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Antiviral Substances in Plants of the Mint Family (Labiatae). III. Peppermint *(Mentha piperita)* and other Mint Plants.* (31874)

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One member of the mint family, *Melissa officinalis* (lemon balm) has been studied in detail and found to contain both tannin and nontannin polyphenol antiviral fractions(1-4). It has been reported that two other mint plants, peppermint and sage, contain the same condensed tannin, consisting of trimers of caffeic acid (3,4-dihydroxycinnamic acid), found in melissa(5). Because this tannin was demonstrated to be a potent inhibitor of Newcastle disease virus (NDV), studies were undertaken to determine if extracts of other plants of the mint family (Labiatae) also had antiviral activity. The following report will show that peppermint contains antiviral substances quite similar to those of melissa and, further, that a number of other mint plants also contain substances with antiviral activity.

Materials and methods. All plants used in this investigation were kindly supplied by S. B. Penick and Co., New York, as dried leaf preparations, with the exception of hyssop which was supplied as branches and seeds. Hot-water extracts of the plant materials were prepared exactly as described for melissa(2). The preparation of tannin and tannin-free fractions by gelatin precipitation or hide-powder adsorption has been reported previously(2,3). The antiviral test procedures have been fully described in previous reports, including methods for infecting, treating, and incubating embryonated chicken eggs(1,2) and the preparation of and medium for chick embryo fibroblast cell cultures and their use in the disc-plate plaque-suppression test(1,2, 6) and hemagglutination tests(2). Source, strain, and maintenance of viruses used in this study have also been previously described(1-3).

Results and discussion. Aqueous extracts of peppermint leaves produced virtually the same antiviral effects observed with extracts of melissa leaves(1) (Table I). The effects were noted in eggs only when the preparation was injected into the allantoic sac 3 to 24 hours prior to virus given by the same route, but there was no effect in influenza virus-infected eggs. However, peppermint extracts exhibited some activity in suppressing plaques of influenza A virus. This borderline activity was not confirmed in egg tests, was not observed with melissa extracts, and is unexplained at present. It is possible that this activity is the result of still another antiviral component at low concentration or of very low potency.

The antiviral effects of peppermint extracts were so similar to those of melissa that experiments were undertaken to determine if both tannin and nontannin antiviral substances were present, as has been found with melissa(4). The antiviral effect against NDV was concentrated in the tannin fraction while the tannin-free fraction showed activity against herpes simplex virus (Table II). Although the nontannin fraction of peppermint is not as potent as that of melissa, there clearly is a second antiviral substance inactive against NDV but active against herpes simplex. Investigations with melissa established that this second antiviral material

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TABLE I. Antiviral Activity of Aqueous Extracts of *Mentha piperita*.

Virus	Egg experiments: Virus dose (LD_{50}/0.3 ml)	Hr injected before (—) or after (+) virus	No. of prep. tested	Survivors/total eggs*: Dilution: UD	½	¼	UD, no virus	Virus only	Disc-plaque suppression test: No. of prep. tested	PFU†	Avg plaque-free zone, mm‡
NDV (11914)	32-80	—24	3	12/16	19/27	13/26	27/37	0/22			
	63-80	— 3	2	14/19	12/19	8/18	—	0/6	5	6 to 8 × 10^2	16 (11)
	80	+ 3	1	0/7	0/6	0/6	—	—			
Herpes simplex (HF)	40	—24	1	7/8	5/9	7/8	8/8	0/9	10	9 × 10^2 to 2 × 10^3	22 (24)
	40-200	— 3 to —6	2	13/16	13/17	13/16	13/15	0/17	3	2 × 10^4	20 (3)
Vaccinia (WR)	100	—24	2	9/16	6/14	5/15	14/15	0/16			
	100	— 3	2	8/14	6/12	1/13	11/13	—	5	1 to 2 × 10^3	16 (7)
	100	+ 3	2	0/14	1/13	0/15	—	—			
Semliki Forest	10-126	—24	8	34/53	32/55	16/55	49/57	1/27	5	2 × 10^3	0 (8)
	250-364	—24	4	3/22	1/21	0/23	7/7	0/9	1	1.4 × 10^3	18 (2)§
West Nile (E-101)	10	—24	1	3/4	—	—	—	0/6	2	5 × 10^2	14 (1), 19 (1)§
Inf. A (WSN)	50	—24	1	0/8	0/9	0/8	9/9	0/8	7	5 × 10^2 to 5 × 10^3	10 (12)
Inf. A (PR8)	100	—24	1	0/7	0/9	0/9	—	0/9			
Inf. B (GL)	50	—24	1	1/6	1/10	0/10	—	0/10			

* Results at the end of incubation for 8 to 10 days at 36°C. UD = undiluted.
† PFU = plaque-forming units.
‡ Numbers in parentheses indicate number of separate tests. Except as indicated, all results were for ¼-inch discs.
§ These data from tests with ½-inch discs.

TABLE II. Antiviral Activity of Tannin and Tannin-Free Fractions of *Mentha piperita*.

Preparation	Virus; dose (LD_{50}/0.3 ml)	No. of prep. tested*	Survivors/total eggs — Dilution — UD	½	¼	UD, no virus	Virus only
Tannin†	NDV; 32	2	11/14	9/11	7/14	8/9	0/15
	Herpes simplex; 50	2	9/14	3/10	2/8	3/3	1/4
Tannin-free‡	NDV; 32-80	2	1/20	1/11	0/6	12/13	0/19
	Herpes simplex; 50-63	3	9/22	3/9	3/10	5/5	1/10

* Preparations injected 24 hr prior to virus injection, via allantoic sac.

† Tannin fraction prepared by gelatin precipitation from aqueous extract and then recovered by trypsin digestion; fraction was diluted to the same volume as the original aqueous extract from which it was derived and 0.3 ml was injected per egg via allantoic route.

‡ Tannin-free fraction was supernate after gelatin precipitation, essentially diluted ½ because of added 2% gelatin solutions; hence, 0.6 ml of this preparation was injected into eggs.

TABLE III. Hemagglutination-Inhibition by Aqueous Extracts and Tannin and Tannin-Free Fractions of *Mentha piperita*.

Preparation	Avg of reciprocal of highest dilution inhibiting hemagglutination* NDV	Mumps
Aqueous extract	32 (7)	128 (2)
Tannin (via gelatin)	32 (2)	16 (1)
Tannin-free (via gelatin)	<2 (2)	<2 (2)
Tannin-free (via hide-powder adsorption)	<2 to 2 (3)	—

* Both viruses used at 8 hemagglutinating units with chicken erythrocytes. Numbers in parentheses indicate number of preparations tested.

was a nontannin polyphenol or mixture of polyphenols that behaved much like caffeic acid. Although caffeic acid itself was not readily detected in melissa preparations, it would seem reasonable that derivatives of caffeic acid, perhaps trimers or dimers of the compound, are present as precursors of the condensed tannin.

To confirm further that the tannin of peppermint contained the antiviral effect against NDV while the tannin-free preparations did not, hemagglutination-inhibition experiments were performed. In these tests the activity against NDV and against mumps virus was only in the fractions containing tannin (Table III). As previously reported (3), the tannin of melissa has an affinity for myxoviruses of subgroup 2 (NDV, mumps, and parainfluenza 1, 2, and 3 viruses) but does not readily react with the true influenza viruses. The data for peppermint tannin suggest that it, too, may have this property.

A brief investigation was undertaken to determine if other plants of the mint family have antiviral components. Largely by using hemagglutination-inhibition tests with NDV and plaque-suppression tests with herpes simplex virus, it was found that a number of plants did have antiviral components which could also be detected in egg and cell-culture systems (Table IV). However, none of the plant extracts seemed to have antiviral potency equivalent to that of melissa. With 3 plant extracts, attempts were made to determine if they also had an antiviral nontannin fraction. The tannin-free fraction of at least two plants exhibited some activity in herpes simplex virus-infected eggs (Table V).

Since plants of the mint family are classified on a morphologic basis, it is of interest that some of them also seem to be related biochemically. Apparently, some of these plants have the same or very similar tannins and, perhaps, the small polyphenols that are precursors of this tannin, as detected by their antiviral effects. It is possible that even those mint plants that seemed inactive in these brief studies also have the same com-

TABLE IV. Antiviral Activity of Aqueous Extracts of Various Plants of Mint Family.

Plant	HAI*	Disc-plaque suppression test				Egg experiments (survivors/total eggs)							
		NDV		Herpes simplex		NDV				Herpes simplex			
	NDV	PFU	Plaque-free zone, mm	PFU	Plaque-free zone, mm	Dose LD_{50}	Extract	Extract, no virus	Virus only	Dose LD_{50}	Extract	Extract, no virus	Virus only
Dalmation sage (*Salvia cyprea*)	8	—	—	3×10^3	20, 21	80	5/6	6/6	0/6	200	6/7 †	5/6	0/8
Crea monda (*Satureia* sp.)	16	8×10^2	26, 30‡	10^3	17, 18 32, 33‡	200	10/17	5/5	0/10	25	5/8 †	5/5	1/4
Wild thyme (*Thymus serpyllum*)	4	8×10^2	31, 30‡	4×10^3 10^3	18, 20 31, 33‡	80	5/5	5/5	0/6	6	10/10†	6/6	2/10
Marjoram (*Origanum majorana*)	—	8×10^2	25, 31‡	10^3	25, 26‡	60	3/9	4/5	0/10	25	4/9 †	3/3	1/4
American pennyroyal (*Hedoma pulegioides*)	4	—	—	10^3	14, 11	—	—	—	—	500	3/9	4/4	0/5
Spanish thyme (*Thymus* sp.)	4	4×10^3	0, 0	4×10^3	14, 14	60	4/7	3/3	0/5	—	—	—	—
French thyme (*Thymus* sp.)	4	4×10^3	Trace, 0	4×10^3	14, 14	60	1/7	3/3	0/5	—	—	—	—
Horehound (*Marrubium vulgare*)	<2	8×10^2	0, 0	10^3	0, 0	—	—	—	—	—	—	—	—
Catnip (*Nepeta cataria*)	<2	5×10^3	0, 0	4×10^3	0, 0	—	—	—	—	—	—	—	—
Rosemary (*Rosmarinus officinalis*)	2	—	—	10^3	0, 0	—	—	—	—	—	—	—	—
Hyssop (*Hyssopus officinalis*)	—	—	—	—	—	—	—	—	—	20	3/7	8/10	0/10

* Hemadsorption inhibition, shown as reciprocal of dilution inhibiting 8 units of NDV.
† Undiluted extracts, 0.3 ml injected 2 hr prior to virus; all other results were with injection of extract 24 hr before virus.
‡ Results with ½-inch discs; all other results were with ¼-inch discs.

TABLE V. Antiviral Activity of Tannin and Tannin-Free Fractions of Various Mint Plants in Herpes Simplex-Infected Eggs.

Plant	Virus dose (LD_{50}/0.3 ml)	Survivors/total eggs		
		Prep.*	Prep., no virus	Virus only
	Tannin fraction†			
Crea monda	25	5/9	5/5	1/4
Wild thyme	6	5/10	5/5	2/10
Marjoram	25	4/9	5/5	1/4
	Tannin-free fraction‡			
Crea monda	25	5/10	3/3	1/4
Wild thyme	6	8/10	5/5	2/10
Marjoram	25	0/4	4/4	1/4

* All plant preparations were injected 2 hr prior to virus.
† Tannin fractions from gelatin precipitation were diluted to original volume of aqueous extract and injected in 0.3 ml.
‡ Tannin-free preparations from gelatin precipitation were injected in 0.6 ml.

ponents but at concentrations too low to detect in antiviral tests.

Summary. Extracts of various plants of the mint family (Labiatae) were studied for antiviral activity. Peppermint *Mentha piperita* extract had antiviral activity against Newcastle disease (NDV), herpes simplex, vaccinia, Semliki Forest, and West Nile viruses in egg and cell-culture systems. It contains a tannin with an affinity for NDV and mumps virus and a nontannin fraction with antiviral effects against herpes simplex virus. Aqueous extracts of sage (*Salvia cyprea*), marjoram (*Origanum majorana*), wild thyme (*Thymus serpyllum*), American pennyroyal (*Hedeoma pulegioides*), Crea monda (*Satureia* sp.) and Spanish and French thymes (*Thymus* sp.) all exhibited some antiviral effects against NDV. The first 6 also exhibited some antiviral effects against herpes simplex. Hyssop (*Hyssopus officinalis*) extracts had activity against herpes simplex virus while rosemary (*Rosmarinus officinalis*), horehound (*Marrubium vulgare*), and catnip (*Nepeta cataria*) extracts were not detectably antiviral. None of these plant extracts produced an antiviral effect superior to that of melissa (*Melissa officinalis*) extracts. The data suggest the existence of some biochemical relationships among plants of the mint family, especially the presence of a common tannin.

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Influence of Cholesterol on Estrogen Induced Aortic Ruptures in Turkeys.* (31875)

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Aortic ruptures have been induced in turkeys using 2 synthetic compounds with estrogenic activity. The syndrome was produced when diethylstilbestrol (DES) was administered parenterally(1), or when incorporated in the feed(2). A high incidence of aortic rhexis has also been produced when dienes-

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