



AFFIDAVIT OF ACCURACY

I, Joy Seletsky, hereby certify that the following is, to the best of my knowledge and belief, a true and accurate translation of the following document, "Synthèse et mises au point", from French into English.



Joy Seletsky
TransPerfect Translations, Inc.
1101 Pennsylvania Avenue, NW
8th Floor
Washington, D.C. 20004

Sworn to before me this
15th day of November, 2002


Signature, Notary Public

Irma B. Gamboa
Notary Public, District of Columbia
My Commission Expires 10-14-2006

Stamp, Notary Public

District of Columbia

- ATLANTA
- BOSTON
- BRUSSELS
- CHICAGO
- DALLAS
- FRANKFURT
- HONG KONG
- HOUSTON
- LONDON
- LOS ANGELES
- MIAMI
- MINNEAPOLIS
- NEW YORK
- PARIS
- PHILADELPHIA
- SAN DIEGO
- SAN FRANCISCO
- SEATTLE
- WASHINGTON, DC

Synthesis and Clarification

DESCRIPTION, IDENTIFICATION AND THERAPEUTIC USES OF CHRYSANTHELLUM "AMERICANUM" CHRYSANTHELLUM INDICUM DC. SUBSP. AFROAMERICANUM B.L. TURNER (*)

by

D. Honore-Thorez

[summary in English]

INTRODUCTION

Practically unknown for two decades, *Chrysanthellum "americanum"* could appear, in the near future, as a major hepatovascular remedy, endowed with not-insignificant antilithic, lipid-lowering and anti-inflammatory properties, among others.

While this plant seems to have already achieved a certain amount of success in France, it is still little known in Belgium, and it seems useful to summarize a body of data that we have been able to gather, without this monograph being exhaustive, since science is still far from knowing everything about *Chrysanthellum "americanum"*.

*Summary of a bibliographic work on *Chrysanthellum "americanum"*, of which a copy is on file at the Institute of Pharmacy, 5, rue Fusch, B-4000, Liege.

ETHNOBOTANY

1. Definition

This herbaceous plant, common in the old and new worlds, has been confused for three years with *Chrysanthellum americanum* (L.) Vatke or *Chrysanthellum procumbens* Rich. ex. Pers. which is a neighboring species, of which the geographic area is limited to Cuba, Honduras and Jamaica and other neighboring islands (2).

It is to Professor B.L. Turner, of the University of Austin, Texas that we owe the description of *Chrysanthellum indicum* DC. subsp. *Afroamericanum* (1,2) which is the African plant that we are interested in, and which Professor Turner clearly differentiates from *Chrysanthellum americanum* (L.) Vatke in a worldwide study of the genus *Chrysanthellum* that will appear at the end of 1985.

Chrysanthellum indicum DC. subsp. *Afroamericanum* B.L. Turner is an annual herbaceous plant which is part of the *Asteraceae* family, which is of the *Helianteeae* order, of the sub-order of *Coreopsidineae*, and of the genus *Chrysanthellum*.

Today a dozen species of *Chrysanthellum* are known, mainly American (1,3). The plant that we will study is a subspecies of *Chrysanthellum indicum*, described in 1836 by De Candolle

(4). According to Turner, it is not represented except in a single taxon, the variety *afroamericanum* (1).

Synonyms of *Chrysanthellum indicum* DC. subsp. *Afroamericanum* B.L. Turner are *Hinterhuberia kotschyi* Sch.-Bip. ex Hoscht, *Adenospermum tuberculatum* H. & A. and *Plangiocheilus erectus* rusby (1).

Since all the bibliographic references have the name “*Chrysanthellum americanum*” and it is by this name that the plant is known in the pharmaceutical area, we have opted to call it *Chrysanthellum “americanum”* in this work.

2. History

The name of the genus *Chrysanthellum* is a diminutive of *Chrysanthemum*, deriving from the Greek word “*chrusos*,” gold, and “*anthesis*,” chamomile. Called “golden chamomile,” *Chrysanthellum “americanum”* has a yellow flower, reminiscent, while smaller, of that of chamomile romaine (5).

Originating in the mountains of Peru and Bolivia, it was not introduced into Africa until the recent past, then naturalized.

Up to the middle of the 20th century, it was used empirically to treat various gastrointestinal complaints, as an indigenous medication, it served, in decoction to wash the tattoos made in the skin in order to avoid inflammation and to obtain proper scars in relief (6).

Berhaut (5) also cited the plant, always in decoction, employed as a mouthwash, to calm toothaches and to wash the head in cases of migraine.

It was Dr. Couderc, a physician from Bordeaux who had lived for a long time in Africa, who was the first to be interested in the plant scientifically in 1960 (6) and it was not long after that when several laboratories became involved in chemical and pharmacological studies of *Chrysanthellum “americanum.”*

It was necessary to wait until 1983 before a first study on the therapeutic properties of the plant appeared in the medical press (7).

3. Description of the plant

Chrysanthellum indicum DC. subsp. *Afroamericanum* is an annual plant 10 to 30 cm high, with branches that are prostrate, ascending or erect (8).

The leaves, mainly bipenniform or tripenniform, with long petioles partially surrounding the stem, are alternate, the lower ones in basilar rosettes in the young specimens (9).

The flowers, of radiating type, are grouped in small heads of yellow to yellow-orange, axillary or terminal, with 1 or 2, rarely more, underlying linear bracts (1).

The heads, with long peduncles, are heterogamous.

The fruits are achenes from 2 to 4 (5) mm long, 1 to 2 mm around, stocky obovates when they come from ligulated flowers or ovilinear and compressed with a cartilaginous wing or a suberose edge when they come from tubular florets (1,8).

The thin root is twisted.

It is important to note that *Chrysanthellum americanum* (L.) Vatke, with which the African plant has long been confused, differs from it with whole leaves with various lobes, more numerous involucrel bracts, external flowers with longer ligulas and more closely winged achenes (1).

4. Localization

A pantropical plant, *Chrysanthellum "americanum"* is found mainly in mountainous regions or those of moderate altitude in Africa and South America. Very frequent in the African high plains, it has also spread to the Black African savannas.

Generally, in ruderal state, it is found along paths and roads, on fallow or uncultivated land at watering holes, generally shallow (9).

It prefers lateritic and silico-granite soil (6). Since its flowering is controlled by the rainy seasons of the different regions where it grows, it blooms at different times of the year, at the end of the rainy season.

Growing wild, *Chrysanthellum "americanum"* is not cultivated anywhere.

5. Description of the drug (10)

The drug is made up of the whole plant, gathered after flowering, sorted and dried in the sun or in well-ventilated areas, protected from moisture.

It contains fragments of the plant, coarsely chopped, including pieces of the stems, also including the roots and the floral peduncles, forming a fairly loose mass.

Flowers, floral heads and achenes make up the finer fragments, separating from the rest of the plant and frequently found in greater quantity in the bottom of the containers.

It has been found useful since then to check the homogeneity of the drug at the time of preparation of a galenic form.

The odor is mild, while the flavor, at first sickeningly sweet, becomes progressively bitter.

We have noted the presence, in certain French pharmacies, of forgeries of *Chrysanthellum "americanum"*, which have been identified by the National Botanical Garden of Meise as being *Parthenium integrifolium* (L.), another *Asteraceae*, endowed with anti-fever properties, but totally dissimilar from *Chrysanthellum "americanum"*, both in the appearance of the leaves and of the fruits (34).

PHYTOCHEMISTRY

Chemical study of the drug reveals flavonoid and saponoside derivatives, but the uniqueness of *Chrysanthellum "americanum"* rests in the fact that three types of flavonoids are found in this plant, which generally occur individually in the plant kingdom: one aurone, one chalcone and flavonones, a fairly rare combination, which we have not found except in the genus *Bidens* (11) and the genus *Coreopsis* (12), which are fairly close to *Chrysanthellum "americanum"* from the chemico-taxonomic point of view.

[see original for diagram]

Chrysanthellum indicum DC. subsp. *Afroamericanum* B.L. Turner

[see original for illustration]

Whole Plant

Achenes

In addition, the saponosides exhibited a structure never found to date in vegetable species.

1. Polyphenolic constituents

The study of these derivatives does not seem to have been the object of any scientific publication. Thanks to the kindness of a French laboratory, we are able to communicate the composition of the polyphenolic fraction (13).

1.1 Derivatives of caffeic acid

These are chlorogenic acid, caffeic acid depside and quinic acid.

The presence of one or several analogs of caffeic acid has already been reported. In pharmacology, the derivatives of caffeic acid and of chlorogenin are cholagogic substances.

1.2 Flavonoids

These consist of:

- two flavanones:

o eriodictyol-7-0-glucoside, the most significant in quantity (14);

o isookanin-7-0-glucoside, also called flavonomarein (12);

- one chalcone: okanin-4'-0-glucoside or marein (12,15). (We need to keep in mind that the numbering of the chalcones is carried out in reverse to the other flavonoids, the node A being number from 2' to 6').

- one aurone: maritimetin-6-0-glucoside or maritimein (12,15);

- one flavone: luteolin-7-0-glucoside;

[see original for structural diagram]

Flavonomarein, marein and maritimein have been studied by M. Shimokoriyama (12), using *Coreopsis tinctoria*, a very common annual plant cultivated in Japan, and the methods of isolation, identification and the mode of distribution of the three glucosides in various parts of the plant have been described in detail.

[see original for structural diagram]

2. Saponosides

Two saponosides of original chemical structure have been isolated using *Chrysanthellum "americanum"*. These include chrysanthelline A, a derivative of echinocystic acid and chrysanthelline B, found in lesser quantity in the plant and which derives from caulophyllogenin. Echinocystic acid has not been found except in around twenty natural saponins (17) and caulophyllogenin has not been found at all to date except in a single saponoside, the cauloside B of *Caulophyllum robustum* (18), which has a structure that is simpler than that of chrysanthelline B.

These two saponins were obtained using methanolic extraction of the whole plant and fractioning of the different extracts (19,20).

The structures have been established using the modern techniques of spectral identification (19,20,21).

The only difference between the two saponins is a α -0-xylosidic bond for the chrysanthelline A and a β -0-xylosidic bond for the chrysanthelline B, of the tetrasachharide, with carboxyl grouping of the aglycon at C28, and an alcohol function at C23 in the case of chrysanthelline B (20).

PHARMACOLOGY

1. Various pharmacological and toxicological studies have been carried out to date, however many of them have not been published.

The pharmacological study essentially focused on three modes of action: hepatotropic activity, hypolipemic action and the vasculotropic activity of *Chrysanthellum "americanum"*.

In the first case, the hepatoprotective action with respect to ethyl alcohol and carbon tetrachloride and the choleric action with respect to sodium dehydrocholate in rats, were proven from the rejuvenation of hepatic functions, while a parallel study carried out in man showed significant results in improvement of the hepatic condition (22).

Chrysanthellum "americanum" has also been shown to be a hypolipemic with respect to clofibrate used as a reference medication, but with more spectacular results on triglycerides than on cholesterol (23).

Although in the study of the plant's vasculotropic properties, it demonstrated little activity administered by mouth in the rat, although it was superior to rutin and its derivatives; the result in humans was definitely superior (22,24,25).

In clinical pharmacology studies, carried out in the scope of this study, in the office of Professor Cloarec at the Saint-Antoine Hospital in Paris, the drug was shown to have significant effects on microcirculation (22,23).

The action of *Chrysanthellum "americanum"* is analogous to that of vitamin P, combined with vasodilatory activity, acting directly on the vascular walls, as is shown by the increase in walking distances and Doppler tests (26).

Anti-edemic activity, evaluated in rats, is fairly low when the drug is administered by mouth, but proved to be superior to that of phenyl-butazone and aspirin with peritoneal administration (22,24,25).

We again make reference to the antilithic action of *Chrysanthellum "americanum"*, demonstrated in an undisputable way by outpatient medicine for calculi of every type, and in any location, as well as hypotensive and bradycardiac effects. although these are obtained by intravenous administration (22).

2. Clinical Information

Dr. Couder has obtained very conclusive results in the scope of lithofuge activity of *Chrysanthellum "americanum"*, both with salivary lithiasis, as well as renal or biliary (22,28,29).

This activity was then confirmed by other physicians (30,31).

There have been accounts, on several occasions, of significant results obtained on subjects with chronic enterocolital disturbances as well as chronic colibacillosis. *Chrysanthellum "americanum"* will also be recommended in chronic cholecystitis, whether involving calculi or not, cirrhosis, chronic pancreatitis, as well as alcohol intoxication (28).

The drug can also be used in vascularopathies characterized by vascular weakness, as well as the high-risk vascular complaints, such as varicose veins, hemorrhoids, purpura, arteritis of lower extremities, retinal and choroidal diseases (16,22).

The studies may have neglected the observations of indigenous medicine, both African and American, in the area of dermatology, notably the remarkable scarring action of the plant, but in the absence of clinical certainty, we will disregard this subject at the moment.

3. Toxicology

Various studies on acute and toxicity have been carried out on the rat, the guinea pig and the beagle, with plant extracts of different concentrations (16,22,25,27).

These investigation have resulted in the conclusion of total innocuousness of the drug at doses of 100 g of dry plant per kilo.

There has also been no evidence of any manifestation of mutagenicity (22).

4. Galenic forms

Chrysanthellum "americanum" is supplied on the pharmaceutical market, as a tea, as a hydro-alcohol fluid extract, of the Codex type, as 1:5 nebulisate, as homeopathic tincture and as capsules of micronized powder, generally with doses of 200 mg.

5. Dosages and tolerance

5.1 The recommended doses are, for tea, an average of 12 g of dry plant per day, i.e. 6 rounded teaspoons in infusion or decoction in one-half liter of water; for fluid extract, 6 teaspoons per day; and for nebulisate, 6 – 400 mg capsules per day (23).

We have not been able to obtain, from various homeopathic laboratories, recommended doses for the homeopathic tincture. However, we must remember that this is prepared at 1:10 which presumes a higher dosage than that of the fluid extract.

As regards capsules of micronized powder, prepared using the whole plant, and thus full of inert substances, such as roots, cellulose, etc., it would be useful to obtain an identical absorption of active ingredients that the patient should be given, which would be a fairly large number of capsules if it is known that the active ingredients are found in greater concentration in the aerial parts of the plant (32).

5.2 *Chrysanthellum "americanum"*, no matter what the method of administration, is relatively well tolerated.

For two or three days at the most in hepatic patients suffering from gastro-intestinal troubles, there may be a temporary exacerbation of symptoms; in such cases it is adequate to reduce the doses by half (28).

In sensitive subjects a slight nervousness may also be noted, presumably due to the tonic action that the plant seems to possess.

QUALITATIVE STUDY

1. Qualitative control

The methods for macroscopic and microscopic identification are not adequate for identification of the drug, but still it will be possible with the concomitant presence of the flower heads or the achenes of the type described above.

2. Chromatographic methods

2.1 The characterization of the caffeic acids is carried out using thin layer ascending chromatography with 5% acetic acid as eluent and developing with U.V. (13).

2.2. The proof of flavonoids is carried out using ascending chromatography on paper or on silica gel, with a butanol-acetic acid-water-415 mixture and development, either using an alkaline solution or with a solution of sodium hydroboride and aluminum chloride, after heating, and reading in U.V. at 365 nm (16-13).

A more rapid technique for detecting flavonoids, which was communicated to us (33) is CCM on silica gel, using as the mobile phase a 6:1:1 or 8:1:1 ethyl acetate-formic acid-water mixture, and as a developer a methanolic solution with 1% aminoethanol diphenylborate and 5% polyethylene glycol.

The observation is carried out with visible light and with U.V. several hours after the reagent is sprayed.

In visible light, we observe a violet spot ($R_f \pm 0.37$), a red-orange spot ($R_f \pm 0.4$), topped by a violet strip, two light yellow-gold spots ($R_f \pm 0.47$ and ± 0.52), a red-violet spot ($R_f \pm 0.56$) and two pale yellow spots ($R_f \pm 0.87$) and in ultraviolet, at 366 nm, several less intense spots at the ends of the chromatograph and much less intense patches, colored dark violet ($R_f \pm 0.40$), pale yellow ($R_f \pm 0.45$), orange ($R_f \pm 0.52$), red ($R_f \pm 0.56$) and two fluorescences, green ($R_f \pm 0.75$ and $R_f \pm 0.87$), the R_f indicated, being those obtained in the 8:1:1 ethyl acetate-formic acid-water phase.

2.3 The chromatographic separation and identification techniques for saponosides have been clarified by M. Becchi et al. (19-20-21).

CONCLUSIONS

We have seen how varied the reports on the action of *Chrysanthellum "americanum"* have been.

The active ingredients are known in practice, their structure clarified, but the pharmacological area need to be explored further.

It would be wrong to attribute various virtues of the plants to a single chemical component and we dare to think, although it would be premature to formulate a theory on the method of action of the drug, that the two chemical families act in a synergistic manner, while playing their own roles.

The future will show whether *Chrysanthellum "americanum"*, which has been given to us by folk medicine, may be able rival traditional medications in this era when plants are coming

on the scene where the medical artillery sometimes seems disproportionate with regard to the desire to cure.

ACKNOWLEDGEMENTS

We express our sincere gratitude to Professor L. Angenot, Director of the Office of Pharmacognosia, University of Liege, to Mrs. M. Tits, Pharmacist and Director of the Dolisos Laboratory of Liege; to Mr. B. Guillot, Pharmacist, Director of the Iphym-Santane of Miribel, France; to Professor B.L. Turner, Department of Botany, University of Austin, Texas; to Mr. P. Bamps, Botanist, of the National Botanical Garden of Belgium in Meise; as well as to the personnel at the Library of Meise; to Mr. P. Maquet, Botanist, Department of Systematics, Botanical Institute of Botany, University of Liege; as well as to personnel of the library, to Mrs. M. J. Guillaume and Mr. Vilain, Institute of Chemistry, University of Liege, as well as to Mr. Carol Gerard, of Brussels for their help, which they graciously provided as we carried out this work.

Received in July 1985

Pharmacist D. Honore-Thorez
18, rue Général Molitor
B-6700 Arlon (Belgium)

[Page 1 English summary presented in French and German]

Bibliography

- (1) B.L. Turner, New species and combinations in *Chrysanthellum* (Asteraceae – Coreopsidae), *Phytologia*, 52, (4), 291-293 (1982).
- (2) B.L. Turner, Manuscript for a world review study of the genus *Chrysanthellum* (Coming end of 1985.)
- (3) U. Eliasson, Studies in Galapagos Plants: the genus *Chrysanthellum*. *Sv. Bot. Tidskr.* 61, (1), 88 (1967).
- (4) De Candolle, *Chrysanthellum indicum*, 5, 631 (1836).
- (5) J. Berhaut, *Chrysanthellum americanum* Vatke, *Illustrated Flora of Senegal*, 2, 475-476 (1974)
- (6) P. Couder (Dr.), New medication on the basis of *Chrysanthellum procumbens*. French patent no. 979 M (1961).
- (7) B. Guillot, A new conquest of phytotherapy: *Chrysanthellum americanum*, *Plants and Medicines*, 5, (1983).
- (8) Kirkia, *Chrysanthellum* Rich., *Journal of the Government Herbarium*, Salisbury, Rhodesia, 5, (10), 334-35 (1967). Eds. H. Wild-R.B. Drummond.
- (9) J. Hutchinson and J.M. Dalziel, *Chrysanthellum* Rich., *Flora of West Tropical Africa*, 2, 234-235 (1963), 2nd Edition. Ed. F.N. Hepper. Published by the crown agents for overseas governments and administrations, London.
- (10) P. Andriane, Microscopic study of *Chrysanthellum americanum*. Unpublished personal communication. Unda Laboratory, Harzee.
- (11) C.R. Hart, The systematics of the *Bidens ferulaefolia* complex., *Syst. Bot.*, 4, (2), 130-147 (1979).
- (12) M. Shimokoriyama, *Anthochlor pigments of Corespsis tinctoria*, *J. Am. Chem. Soc.*, 79, 214-220 (1956).

- (13) Iphym, Analysis protocol for *Chrysanthellum*. Unpublished. Iphym Laboratory, Miribel, France.
- (14) B.A. Bohm, The Minor Flavonoids in the Flavonoids: Advances in Research. Chapt. 6, 332. Ed. By J.B. Harborne-T.J. Marbry, Chapman & Hall, London-N.Y. (1982).
- (15) B.A. Bohm, Chalcones, aurones and dihydrochalcones in the flavonoids. Chapt. 9, 460 and 472. Ed. by J.B. Harborne, T.J. Marbry, H. Marbry, Chapman & Hall, London (1975).
- (16) SOCIETE CORTIAL, Purification products for *Chrysanthellum* and resulting products. French patent no. 7422371. Applied for by Société Cortial, 1974.
- (17) K. Hiller and G. Voigt, New Results in Research of Triterpensaponine, *Die Pharmazie*, 32, 365-393 (1977).
- (18) L.I. Strigina, N.S. Cheterina, V.V. Isakov, A.K. Dzizenko and G.B. Eliakov, Structure of Caulosid B, a glycoside of the new Trierpenoid Caulophyllogenin from *Caulophyllum robustum*. *Khim. Prir. Soedin*, 6, 733-739 (1974), in *Chem. Abstr.*, 82, 580-125562.
- (19) M. Becchi, M. Bruneteau, M. Trouilloud, H. Combier, J. Sertou and G. Michel, Structure of a new saponin: Chrysanthellin A from *Chrysanthellum Rich.*, *Eur. J. Bioch.*, 102, 11-20 (1979).
- (20) M. Becchi, M. Bruneteau, M. Trouilloud, H. Combier, J. Sertou and G. Michel, Structure of Chrysanthellin B, a new saponin isolated from *Chrysanthellum Rich.*, *Eur. J. Bioch.*, 108, 271-277 (1980).
- (21) M. Becchi, M. Bruneteau, H. Pontagnier and G. Michel, Confirmation of the structure of the oligosaccharide chain of chrysanthelline A, by RMH 13C, *Planta Media*, 42, 265-266 (1981).
- (22) H. Lievre and B. Guillot, *Chrysanthellum americanum*: a plant in the service of the liver and of lipoprotein metabolism. *Young Physician's Review*, June 1983.
- (23) H. Lievre, B. Guillot and E. Reymond, : a hepatoprotective, lipid-normalizing and vascularprotective agent. Confirmations and acquisitions, *Journal of the Young Practitioner*, 7, (1984).
- (24) R. Glawe, W. Moll, U. Mengdehl, H. Nieburh, Glucosycaulophyllogeninester, Processes for its manufacture and medications containing it. German patent no. 30 15 363 (1979). Sarget Laboratory (France).
- (25) H. Combier, C. Mouries, A. Thibault, G. Prat and F. Fauron, New vegetable extract using varieties of *Chrysanthellum*. French patent no. 7701488 (1977). Sarget Laboratory (France).
- (26) B. Guillot, A new conquest of phytotherapy: *Chrysanthellum americanum*, *Plants and Medicines*, 1983
- (27) P. Couderc, Therapeutic *Chrysanthellum americanum* extract. French patent no. 7025949 (1970).
- (28) P. Couderc, *Chrysanthellum americanum*: Conference given at the third meeting of Phytotherapy-Aquitaine, December 1983. *Phytotherapy*, 10, (1984).
- (29) P. Couderc, Contribution of the studies of calculus disease: its treatment with extract of *Chrysanthellum*. Gerba-Bordeaux Publication 1981.
- (30) M. DeGrelan, Contribution to the study of oxalic lithiasis with an extract of *Chrysanthellum*. Physician thesis, Bordeaux 1967.
- (31) P.L. Barrail, Pathogenesis of salivary lithiasis, defense of a hypothesis of lithogenic unity. Thesis of dental surgeon, Bordeaux 1972.
- (32) Iphym-Santane documentation.
- (33) M. Tits, Chromatographic identification technique for flavonoids. Personal communication. Dolisos-Liege Laboratory.
- (34) P. Bamps, Personal communication. National Botanical Garden of Belgium, Meise.