 23 seed germination in Petri dishes with methanolic extracts from .1, 1 and 5 g of green leaves of E. camaldulensis (N = 20 for all means).

Hours after		Dose					
		.1		1		5	
	Means ± S.D.	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
24	0.00 ± 0.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00
48	20.00 ± 0.00	19.67 ± 0.58	98.35	16.33 ± 3.79	81.65	1.33 ± 2.31	6.65
72	20.00 ± 0.00	19.67 ± 0.58	98.35	16.67 ± 0.58	83.45	10.33 ± 0.58	51.56
90	20.00 ± 0.00	20.00 ± 0.00	100.00	19.67 ± 0.58	88.36	14.33 ± 0.58	71.65

Table 11 Growth of M. pigra seedlings when treated with methanolic extracts from .1, 1 and 5 g of green leaves of E. camaldulensis (N = 18 for all means). Control for hypocotyl = 12.61 ± 2.73 mm; for longest root = 28.83 ± 5.11 mm.

	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hypocotyl	10.67 ± 1.64	84.62	3.22 ± 2.29	25.54	0.39 ± 0.50	3.09
Longest root	32.72 ± 12.38	113.43	1.50 ± 1.15	5.20	0.39 ± 0.50	1.35

Table 12 Growth of *M. pigra* seedlings when treated with methanolic extracts from .1, 1 and 5 g of fallen leaves of *E. camaldulensis* (N = 18 for all means). Control for hypocotyl = 16.56 ± 3.01 mm; for longest root = 27.67 ± 7.74 mm.

	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hypocotyl	8.39 ± 4.45	50.66	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
Longest root	6.06 ± 5.26	21.90	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00

Table 13 Hypocotyl growth of M. pigra seedlings when treated with various solvent extracts from .1, 1 and 5 g of green leaves of E. camaldulensis (N = 18 for all means). Control = 12.61 ± 2.73 mm.

Solvent	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hexanc	11.72 ± 1.41	92.94	9.06 ± 1.55	71.85	7.56 ± 2.51	59.84
EtOAC	11.56 ± 1.76	91.66	10.94 ± 1.59	86.67	8.94 ± 1.51	71.90
Water	12.78 ± 1.73	101.35	9.33 ± 1.94	73.99	0.94 ± 0.80	7.45

Table 14 The longest root growth of M. pigra seedlings when treated with various solvent extracts from .1, 1 and 5 g of green leaves of E. camaldulensis (N = 18 for all means). Control = 28.83 ± 5.11 mm.

Solvent	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hexane	23.33 ± 9.36	80.92	34.11 ± 10.40	118.31	13.83 ± 8.32	49.93
EtOAC	23.06 ± 4.71	79.99	20.28 ± 8.50	71.33	13.06 ± 3.56	45.30
Water	35.10.30	121.61	8.83 ± 6.96	30.63	0.72 ± 0.57	2.50

Table 15 Growth of *M. pigra* seedlings when treated with methanolic extracts from .1, 1 and 5 g of bark of *E. camaldulensis* (N = 18 for all means). Control for hypocotyl length = 16.56 ± 2.01 mm; for longest root length = 27.06 ± 7.74 mm.

	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hypocotyl	17.33 ± 3.76	104.67	7.44 ± 3.71	44.33	4.28 ± 2.61	25.85
Longest root	25.89 ± 7.90	95.68	2.17 ± 1.42	8.02	0.83 ± 0.51	3.07

Table 16 Hypocotyl growth of M. pigra seedlings when treated with various solvent extracts from .1, 1 and 5 g of bark of E. camaldulensis (N = 18 for all means). Control = 16.56 ± 3.01 mm.

Solvent	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hexane	14.72 ± 2.36	88.99	16.28 ± 6.01	98.31	11.50 ± 3.68	69.44
EtOAc	14.50 ± 3.38	87.66	10.33 ± 3.31	62.38	4.67 ± 2.89	28.20
Water	16.00 ± 2.33	94.62	6.61 ± 5.71	39.92	2.11 ± 1.53	12.74

Table 17 Growth of the longest root of M. pigra seedlings treated with various solvent extracts from .1, 1 and 5 g of bark of E. camaldulensis (N = 18 for all means). Control = 27.06 ± 7.74 mm.

Solvent	Dose					
	.1		1		5	
	Means ± S.D.	% of control	Means ± S.D.	% of control	Means ± S.D.	% of control
Hexane	28.89 ± 8.22	106.76	26.00 ± 8.27	96.08	29.16 ± 8.35	107.76
EtOAC	31.44 ± 9.95	116.19	13.28 ± 2.93	49.08	1.67 ± 1.19	6.17
Water	33.94 ± 11.25	125.42	13.83 ± 6.33	51.19	0.94 ± 0.48	3.47

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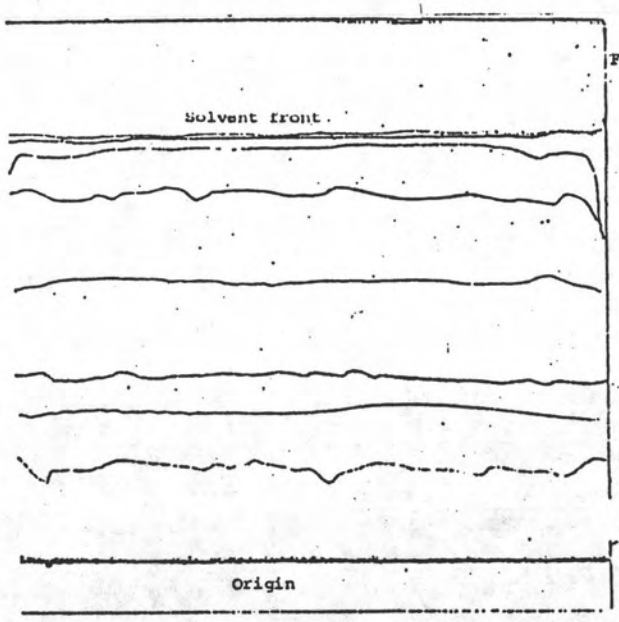
Table 18 Growth of M. pigra seedlings (means with standard deviations) treated with various fractions from charcoal-celite column chromatography of methanolic extracts from .1, 1 and 5 g of green leaves of E. camaldulensis. Control for hypocotyl length = 13.61 ± 2.43 mm; for the longest root length = 32.72 ± 8.66 mm. N = 18 for all means.

Length	Does (g)	Eluate (water/acetone, v/v)					
		50:50	40:60	30:70	20:80	10:90	0:100
Hypocotyl	.1	10.44 ± 1.65	9.89 ± 1.23	12.39 ± 2.43	10.22 ± 1.70	10.65 ± 2.53	11.06 ± 9.55
	1	1.67 ± 1.03	5.67 ± 2.93	7.33 ± 1.75	9.89 ± 1.68	9.78 ± 1.22	11.44 ± 2.15
	5	0.00 ± 0.00	1.22 ± 0.73	1.56 ± 0.70	4.28 ± 2.49	6.56 ± 3.01	9.72 ± 2.14
Longest root	.1	25.39 ± 7.64	34.22 ± 14.14	28.78 ± 6.75	31.72 ± 6.28	32.83 ± 10.20	32.35 ± 9.55
	1	1.00 ± 0.00	11.83 ± 12.99	34.61 ± 12.19	29.11 ± 14.48	28.83 ± 13.53	37.11 ± 10.80
	5	0.00 ± 0.00	0.83 ± 0.73	0.94 ± 0.24	5.44 ± 6.86	19.50 ± 10.61	32.83 ± 15.90

Table 19 Growth of M. pigra seedlings (percent of control) treated with various fractions from charcoal-celite column chromatography of methanolic extracts from .1, 1 and 5 g of green leaves of E. camaldulensis. Control for hypocotyl length = 13.61 ± 2.43 mm; for the longest root length = 32.72 ± 8.66 mm. N = 18 for all means.

Length	Dose (g)	Eluate (water/acetone, v/v)					
		50:50	40:60	30:70	20:80	10:90	0:100
Hypocotyl	.1	76.71	72.67	91.04	75.09	77.60	81.26
	1	12.27	41.66	53.86	72.67	71.86	84.06
	5	0.00	8.96	11.46	31.45	48.20	71.42
Longest root	.1	77.60	104.58	87.06	96.94	100.34	98.81
	1	3.06	36.16	105.78	88.97	88.11	113.42
	5	00.00	2.54	2.87	16.63	59.60	100.34

Table 20 Thin-layer chromatogram and toxicity data for the water-acetone (50:50) fraction from charcoal-celite column chromatography towards M. pigra growth (means with standard deviations). Control for hypocotyl = 13.67 ± 1.97 mm; for the longest root = 24.67 ± 4.55 mm. Percent of control is shown in parenthesis. N = 18 for all means.



Fraction	Hypocotyl length (mm.)	Length of longest root (mm.)
1.	7.83 ± 2.23 (57.28%)	6.83 ± 3.54 (27.69%)
2.	11.50 ± 3.27 (84.13%)	20.50 ± 6.53 (83.10%)
3.	15.17 ± 2.23 (110.97%)	20.50 ± 2.95 (83.10%)
4.	16.83 ± 3.31 (123.12%)	21.67 ± 6.02 (87.84%)
5.	6.00 ± 2.28 (43.89%)	4.67 ± 4.32 (18.93%)
6.	15.50 ± 2.07 (113.39%)	19.83 ± 2.23 (80.33%)
7.	17.83 ± 1.94 (130.43%)	23.33 ± 10.48 (94.57%)
Origin	19.33 ± 2.34 (141.40%)	35.33 ± 8.26 (143.21%)

Table 21 Effect of two substances from the thin-layer chromatogram showing toxicity towards *M. pigra* seedlings on *O. sativa* cv. RD 23 seedling growth (means with standard deviations). Control for second leaf sheath = 33.17 ± 2.48 mm; for longest root = 58.17 ± 5.60 mm. Percent of control is shown in parenthesis. N = 18 for all means.

Fraction number	Length of second leaf sheath (mm)	Length of longest root (mm)
1 (Rf = 0.98)	25.83 ± 4.02 (77.87%)	5.83 ± 0.98 (10.02%)
5 (Rf = 0.39)	31.17 ± 1.94 (93.97%)	24.17 ± 4.83 (41.55%)

Table 22 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the hypocotyl length of M. pigra seedlings (means with standard deviations). Control = 25.65 ± 1.78 mm. N = 18 for all means.

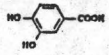
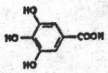
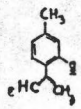
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		5.83 ± 1.03	5.42 ± 0.67	5.16 ± 1.19	1.33 ± 0.82	0.00 ± 0.00
Salicylic acid		11.42 ± 2.83	13.42 ± 1.66	13.75 ± 2.49	1.67 ± 1.97	0.00 ± 0.00
Gentisic acid		5.83 ± 0.72	6.08 ± 1.00	5.33 ± 0,89	4.58 ± 1.78	0.00 ± 0.00
Protocatechuic acid		19.08 ± 3.23	14.67 ± 1.97	9.92 ± 2.50	6.92 ± 1.31	0.00 ± 0.00
Gallic acid		26.58 ± 3.30	21.67 ± 4.14	12.50 ± 2.07	11.00 ± 1.48	3.92 ± 1.16
Vanillin		14.25 ± 2.83	13.75 ± 2.48	15.00 ± 1.13	11.33 ± 1.83	5.75 ± 7.12
Thymol		14.67 ± 2.27	13.17 ± 2.48	12.33 ± 1.78	5.75 ± 1.29	0.00 ± 0.00

Table 22 (cont.)

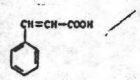
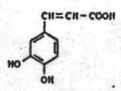
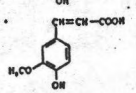
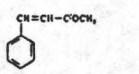
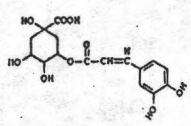
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		12.83 ± 1.80	9.42 ± 2.27	10.75 ± 2.30	0.00 ± 0.00	0.00 ± 0.00
p-Coumaric acid		29.92 ± 1.83	25.50 ± 7.34	25.00 ± 0.61	19.29 ± 6.51	0.00 ± 0.00
Caffeic acid		23.00 ± 3.19	25.33 ± 4.21	29.33 ± 8.68	17.67 ± 4.14	10.67 ± 7.14
Ferulic acid		5.25 ± 0.97	4.67 ± 1.37	3.58 ± 0.67	2.83 ± 1.95	0.00 ± 0.00
Methyl cinnamate		13.58 ± 1.73	12.67 ± 2.15	13.17 ± 2.82	12.75 ± 2.67	0.00 ± 0.00
Chlorogenic acid		6.08 ± 0.51	5.33 ± 0.49	5.83 ± 0.72	7.50 ± 2.68	5.42 ± 1.08

Table 23 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the hypocotyl length (% of control) of *M. pigra* seedlings. N = 18 for all means.

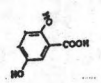
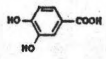
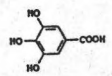
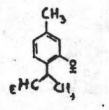
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		22.73%	21.13%	20.12%	5.19%	0.00%
Salicylic acid		44.52%	52.32%	53.61%	6.51%	0.00%
Gentisic acid		22.73%	23.70%	20.78%	17.86%	0.00%
Protocatechuic acid		74.39%	57.19%	38.67%	26.98%	0.00%
Gallic acid		103.63%	84.48%	48.73%	42.88%	15.28%
Vanillin		55.56%	53.61%	58.48%	44.17%	22.42%
Thymol		57.19%	51.35%	48.07%	22.42%	0.00%

Table 23 (Cont.)

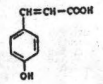
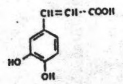
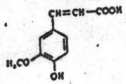
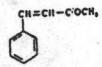
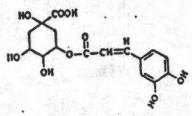
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		50.02%	36.73%	41.91%	0.00%	0.00%
p-Coumaric acid		116.65%	99.42%	97.47%	75.20%	0.00%
Caffeic acid		89.67%	98.75%	114.35%	68.89%	41.60%
Ferulic acid		20.47%	18.21%	13.96%	11.03%	0.00%
Methyl cinnamate		52.94%	49.40%	51.35%	49.71%	0.00%
Chlorogenic acid		23.70%	20.78%	22.73%	29.24%	21.13%

Table 24 Effect of phenolic compounds (.1, 1, 10, 100 and 1,000 ppm) on the length of the longest root of M. pigra seedlings. (means with standard deviations). Control = 37.83 ± 5.27 mm. N = 18 for all means.

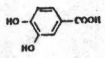
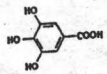
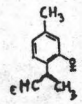
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		11.83 ± 3.27	8.42 ± 2.35	0.33 ± 1.78	1.33 ± 0.82	0.00 ± 0.00
Salicylic acid		35.50 ± 7.79	29.67 ± 13.64	21.83 ± 2.71	0.93 ± 1.31	0.00 ± 0.00
Gentisic acid		8.92 ± 3.12	7.25 ± 2.99	8.08 ± 3.37	7.17 ± 4.71	0.00 ± 0.00
Protocatechuic acid		27.42 ± 6.23	16.00 ± 2.89	14.00 ± 2.37	14.33 ± 2.74	0.00 ± 0.00
Gallic acid		34.17 ±	28.75 ± 6.25	21.50 ± 4.31	20.33 ± 3.77	5.57 ± 2.56
Vanillin		30.42 ± 9.28	28.17 ± 7.70	26.17 ± 6.10	24.33 ± 7.35	1.92 ± 2.57
Thymol		30.75 ± 11.23	33.17 ± 10.16	23.58 ± 5.26	19.33 ± 6.86	0.00 ± 0.00

Table 24 (cont.)

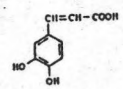
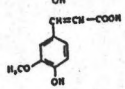
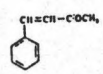
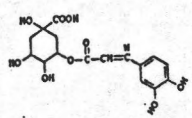
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		36.50 ± 10.08	24.25 ± 15.53	10.92 ± 6.97	0.00 ± 0.00	0.00 ± 0.00
p-Coumaric acid		36.92 ± 13.56	33.00 ± 4.20	31.92 ± 10.01	34.25 ± 12.96	0.00 ± 0.00
Caffeic acid		41.50 ± 6.50	30.25 ± 7.05	35.25 ± 6.62	42.67 ± 12.21	19.71 ± 1.00
Ferulic acid		5.83 ± 2.44	6.42 ± 3.42	3.67 ± 1.15	1.75 ± 1.29	0.00 ± 0.00
Methyl cinnamate		44.92 ± 10.12	22.50 ± 12.78	20.67 ± 7.56	11.92 ± 3.63	0.00 ± 0.00
Chlorogenic acid		8.33 ± 2.93	7.42 ± 2.15	8.50 ± 2.50	8.07 ± 2.19	5.75 ± 2.96

Table 25 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the length of the longest root (percent of control) of M. pigra seedlings. N = 18 for all means.

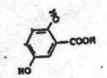
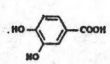
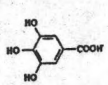
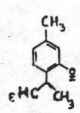
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		31.27%	22.56%	16.73%	3.52%	0.00%
Salicylic acid		93.84%	78.43%	57.71%	2.46%	0.00%
Gentisic acid		23.58%	19.16%	21.36%	18.95%	0.00%
Protocatechuic acid		72.48%	42.29%	37.01%	37.88%	0.00%
Gallic acid		90.33%	76.00%	56.83%	53.74%	17.72%
Vanillin		96.27%	74.46%	69.18%	64.31%	5.08%
Thymol		81.28%	87.68%	62.33%	51.07%	0.00%

Table 25 (cont.)

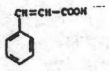
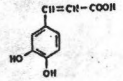
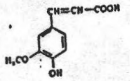
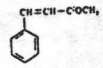
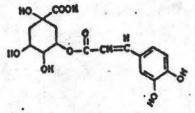
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		96.48%	64.10%	28.87%	0.00%	0.00%
p-Coumaric acid		97.59%	87.23%	84.38%	90.5%	0.00%
Caffeic acid		109.70%	79.96%	93.18%	112.79%	52.10%
Ferulic acid		18.41%	16.97%	9.70%	4.63%	0.00%
Methyl cinnamate		118.74%	59.48%	54.64%	31.51%	0.00%
Chlorogenic acid		22.02%	19.61%	22.47%	21.33%	15.20%

Table 26 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the length of the second leaf sheath of O. sativa cv. RD 23 seedlings (means with standard deviations). Control = 33.83 ± 5.98 mm. N = 18 for all means.

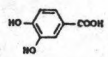
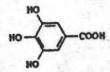
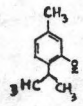
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		29.67 ± 2.81	34.50 ± 3.34	30.83 ± 3.16	33.92 ± 5.09	0.00 ± 0.00
Salicylic acid		31.50 ± 5.32	33.83 ± 4.17	33.67 ± 4.66	32.83 ± 1.95	0.00 ± 0.00
Gentisic acid		32.42 ± 5.74	33.25 ± 4.49	36.50 ± 3.85	34.17 ± 4.09	0.00 ± 0.00
Protocatechuic acid		43.08 ± 5.35	46.33 ± 6.10	45.83 ± 5.13	43.33 ± 6.26	22.92 ± 3.26
Gallic acid		46.17 ± 3.51	43.08 ± 5.11	47.00 ± 3.69	41.58 ± 4.70	32.25 ± 4.25
Vanillin		32.33 ± 2.74	33.33 ± 4.36	29.17 ± 9.36	34.08 ± 8.87	9.42 ± 13.21
Thymol		30.92 ± 6.87	31.00 ± 6.37	33.00 ± 3.69	25.50 ± 5.42	0.00 ± 0.00

Table 26 (cont.)

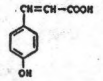
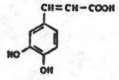
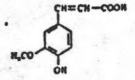
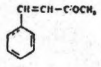
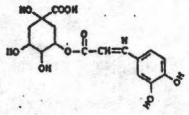
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		33.83 ± 4.28	34.08 ± 8.15	33.83 ± 6.63	11.50 ± 12.12	0.00 ± 0.00
p-Coumaric acid		47.08 ± 2.27	44.17 ± 5.18	43.83 ± 2.59	37.42 ± 7.79	2.42 ± 8.37
Caffeic acid		43.67 ± 5.23	51.83 ± 19.04	41.92 ± 9.92	46.00 ± 3.00	24.83 ± 3.41
Ferulic acid		32.08 ± 6.10	33.25 ± 3.65	33.75 ± 4.65	32.83 ± 3.66	18.42 ± 13.67
Methyl cinnamate		32.92 ± 9.25	36.42 ± 2.50	37.08 ± 2.61	31.58 ± 4.14	2.25 ± 5.36
Chlorogenic acid		33.67 ± 6.77	35.00 ± 6.30	35.33 ± 6.67	34.00 ± 5.34	31.92 ± 3.80

Table 27 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the length of the second leaf sheath (percent of control) of O. sativa cv. RD 23 Seedlings. N = 18 for all means.

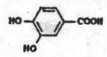
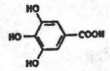
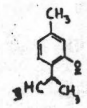
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		79.72%	101.98%	91.13%	100.27%	0.00%
Salicylic acid		93.11%	100.00%	99.53%	97.04%	0.00%
Gentisic acid		95.83%	98.29%	107.89%	101.01%	0.00%
Protocatechuic acid		127.34%	136.95%	135.47%	128.08%	67.75%
Gallic acid		136.48%	127.34%	138.93%	122.91%	95.33%
Vanillin		95.57%	98.52%	86.23%	100.74%	27.85%
Thymol		91.40%	91.63%	97.55%	75.38%	0.00%

Table 27 (cont.)

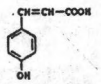
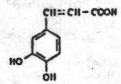
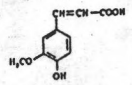
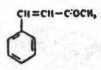
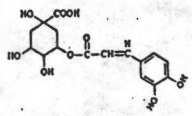
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		100.00%	100.74%	100.00%	33.99%	0.00%
p-Coumaric acid		139.17%	130.56%	129.56%	110.61%	7.15%
Caffeic acid		129.09%	153.21%	123.91%	135.97%	73.40%
Ferulic acid		94.83%	98.29%	99.76%	97.04%	54.45%
Methyl cinnamate		97.31%	107.66%	109.61%	93.35%	6.65%
Chlorogenic acid		99.53%	103.46%	104.43%	100.50%	94.35%

Table 28 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the length of the longest root of O. sativa cv.. RD. 23 seedlings. (means with standard deviations). Control = 54.67 ± 14.61 mm. N = 18 for all means.

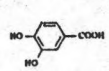
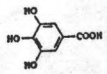
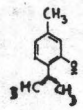
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		69.08 ± 11.61	70.75 ± 9.04	66.83 ± 15.21	60.25 ± 15.81	0.00 ± 0.00
Salicylic acid		64.92 ± 21.12	50.00 ± 9.95	49.08 ± 14.13	49.75 ± 25.63	0.00 ± 0.00
Gentisic acid		51.67 ± 12.61	53.25 ± 17.29	71.50 ± 11.51	59.25 ± 10.90	0.00 ± 0.00
Protocatechuic acid		68.08 ± 12.08	67.58 ± 16.38	44.42 ± 7.91	28.08 ± 3.45	0.50 ± 0.80
Gallic acid		58.42 ± 16.08	63.17 ± 10.37	60.33 ± 9.08	57.00 ± 12.04	16.25 ± 8.36
Vanillin		65.42 ± 15.57	56.92 ± 21.89	48.00 ± 20.92	54.25 ± 24.45	3.08 ± 6.88
Thymol		58.42 ± 20.46	64.17 ± 22.04	63.75 ± 17.45	36.17 ± 25.25	0.00 ± 0.00

Table 28 (cont.)

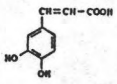
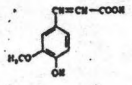
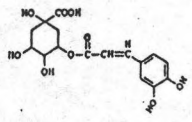
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		60.25 ± 18.77	42.58 ± 18.48	31.08 ± 6.43	5.17 ± 4.37	0.00 ± 0.00
p-Coumaric acid		58.75 ± 14.31	51.75 ± 14.07	43.82 ± 6.49	37.17 ± 7.79	0.00 ± 0.00
Caffeic acid		57.92 ± 8.55	51.83 ± 7.33	49.92 ± 2.48	42.50 ± 7.12	2.83 ± 5.54
Ferulic acid		53.42 ± 13.17	60.25 ± 16.09	53.67 ± 12.63	55.42 ± 18.90	1.92 ± 2.78
Methyl cinnamate		49.58 ± 28.15	59.08 ± 11.32	51.08 ± 14.92	36.58 ± 14.25	2.75 ± 5.31
Chlorogenic acid		56.33 ± 11.90	47.17 ± 11.32	62.33 ± 11.23	57.58 ± 14.25	17.58 ± 4.46

Table 29 Effect of phenolic compounds (.1, 1, 10, 100 and 1000 ppm) on the length of the longest root (percent of control) of O. sativa cv. RD 23 Seedlings. N = 18 for all means.

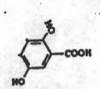
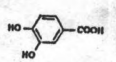
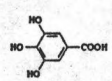
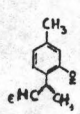
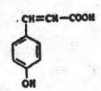
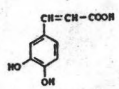
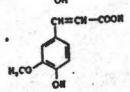
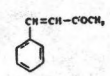
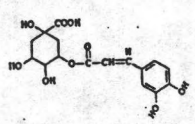
Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Benzoic acid derivatives						
Benzoic acid		126.36%	129.41%	122.24%	110.21%	0.00%
Salicylic acid		118.75%	91.46%	89.78%	91.00%	0.00%
Gentisic acid		94.51%	97.40%	130.78%	108.38%	0.00%
Protocatechuic acid		124.53%	123.61%	81.25%	51.36%	0.91%
Gallic acid		100.86%	115.55%	110.35%	104.26%	29.72%
Vanillin		119.66%	104.12%	87.80%	99.23%	5.63%
Thymol		106.86%	117.38%	116.61%	66.16%	0.00%

Table 29 (cont.)

Phenolic compounds	Chemical structure	Concentration (ppm)				
		.1	1	10	100	1000
Cinnamic acid derivatives						
t-Cinnamic acid		110.21%	77.89%	56.85%	9.46%	0.00%
p-Coumaric acid		107.46%	94.66%	80.15%	67.99%	0.00%
Caffeic acid		105.94%	94.81%	91.31%	77.74%	5.18%
Ferulic acid		97.71%	110.21%	98.17%	109.37%	3.51%
Methyl cinnamate		90.69%	108.07%	93.43%	66.91%	5.03%
Chlorogenic acid		103.04%	86.28%	114.01%	105.32%	32.16%