PHARMACOGNOSTIC STUDIES OF MENTHA SPICATA L. IN RELATIONSHIP TO TWO OF ITS HYBRIDS M. PIPERITA L. AND M. GENTILIS L.

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The essential oil extracted by microdistillation from *M. spicata* L. and two of its hybrids *M. piperita* L. and *M. gentilis* L. was compared with Oleum menthae piperitae obtained from the market. The yield acquire per plant was found to be very low i.e. not exceeding 0.01–0.03 ml. The analysis of all the samples was made by gas-chromatograph. Chromosome counts showed *M. spicata* having a somatic number of 54 while *M. piperita* and *M. gentilis* had 48 and 72 chromosomes, respectively. An attempt has been made to establish whether there is any relationship between polyploidal class and their essential oil content.

Introduction

Mentha is known for its economic importance in perfumery and pharmaceutical industry as the main source of Menthol and Piperiton. The major components of Mentha oil are a principal ketone (menthon) and its related alcohol (menthol) and ester. This genus displays a very high degree of interspecific and intraspecific hybridization resulting into series of intermediate hybrids ranging between two parental pure species and distinguishing from one another in morphological habits, cytogenetical behaviour, polyploidy level and essential oil content.

In the light of the above fact it was found interesting to compare the essential oil of a pure species with that of its hybrids to see whether there is any relationship between polyploidal class and their essential oil content. *M. spicata* L. which produces two well-established natural hybrids namely *M. piperita* and *M. gentilis* was chosen for this study.

The essential oil was extracted from various individual plants of these species by microdistillation. These samples and the standard Oleum menthae piperitae obtained from the market were analysed by gas chromatography.

Materials and Method

The essential oil was extracted by microdistillation.[1] The classical methods of distillation were not useful as the oil content available was not more than 0.01–0.03 ml per plant. The oil drop suspended in water was dissolved in pentane which was later separated in a separating funnel and used for gas-chromatographic analysis. The same plant material was used for the determination of the chromosome number which was used for the extraction of the essential oil. Chromosome counts were made from root-tips fixed in Navashin (1% chromic acid, 95% acetic acid and 40% formalin in the ratio of 10:1:4). The percentage of various substances present in the oil was calculated by measuring individual peaks of the Fractogram by means of planimeter. Peaks III/IV and VII/VIII comprised two peaks each which overlapped each other thus preventing their separation and making it difficult to say with certainty whether only one or the other or both substances are present in an individual. In certain cases, however, where it was possible to identify the substances, for example in Oleum menthae piperitae, it was not possible to evaluate their percentages separately. Peaks Ia, IIa, VIa, XIIa, XIIIa, XIV and XV are unknown while rest of the peaks have been identified by comparison with porsh and Farnow.[2] A Perkin-Elmer Fractometer (Type 116 E) with a polypropyleneglycol column (Type R) was used for microanalysis under the conditions described by Baquar and Reese.[3]

Systematic Position and Chromosome Number

Mentha spicata is one of the six pure species of the Eurasian continent, the others being *M. pulegium* L., *M. aquatica* L., *M. arvensis* L., *M. longifolia* (L) Hud. and *M.rotundifolia* L. *M.spicata* produces hybrids with practically all the other species, but of these two interspecific hybrids namely *M. piperita* L. and *M.gentilis* L. are well established in nature and were therefore selected for the present study. *M. piperita* is a cross between *M. spicata* and *M. aquatica* while *M. gentilis* is a hybrid between *M. spicata* and *M. arvensis*.

Results and Discussion

The gas-chromatographic study in the present investigation has been used less as a medium for

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the analysis of chemically defined substances but more as a criteria to compare, from the morphology of the fractograms, the phytochemical characters of the pure species with its hybrids. The investigation has been made from two different aspects. Firstly, the essential oil of various individuals of the same species have been compared among themselves and secondly, the typical representative of *M. spicata*, *M. piperita* and *M. gentilis* have been compared with one another.

The fractograms (Fig. 1) obtained reveal that the oil composition of *M. gentilis* resembles closely with *M. spicata* while *M. piperita* deviates from it.

TABLE 1.—SOMATIC CHROMOSOME NUMBERS.

Species	Somatic chrom. number	Possible ploidy
M. gentilis L. ..	72	12 x
M. spicata L. ..	54	9 x
M. piperita L. ..	48	8 x

Practically all the substances are present in both, *M. gentilis* and *M. spicata* except peak XII which is totally absent in *M. spicata* but is present in *M. gentilis*. Peak VI (sabinen hydrate) is present in *M. spicata* only in traces but in *M. gentilis* it is in considerably high quantity (10.25%). Peak VIa on the other hand was present in *M. gentilis* only in traces whereas in *M. spicata* it is about 1.82%. The main constituent of *M. spicata* is peak XIII (piperitone) which is as high as 57.03%. Piperitone in *M. gentilis* is 29.40% which is also a high quantity. Normally it may be presumed that a substance present in one of the two parents is present in the hybrid also, if it is supposed to be governed by the genetical factors.

Fractograms of *M. piperita* deviates from *M. spicata* mainly in having peaks VII/VIII, IX, XI, XIIa and XIV (Table 2) prominently represented while these peaks are totally missing in *M. spicata*. These substances appear to have come from the other parent. The oil content of *M. piperita* which is characterized by peak VII/VIII (menthofuran, menthone and isomenthone) was compared with that of standard Oleum menthae piperitae. It showed a considerable qualitative variation apart from quantitative difference. In fractograms of Oleum menthae piperitae which is very much identical to that of Brazilian pepermint oil[2] peaks

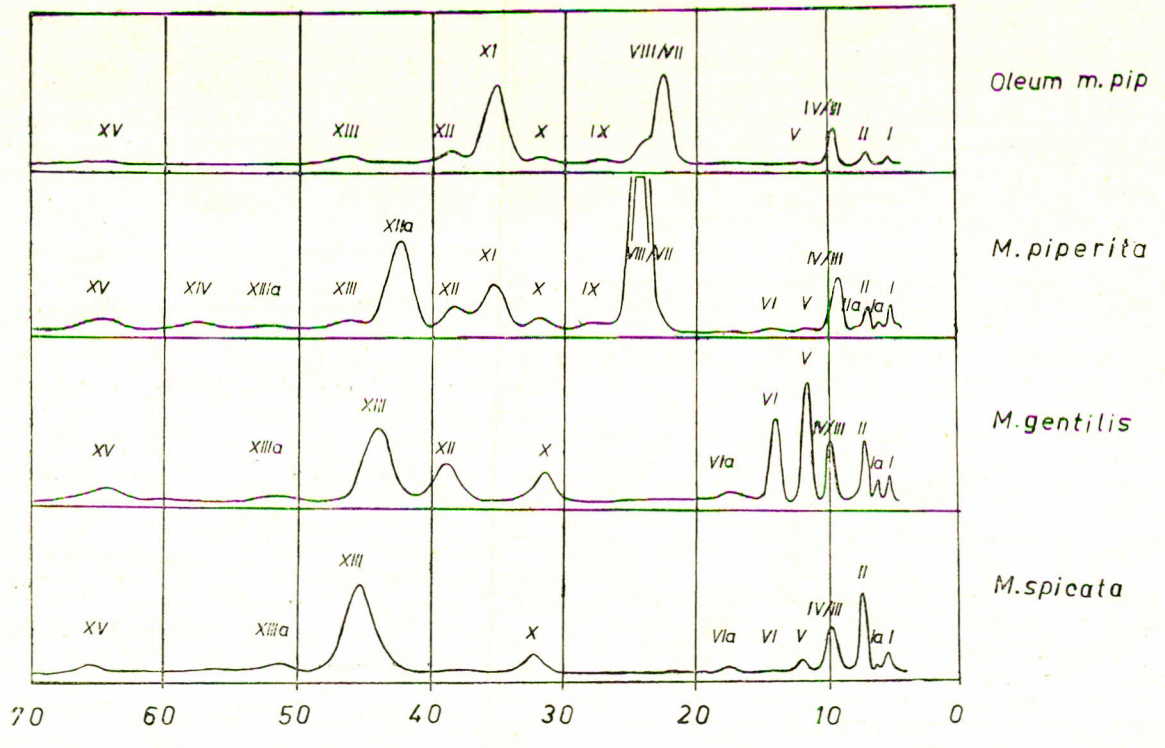

Fig. 1.—Fractogram of *S. spicata*, *M. gentilis*, *M. piperita* and Oleum menthae piperitae showing various peaks.

TABLE 2.—OIL COMPOSITION.

Peaks	Substances	*M.spicata*	*M.gent.*	*M.piper.*	Ol.m. pip.
I	α- Pinene	2.16	1.92	1.44	0.75
Ia	Unknown	0.76	1.43	0.50	—
II	β- Pinene	7.92	5.55	1.41	1.79
IIa	Unknown	—	—	+	—
III/IV	Limonene and cineol	10.43	7.46	5.60	5.63
V	Ethylamylcarbinol	2.24	13.40	+	+
VI	Sabinene hydrate	+	10.25	+	—
VIa	Unknown	1.82	+	—	—
VII/VIII	Menthofuran, menthone and isomenthone	—	—	43.71	37.00
IX	Isopulegol	—	—	2.04	1.86
X	Neomenthol and neoiso-isopulegol ..	8.93	9.40	1.81	3.10
XI	Menthol and neoisomenthol ..	—	—	10.44	40.60
XII	Menthyl acetate and isomenthol ..	—	14.00	5.35	5.68
XIIa	Unknown	—	—	20.20	—
XIII	Piperitone	57.03	29.40	2.54	3.27
XIIIa	Unknown	4.91	1.86	+	—
XIV	Unknown	—	—	1.89	—
XV	Unknown	3.14	5.37	2.94	+

+, Present in traces; —, Missing.

XIIa and XIV are missing, while these are 20.2% and 1.89% respectively in *M. piperita* according to the present study. The slight qualitative variation in the oil content of *M. piperita* as shown by Porsh and Farnow[2] from various, wide-apart origin as American, Bulgarian, Japanese, Chinese, French, Hungarian and Brazilian is governed, probably, more by the climatic and the soil condition than anything else. This variation is also due to the fact that the analysis done by them was not from the oil extracted from a single plant but from a large number of individuals together from a big population. Reitsema, Cramer and Fass [4] found considerable difference in the oil content of different parts of a single plant. The younger leaves of the apex have a much higher percentage of oil per unit leaf area than the older ones below. The reason for this is not very clear. However, they beleived that the major portion of oil may be present already in the first stage of leaf growth and the subsequent increase in leaf size does not effect the oil content. The other reason for a lesser quantity of oil in the older leaf, which appears to be more appropriate, is that oil constantly evaporates off from the leaf surface.

A considerable quantitative variation exists in the oil composition within various individuals of the same species, although the ratio of the quantity of these substances in all the individuals was more or less the same. The difference from individual to individual plant belonging to the same specific status does not effect its qualitative balance. The fractograms of various species appear to be very characteristic of their own and thus, with certain reservation, could be of considerable value in finding out relationship of various alied species. This of course could be said with certainty only about plants from northern Germany. Investigation on these lines on the plants of the other geographical region will throw more light on the subject. It is quite possible that a qualitative difference may be recorded from the individuals of the same species from other parts of the world. It is because of this inter–and intraindividual variation in plants and their oil composition that there does not appear to be any correlation between various numerical polyploidal classes and their oil content. From Table I it would be clear that the somatic number of 48 has been found in *M. piperita*, 54 in *M.s picata* and 72 in *M. gentilis* but the amount of various substances in these polyploidal classes increases or decreases irrespective of the chromosome number and is far from uniform. It does not show any direct or indirect relationship.

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