Pharmacognostic Studies at the College of Pharmacy University of the Philippines

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Pharmacognostic studies at the College of Pharmacy, University of the Philippines, are limited by three factors, namely, manpower, funding, and facilities. The field is not attractive to pharmacy students today as industrial pharmacy and clinical pharmacy which are the current thrusts of the College. There is only one pharmacognosist in the College and only one graduate student so far has majored in the field, while another faculty member is abroad who obtained his MS in pharmacognosy but is now branching into another field. A deterrent is the need for an adequate background in such cognate courses as plant morphology, anatomy, physiology and biochemistry, which the average pharmacy graduate lacks. Two small greenhouses and a garden, approximately 20m×60m, are available for physiological and field studies. Chemical analyses are conducted in a laboratory in the College. Samples for instrumental analysis sometimes have to be sent abroad. However, work has proceeded in spite of the constraints presently existing.

Physiological and chemical investigations on the following plants have been and being conducted (T-1):

1. *Mentha cordifolia* Opiz (Labiatae)
2. *Mentha arvensis* L. var. *villosa* (Benth.) Stewart f. *villosa* (Labiatae) (Philippine variety; nomenclature still pending)
3. *Mentha arvensis* L. var. *piperascens* Malinvaud (Labiatae)
4. *Zingiber zerumbet* (L.) Sm. (Zingiberaceae)
5. *Zingiber zerumbet* (L.) Sm. var. (Zingiberaceae) (New variety; nomenclature still pending)
6. *Piper methysticum* Forst. (Piperaceae)
7. *Datura innoxia* Mill. (Solanaceae)
8. *Catharanthus roseus* (L.) G. Don (Apocynaceae)

This paper will present the work that has been done and is still being done on these plants.

I. *Mentha* spp.

Philippine mint or *yerba buena* was formerly reported in Philippine taxonomic lists as *Mentha arvensis* L., together, in some instances, with pictures of the vegetative plant and descriptions of the inflorescence. The plant does not flower under Philippine conditions and only vegetative specimens were available in the Philippine National Herbarium. The correct name of the plant prior to physiological and chemical studies was, therefore, open to doubt.

The plant was subjected to three different photoperiods in the darkroom of the physics department of the University. The photoperiods used were 9 hours, 18 hours, and 9 hours supplemented with 2 hours in the middle of the dark period. Plants were grown in the field and subjected to natural daylengths with the dark period interrupted by two hours of light in the middle.

Meanwhile, the growth of the plant in the

greenhouse under daylengths of 12 and 8 hours was observed and evaluated in terms of fresh and dry weight, number of internodes produced, length of internodes, dry weight of leaves, palisade number of leaves, and oil gland number. Oil yield of the plant under two light intensities in the greenhouse was also determined. Plants grown in the field were observed for monthly variation in oil yield. Samples of the oil from plants grown in the field were submitted for analysis to the Takasago Perfumery Company in Tokyo and to the A.M. Todd Company in Kalamazoo, Michigan. Stolons were sent to the latter company for growing under a temperate climate.

The plant flowered under long-day conditions (i.e., 18-hour photoperiod or 9-hour photoperiod with an interrupted dark period or natural daylengths in the field supplemented with two hours of light in the middle of the night) and on the basis of the inflorescence developed, the plant was identified as *Mentha cordifolia* Opiz. Terminal panicles were formed instead of the axillary cymes produced by *Mentha arvensis*. The chief constituent of the oil was shown to be piperitenone oxide. *M. cordifolia* is a cross between *M. rotundifolia* (L.) Huds. and *M. spicata* L. Piperitenone oxide is the principal constituent of *M. rotundifolia*. The Philippine plant grown in Michigan showed exactly the same growth characteristics as the authentic specimen. The correct name of the plant was thus established to be *Mentha cordifolia* Opiz.

The plant did not flower under the two photoperiods and the two light intensities used in the greenhouse. Vegetative characteristics were duly recorded. Under the treatments used, responses were better under the longer photoperiod and the higher light intensity. Oil yield varies monthly with a principal peak lagging slightly behind the peak of maximum growth as measured by dry-weight production. Heavy rains affect the growth of the plants and oil yield adversely. Analysis of the oil at different times confirm the presence of piperitenone oxide as the principal component.

A plant known as *polius* was collected and cultivated for study. It produced axillary cymes characteristic of *Mentha arvensis* L. and its oil showed the presence of pulegone (59.8%) and menthone (18.7%) as the principal components (T-2). Growth characteristics indicate that the plant is most probably a variety of *Mentha arvensis*. It resembles most closely the description of *Mentha arvensis* L. var *villosa* (Benth.) Stewart f. *villosa*.[1)]

These two plants are good examples of "chemical or physiological races". The American *Mentha cordifolia*, grown in the Philippines, looks exactly like the Philippine plant, but an analysis of the oil showed that it its principal constituent is carvone. This compound is characteristic of the oil of *Mentha spicata*, the other parent of *Mentha cordifolia* (T-3). In the North American species of *Mentha arvensis*, four chemical races have been reported[2)] (T-4):

Type 1—high in pulegone, isomenthone, and methone

Type 2—high in linalool, *cis*-ocimene, and *trans*-ocimene

Type 3—high in *cis*-isopulegone and *trans*-isopulegone

Type 4—high in *cis*- and *trans*-ocimene and in 1,8-cineole

The Philippine plant belongs to the first type based on the volatile oil composition. *Mentha*

1) Stewart, Sara R. 1944. *Mentha arvensis* and some of its North American variations. *Rhodora* 46:331-5.
2) Gill, L.S., B.M. Lawrence, and Julia K. Morton. 1973. Variation in *Mentha arvensis* L. (Labiatae). I. The North American Populations. *Bot. J. Linn. Soc.* 67:213-32.

arvensis is a cross between *Mentha pulegium* L. and *Mentha aquatica* L.

Stolons of 21 different mint plants representing twelve species were received from the A. M. Todd Company and grown in the greenhouse. Of these, Japanese mint or *Mentha arvensis* L. var. *piperascens* Malinvaud, the source of natural menthol, grew very luxuriantly under Philippine conditions. The current emphasis of our government on the development of local sources of drug substances stimulated the study on the cultivation of this plant in the Philippines. Field studies were done on the plant under two levels of fertilizer and two levels of watering. Under the conditions used, the plant yielded 3.33 to 5.01% of the volatile oil on a moisture-free basis. Differences in oil yield in response to the cultivation treatments were not significant except for the greater oil yield of old plant parts in response to the higher fertilizer level of 120g per 9sq m than to the lower level of 60g per 9 sq m applied every four weeks.

II. *Zingiber* spp.

A study was made to differentiate two species of *Zingiber* used in folk medicine in the Philippines. Results obtained show marked differences between the two plants, one of which was identified as *Zingiber zerumbet* (L.) Sm. On the basis of differences noted, the second plant is being proposed as a new variety of the species.

The two plants are essentially similar except for the following morphological and chemical differences (T-5): *Zingiber zerumbet* (L.) Sm. has ovate lanceolate leaves; a prominent ligule, about 1.5 to 3.5cm long; an ovoid to oblong infloresence with an obtuse apex; green, pink-edged, obovate mucronate bracts; pale yellow rhizomes; and oil cells measuring 30 to 90μ in diameter. The volatile oil isolated from the dried rhizomes is dextrorotatory, congeals at 3°C, and the principal constituent is zerumbone. The proposed variety, on the other hand, has oblong lanceolate leaves; a small ligule, about 1 to 2 mm long; a cone-like inflorescence with an acute apex; brownish-purple, green-edged, obovate acute bracts; yellow orange rhizomes; and oil cells measuring 50 to 136μ in diameter. The volatile oil from the dried rhizomes is levorotatory, congeals at $-27°$, and its principal constituent is 4-terpinenol.

III. *Piper* spp.

A chemical study of Philippine-grown *Piper methysticum* Forst., a plant widely known for its hallucinogenic properties, is being undertaken as a start to the investigation of Philippine species of *Piper*. The roots of two-year old *Piper methysticum* plants were harvested, air-dried, and ground to a coarse powder. The powdered drug was exhaustively extracted with chloroform in a Soxhlet apparatus. The chloroform extract was concentrated and subjected to column chromatography. The sequence of eluting solvents used was petroleum ether, benzene, chloroform, and methanol. Each eluate was spotted on TLC plates for screening. Eluates yielding similar chromatograms were combined and allowed to crystallize.

Six kinds of crystals were obtained and chromatographed on TLC plates together with kava-pyrones using cyclohexane-ethyl acetate as developing solvent and a shortwave UV lamp, iodine vapors, anisaldehyde spray reagent, and vanillin-sulfuric acid, used in succession to locate all the spots. The R_f values of the isolated crystals are: 0.5529, 0.5555, 0.5726, 0.6471, 0.6441, and 0.4839 (T-6). The IR

spectrum of each was taken and it was found that the first three are identical so that, as of now, four crystalline substances have been isolated. None of these compounds appears to be identical with piperine, kawain, methysticin, and yangonin, which have much lower R_f values.

Piper betle L. and *P. nigrum* L. plants are being grown for future study.

IV. *Datura innoxia* Mill. and *Catharanthus roseus* (L.) G. Don

At the end of the rainy season, uniform seedlings of *Datura innoxia* Mill. (Fam. Solanaceae) and *Catharanthus roseus* (L.) G. Don (Fam. Apocynaceae) were set out in the field using a 2^3 factorial experiment in complete randomized design using eight treatment combinations. The plants were distributed according to a field lay-out where sixteen 1.5m×3m plots were prepared for each plant-datura, purple-with-purple-eye catharanthus, and white-with-yellow-eye catharanthus. Each treatment, in duplicates, included three variables, namely fertilizen, density, and water. For density 1 (D_1), 8 plants per plot were spaced 60cm apart and for density 2 (D_2), 14 plants were spaced 30cm apart. Daily water levels were 12 liters (W_1) per plot and 24 liters (W_2) per plot. Fertilizer levels were 5g complete fertilizer per plant every 2 weeks (F_1) and 5g complete fertilizer per plant every weeks (F_2). In the case of datura, flower buds were removed as soon as they appeared in order to eliminate variables in metabolism as the plant enters the flowering. stage

Harvesting of datura plants started 45 days after treatment. Leaves, stems, and roots were separated, washed quickly just sufficiently to remove adhering soil particles, air-dried, and weighed. The total dry weight of each plant was calculated by adding the weights of its leaves, stems, and roots. Since all the plants could not be harvested in one day, one plant per plot was harvested each day. Statistical analysis of the data showed that only three collection dates were valid: 45, 52 and 59 days after start of treatment.

Analysis of the main effect means on the dry weight production showed that differences were significant, with density producing the largest differences, followed by fertilizer, and lastly by water. Differences in the effect of interactions between two variables on the dry weight production were significant for the interaction between fertilizer and density. Dry weight production was higher with plants 60cm apart than with plants 30cm apart under similar fertilizer and water levels.

The catharanthus plants were not harvested because the rainy season started early and the growth of the plants was set back. The plants fell as a result of the strong rains and wind and the branches and leaves that were dragged on the ground rotted. The plants were allowed to grow into the second year and treatment will be resumedon the two year-old plants.

Bibliography

1. Balbin-Olivers, Mildred: Chemical and Morphological Studies on *Zingiber zerumbet* (Linne) Smith and Its Proposed Variety. Ph. D. thesis, University of the Philippines (1977)
2. Cantoria, Magdalena, and D.B. Mabasa: Studies on the physiology of *Mentha arvensis* L. I. Effect of two different daylengths on the vegetative growth. *Plant & Cell Physiol.* **6**:561-4 (1965)
3. Cantoria, Magdalena: Physiological and che-

mical studies for the identification of Philippine mint. *Philippine J. Sci.* **97**:277-84 (1968)
4. Cantoria, Magdalena: Research on mint in the Philippines. *Anais da Academia Brasileira de Ciencias* **44** (Suppl.):64-7 (1972)
5. Cantoria, Magdalena, and Ma. Vicenta T. Cuevas-Gacutan: Studies on the physiology of Philippine mint (*Menta cordifolia* Opiz). II. Effect of two different light intensities on the vegetative growth and oil yield. *Philippine J. Sci.*, **103**:13-9 (1974)
6. Cantoria, Magdalena: Studies on the physiology of Philippine mint (*Mentha cordifolia* Opiz). III. Variation in oil yield. *Philippine J. Sci.* **103**:67-74 (1974)
7. Cantoria, Magdalena: Aromatic and medicinal herbs of the Philippines. *Quart. J. Crude Drug Res.* **14**:97-128 (1976)
8. Cantoria, Magdalena C.: Pharmacognosy in Action. National Research Council of the Philippines, Bicutan, Tagig, Metro Manila (1977)

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