European Scientific Journal December 2013 edition vol.9, No.36 ISSN: 1857 – 7881 (Print) e - ISSN 1857- 7431

USE OF CHRYSANTELLUM AMERICANUM (L.) VATKE AS SUPPLEMENT

Lydia Ferrara
Chair of Hydrology Chemistry, Department of Pharmacy,
University of Naples "Federico II", Naples

Abstract

The Chrysantellum americanum (L.) Vatke is a plant belonging to the family of Compositae long used in traditional medicine as hepatoprotector, biliary drainage, analgesic, rheumatic pains and kidney, as vasoprotector capillary and venous endothelium.The analysis of the protein fraction of Chrysantellum americanum (L.)Vatke revealed a content of crude proteins determined by the method of Kjeldahl equal to 10%. After acid hydrolysis of proteins, by GC-MS, were identified fifteen amino acids: glycine, valine, leucine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, glutamic acid, arginine, lysine, histidine, tyrosine, and also the GABA The action tonic, hepatoprotective, lipid-lowering and venotropic of phytocomplex also makes it suitable as a supplement to the established tolerability devoid of toxic and teratogenic effects

Keywords: Chrysanthellum americanum, hepatoprotective herb, protein analysis

Introduction

The Chrysantellum americanum (L).Vatke is a herbaceous plant of the Compositae family that grows in the tropical zone of Africa from Senegal to Nigeria and tropical America from southern Mexico to northern Brazil preferring the wastelands, dry and rocky. The plant grows up to 30 cm tall, erect or prostrate with very jagged leaves, three or five lobes deeply incised, each lobe is in turn divided and ends in a pointed shape . The flowers are yellow heads with peduncles 3-5 cm up to 6 cm, the fruits are achenes flattened, dark-colored, flared walls at the top and surrounded by a bract whitish. The drug is made from coarsely shredded fragments of the

whole plant harvested after flowering and dried in the sun or in a well ventilated place, protected from moisture, it looks like a mixture of heterogeneous grain with a delicate, while the taste very sweet at first, becomes increasingly bitter. The Crisantellum americanum in Cuban traditional medicine is used for gastro-intestinal pains, rheumatism and kidney, strengthens and increases hair growth, gives force to the whole organism. The plant rich in flavonoids and saponins exerts a protective action against capillary and venous endothelium, reduces the fragility and permeability of capillaries and veins and is therefore indicated for the treatment of venous disease of the lower limbs characterized by weakening of the vessel wall, increased permeability and edema formation.

The alcoholic extract of the plant showed sedative and has been used in various affections of nature intestinal, liver and kidney. The activity is mainly focused on liver detoxification and regeneration of damaged hepatocytes, biliary drainage as it facilitates the elimination of triglycerides and cholesterol. Couderc P.G.(1979,1984) showed that the administration in any form (oral, rectal or parenteral) or dosage of the plant is effective in cases of acute alcohol level, speeding up the process of elimination of alcohol from the body and attenuating disturbances of reflexes, of vision, of equilibrium and therefore the extract may be indicated in cases of chronic alcohol level. According Dubernard (1998) the regeneration of liver tissue compromises occurs through induction of microsomal enzymatic reactions, in particular for the induction of cytochrome P450. Becchi et al (1979,1980) have isolated two saponins called " crisantelline A and B" characterizing the structure. French researchers Cloarec M (1982), Couderc P (1981) have highlighted that the components of the extract, in particular flavonoids and saponins exert hepatoprotective action, angioprotettrice and antiinflammatory, the latter higher than that of phenylbutazone.

Among the flavones identified assumes particular importance Eriodictyol-7-O-glucoside or Pyracanthoside, activator Nrf2 (nuclear factor erythroid 2-related factor) effective in counteracting toxicity induced by cisplatin, one of the most effective chemotherapeutic agents in the treatment of solid tumors. Some serious side effects of cisplatin, such as nephrotoxicity, neurotoxicity, ototoxicity, greatly impede its effectiveness as chemotherapy and the use of extracts of Chrysantellum americanum has proven useful to mitigate these effects (Fram R.J.1992)

Shimokoriyama M.(1956) studied the characteristics of the three main flavonoids present in chrysantellum: isookanin-7-o-glucoside, also

called flavonomarein; one chalcone, okanin-4'-o-glucoside or marein; one aurone, maritimetin-6-o-glucoside or maritimein. The group of yellow pigments containing chalcones and aurones are known as "anthochlor pigment" for the characteristic red color which they undertake after reaction with exposure to ammonia, reaction that distinguishes them from the yellow carotenoid pigments, which do not have this reaction

Materials and Methods

Total protein determination

Our research has been devoted to the analysis of protein fraction. The determination of protein substances crude was performed by the Kjeldahl method reported in AOAC (Association of Official Analytical Chemists Journal, 1990) and one gram of dried drug was used. The data relating to % of total nitrogen average value of three determinations was found to be 1.8%. The % protein calculated by multiplying the value of total nitrogen by a factor of 5.30 as indicated by Jones (Jones, 1941), was 10%.

Amino acids determination

5.0 g of Crysantellum americanum leaves were hydrolyzed with 250 ml of 6 N HCl for 24h. After filtration the aqueous solution was evaporated completely and the residue solubilized in 0.01N HCl (50 mg/10 ml); then it was treated with petroleum ether to remove lipids and with ethyl acetate to remove most of the coloring compounds. 10 ml of the purified hydrochloric solution were loaded into a column of Dowex 50W-exchange sulfonate resin (0.9 i.d. x 4.9 cm) previously activated according to Bauman method (Bauman et al., 1947). After washing the column with 50 ml of distilled water, the amino acids were eluted with a solution of 2N NH_4OH and finally the solution was concentrated to dryness.

HPLC PTH amino acids

The sample containing amino acids after addition of 100 mL of a buffer solution of CH_3COOH 2N/diethyl amine and 100 □L of isothiocyanate was used for the reaction in a closed tube for 3 hours at 25° C., obtaining the phenylthiocarbamyl amino acids derivatives. After removal of the excess of reagents, the phenylthiocarbamyl derivatives were converted into phenylthiohydantoins (PTH) amino acids; then the obtained compounds were dissolved in 100 □L of distilled water and 200 □L of CH_3COOH saturated with HCl and the resulting solution was keep on a water bath at 25° C for six hours. Following the removal of the solvent, the derivatives were purified by washing solution with water and after removal of solvent; the residue was washed twice with ethyl alcohol. PTH amino acids were

solubilized in 1 ml of a solution of 0.1 M sodium acetate/acetonitrile (70:30 v/v) and 10 □L were analyzed by HPLC. A column Ultracarb 5□ODS (20) 250 x 4.60 mm was used and a sodium acetate 0.01 M at pH 4.8 and acetonitrile gradient (from 68:32 to 10:90) total time 33 min. with a flow rate of 1 ml /min at a wavelength of 254 nm were used. The identification of amino acids in the sample was performed by comparing the retention times with those of a series of PTH amino acids standard eluted in the same conditions. The standards were supplied, by Sigma Aldrich (Buchs, Switzerland) as a dry stabilized film in an amber screw cap vial containing 0.1 μmole of each of the following PTH-amino acids: PTH-alanine, PTH-arginine, PTH-asparagine, PTH-aspartic acid, PTH-glutamic acid, PTH-glutamine, PTH-glycine, PTH-histidine, PTH-isoleucine, PTH-leucine, Nα-PTH-Nε-PTClysine, PTH-methionine, PTH-phenylalanine, PTH-proline, PTH-serine, PTH-threonine, PTH-tryptophan, PTH-tyrosine, PTH-valin (FIG.1)

GC-MS Analysis of amino acids

The identification of amino acids was performed by GC-MS using the same procedure of Chaves das Neves (Chaves das Neves et al, 1987) suitably modified. Amino acid analysis was performed on a Trio 2000 mass spectrometer equipped with a gas chromatograph GC 8000 (Micromass, Manchester, UK); a capillary column SPB-1 (30m x 0.25mm; id 0.25□ film thickness, Supelco, USA) was used with helium as carrier gas at flow rate of 1 ml/min. The sample was lyophilized in a vial and derivatized with 400 □L of a mixture of acetonitrile / N-methyl – N - (terbutildimetilsilil) trifluoroacetamide (50/50v/v) at 100° C for 2 h in a nitrogen atmosphere. The sample was diluted with acetonitrile in the ratio 1:9 v/v and then 1□L had been used for analysis. The analytical conditions were as follows: injector temperature 250° C; source temperature 200° C; interface temperature 260° C; column temperature programmed from 80° C to 280°C with increase of 3° C/min. The spectra were acquired in a mass range between 50 and 500u with a scan time of 0.06 sec. and one intersecan time of 0.08 sec. using a program management system GC-MS data acquisition and processing Mass Lynx provided by the manufacturer (FIG 2)

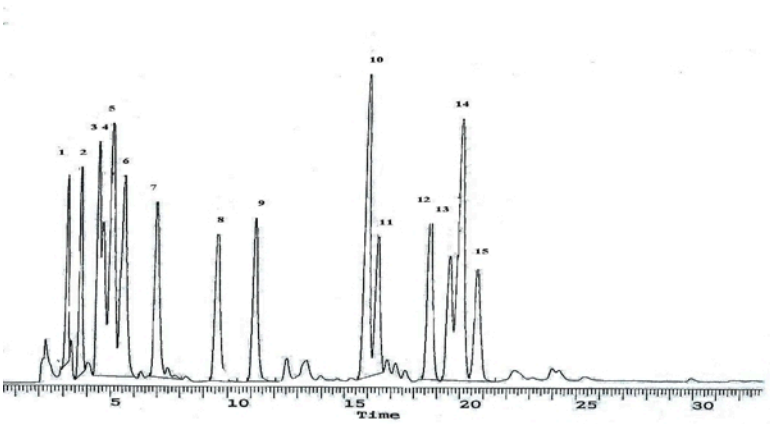

FIG 1 HPLC of PTH –aminoacids: 1-alanine 2-glycine 3-valin 4-leucine 5-isoleucine 6-proline 7-methionine 8-threonine 9-phenylalanine 10-asparagine 11-hydroxyproline 12-glutamine 13-arginine 14-lysine 15-tyrosine

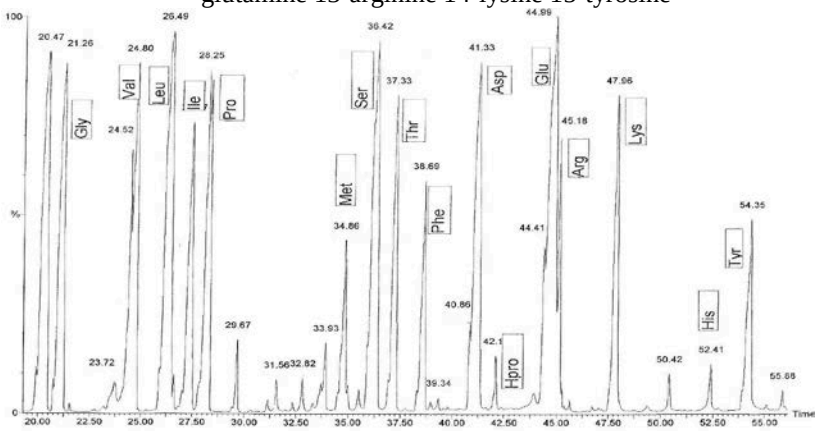

FIG. 2 GC-MS of N, O(S)-tert-butyldimethylsilyl derivatives of amino acids.

Conclusion

The analytical results have revealed a protein content high enough for the plants and the presence of as many as fifteen amino acids including valine, leucine and isoleucine to branched chain of high biological value because they increase muscular endurance, promoting recovery after exercise prolonged.Unlike many other amino acids they have a dual function: plastic, being essential for protein synthesis; energy, in particular conditions could be used as an alternative source of energy. Experimentally it has been noticed that a ratio isoleucine, valine and leucine 1:1: 2 is particularly effective to boost the ergogenic effects.Another peculiarity of the branched chain amino acids in free form regards their ability to be absorbed as such and remain unaltered even during the passage through the liver, where reaching the muscles play an important role in protein synthesis.

The presence of methionine, a sulfur amino acid, is important for its action against free radicals and is of great help in the detoxification of heavy metals. Its action is increased by the proline that counteracts the aging process by antagonizing the formation of free radicals.

Tyrosine promotes the proper functioning of the thyroid, pituitary gland and adrenergic; suppresses appetite, helps reduce body fat and seems to have a beneficial effect on the reduction of anxiety, depression, headaches, and in the treatment of Parkinson's disease.

To the high sensitivity of the analytical method worked it could also note the presence of GABA which is known the antihypertensive action: in fact, while having a retention time similar to that of proline, from the spectrum GC / MS has been possible to identify the fragmentation m / z characteristic of GABA

Pharmacological studies have been directed mainly on three actions: hepatoprotective, hypolipemic action, and venotropic.

As the hepatoprotective agent Chrysanthellum americanum is capable of stimulating experimentally the mechanisms of hepatic detoxification and regeneration of damaged hepatocytes. Its assets biliary drainage facilitates the elimination of triglycerides and cholesterol, lipid-lowering by turning it. Even saponosides and flavonoids behave as hepatoprotectors against hepatotoxic substances such as carbon tetrachloride. Unlike other plant compounds that act as hepatoprotectors only estimate sense, the Chrysanthellum americanum is also active on tissues already partially compromised, inducing the microsomal enzyme reactions that lead to inactivation and removal of toxins. Adding to the protective action that stimulating the potential of detoxification and regeneration

The vasoprotective action is exerted by some components not completely known of the Chrysanthellum americanum, against capillary and venous endothelium, with an activity comparable to that observed with rutin and other flavonoids with vascular tropism. The Chrysanthellum americanum reduces the fragility of small vessels and the permeability of the capillaries and veins, therefore its use has been suggested in the treatment of venous disease characterized by weakening of the vessel wall, increased permeability and perivascular edema formation, such as. venous insufficiency of the lower limbs such as varicose veins, varicophlebitis, edema and even for hemorrhoids

Further investigations are in progress to test other properties of the plant complex, thewhose employment is non-toxic and does not produce genetic alteration and therefore its use as a supplement is safe and well tolerated.

References:

Couderc P.G. Method for enriching chrysantellum with active ingredients by physical processes. e Oct, 1986: France patent 2579907-A1 Eur J Biochem. 1979 Dec;102(1):11-20 Couderc P.G. Herbal medicinal preparations based on chrysanthellum for use in the preparation of a medicament France Patent 2702376-A1 September 1994

Dubernard PM Etude de l'effet du *Chrysanthellum americanum* sur les lithiases rénales. Phytotherapy 1998 2(4) 210

Becchi M, Bruneteau M, Trouilloud M, Combier H, Sartre J, Michel G Structure of a new saponin: chrysantellin A from Chrysanthellum procumbens Rich Eur J Biochem. 1979 Dec;102(1):11-20.

Becchi M, Bruneteau M, Trouilloud M, Combier H, Sartre J, Michel G Structure of chrysantellin B, a new saponin isolated from Chrysanthellum procumbens Rich

Eur.Biochem.J. 1980,108,271-77

Cloarec M. Pharmacologie clinique et clinique d'un extrait de Chrysanthellum,CHU Hopital S.Antoine Paris 1982

Couderc P. Contribution a l'etude de la maladie calculeuse: son traitments par l'extrait de Chrysantellum. Publication Gerbs,Bordeaux,1981

Fram R.J. Cisplatinum and platinum analogues recent advancesCurr. Opin.Oncol 1992, 4, 1073-79

Shimokoriyama M. Anthochlor pigments of Corespsis tinctoria, J Am. Chem. Sot., 1956,79,214-220

AOAC 14th Ed Association of Official Analytical Chemists Washington DC 1990

Jones D.B. Factors for converting percentages of nitrogen in food and feeds into percentage of protein Circ U.S.D.A.1941,.183,1-22

Bauman W.C.,& Eichoorm J Fundamental properties of a synthetic cation-exchange resin .J Am. Chem.Soc.1947, 69, 2830 -36

Chaves das Neves H.J. and Vasconcelos A.M.P., Capillary gas chromatography of amino acids, including asparagine and glutamine: sensitive gas chromatogaphic—mass spectrometric and selected ion monitoring gas chromatographic—mass spectrometric detection of the N,O(S)-tert.-butyldimethylsilyl derivatives J. of Chromatolog 1987, 392,249.-258