

CENTELLA ASIATICA SELECTED TRITERPENES

A HIGHLY STANDARDIZED NATURAL REMEDY
FOR THE MAINTENANCE OF AN HEALTHY VENOUS SYSTEM





BOTANY AND HISTORY

Centella asiatica (L.) Urb. (syn.: *Hydrocotyle asiatica* L.) of the Apiaceae (Umbelliferae) family is a small herbaceous perennial plant, native to India, China, Indonesia, Australia, the South Pacific, Madagascar, and southern and middle Africa. It grows preferably in damp swampy areas, up to 700 meters above sea level.

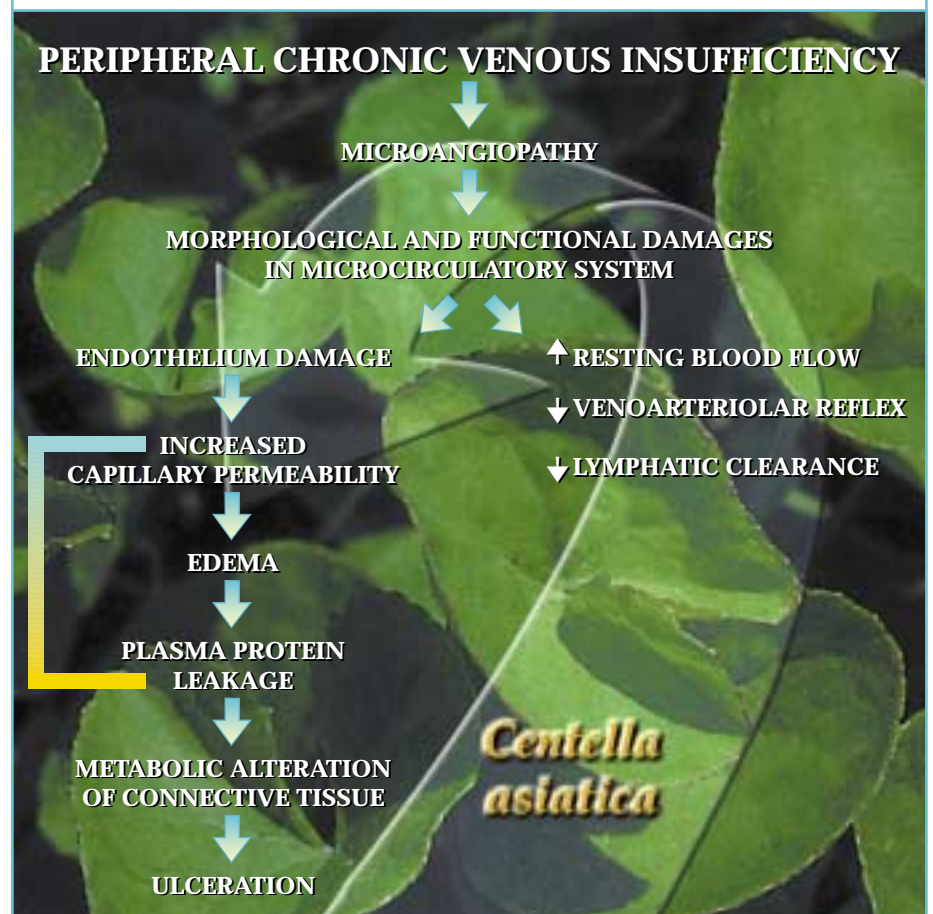
This slender creeping plant has long, prostrate, filiform stems with long internodes, rooting at nodes. The long-petioled leaves, 1-5 in number from each node, are reniform, oval or orbicular, deeply cordate, 1-7 cm in diameter. The small, purple to white-green flowers, 3-6 in number, are arranged in umbels arising from the axils of the leaves. The fruit is 8 mm long, ovoid, hard with strongly thickened pericarp.³ Since ancient times *C. asiatica* has been used in traditional Indian medicine for various pathological disorders and in

particular for the healing of wounds and for leprosis. In the Ayurvedic system of medicine it is also recommended in chronic diseases and as a "brain tonic" in various mental disorders. The wound healing applications have also been known in the folk medicine of Malay Peninsula, Java and other islands, Madagascar, Kenya whereas the psychotropic application of *C. asiatica* has been known in China. It is listed officially in the Chinese Pharmacopoeia and used as an antipyretic and diuretic, and in the treatment of icterus, heatstroke, diarrhea, ulcerations, eczema, and traumatic diseases.³⁻⁷ Today preparations of *C. asiatica* leaves constitute the active principle of pharmaceutical and cosmetic products for the treatment of venous and skin disorders.

Phytopreparations containing terpenes, flavonoids and coumarins have a good therapeutic potential as venoactive drugs and are widely used in the treatment of symptomatology associated with chronic venous insufficiency of the lower limbs. This illness causes severe injuries in the venous wall, metabolic alterations of the vascular and perivascular tissue and morphological and functional changes in the microcirculatory system.^{1,2}

It is reported that an abnormally increased capillary permeability, due to damage to the endothelium, causes tissue edema and the leakage of plasma proteins such as fibrinogen. Fibrinogen, turned into fibrin, induces severe alterations in the metabolism and in the structure of the perivascular connective tissue, which may lead to the onset of venous ulcers and to a worsening of the illness (Fig. 1). An increase in resting blood flow, an impairment of the venoarteriolar reflex, and a decreased lymphatic clearance are the main functional changes in microcirculatory system. The total triterpenic fraction of *C. asiatica* leaves has been found to be effective for chronic venous insufficiency and varicosities, by improving microcirculation and the metabolic activity of the vascular and perivascular connective tissue.

Fig. 1 Main target sites of *Centella asiatica*.



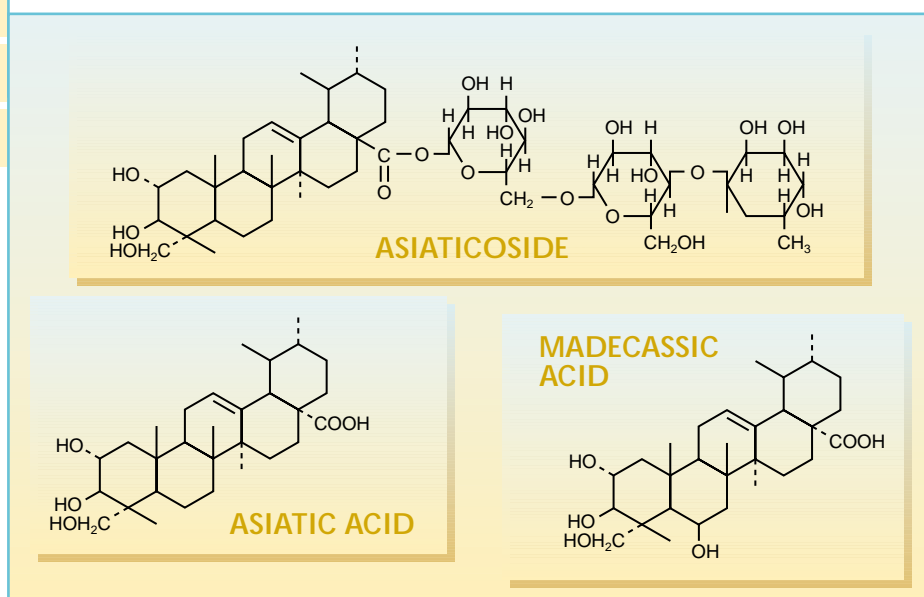
CENTELLA ASIATICA

SELECTED

TRITERPENES

Centella asiatica selected triterpenes (CAST) is a standardized mixture of the most significant active principles of *C. asiatica* leaves (Extract / Drug ratio: \approx 1/30), namely the glycoside asiaticoside (36 to 44%) and the genins asiatic and madecassic acid (56 to 64%), assayed by HPLC (Fig. 2).

Fig. 2 Chemical structures of the CAST constituents.



BIOLOGICAL ACTIVITIES

The first experimental studies, carried out from the late 1950's, highlighted that asiaticoside was endowed with a potent wound and ulcer healing activity.³ CAST and its components have been reported to facilitate the formation of wound tissue and enhanced the tensile strength of the newly made skin after local application on rat wounds.^{8,9} Poizot and Dumez¹⁰ have reported that a standardized extract of *C. asiatica* accelerated the healing of chronic wounds in rats after oral administration of 100 mg/kg. Later, in *in vitro* studies¹¹⁻¹⁶ on cultured human vein and skin fibroblasts, CAST and its triterpenic components were found to increase proline incorporation and stimulate collagen biosynthesis (Table 1). This finding has been

confirmed *in vivo* by Suguna *et al.*¹⁷ with an alcoholic extract of *C. asiatica* leaves.

Tenni *et al.*¹⁶ have also reported that CAST (25 μ g/mL) stimulated also the cell layer fibronectin on human skin fibroblast cultures.

Fowst *et al.*¹⁸ have reported that CAST (15-70 μ g/mL) positively influenced the confluence rate of endothelial cells in

culture, and showed a stimulating activity on fibronectin and PGI₂ production.

CAST proved also to inhibit the platelet aggregation induced by collagen, ADP, and arachidonic acid.

These results suggest that CAST may modulate the metabolism of connective tissue, favoring wound healing and also improving tissue microcirculation.

Table 1 Effect of CAST on collagen and total protein synthesis in human skin fibroblasts.

	Total proteins (dpm x 10 ⁻³ /plate)	Collagen (%)
Control	221 ± 26	8.40 ± 0.43
CAST (25 μ g/mL)	205 ± 32	11.38 ± 0.72 *

Total protein synthesis was determined as the radioactivity incorporated into non dialyzable molecules of the medium and cell layer; collagen percentage was calculated from the radioactivity of proline and hydroxyproline.

* p <0.05 vs control

TOXICOLOGICAL AND PHARMACOKINETIC DATA

Standardized extracts of *C. asiatica* leaves and asiaticoside have been reported to be well tolerated in experimental animals especially by oral route. In toxicological studies asiaticoside did not show any sign of

toxicity up to the dose of 1 g/kg after oral administration, whereas the toxic dose by intramuscular application to mice and rabbits was 40 to 50 mg/kg.³ In teratological studies in rabbits, a standardized extract of *C. asiatica*

leaves did not show any teratogenic effect.¹⁹

The pharmacokinetics of CAST has been investigated in healthy volunteers by Grimaldi *et al.*²⁰ after oral administration in single doses (30-60 mg) or after

Fig. 3 Plasma concentrations of asiatic acid after oral administration of CAST in healthy volunteers.

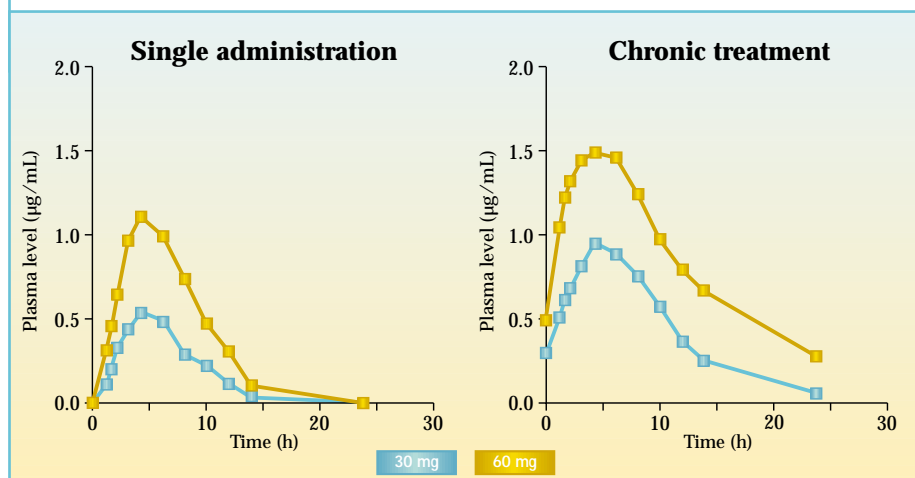


Table 2 Pharmacokinetic parameters.

Parameter	Single dose		7-day treatment	
	30 mg	60 mg	30 mg	60 mg
C_{max} (µg/mL)	0.70 ± 0.11	1.36 ± 0.13	1.03 ± 0.05	1.69 ± 0.07
$t_{1/2}$ (h)	2.20 ± 0.30	3.40 ± 0.68	6.33 ± 1.82	10.28 ± 1.80
AUC_{0-24} (µg/mL·h)	4.16 ± 1.10	9.39 ± 1.02	10.47 ± 1.09	20.83 ± 1.27

Table 3 CAST: main clinical studies performed between 1990-1994.

Number of reference	Number of patients and diagnosis	Dosage (mg/day, p.o.) and duration	Results
28	20 varicosities	60 3 months	↓ serum uronic acids ↓ serum lysosomal enzymes
29	62 postphlebotic syndrome	90-180 4 weeks	↓ capillary filtration rate and edema ↓ symptoms
30	44 chronic venous insufficiency	180 2 weeks	↓ capillary permeability and edema ↓ symptoms
31	50 diabetic microangiopathy	120 6 months	improved microcirculation ↓ capillary permeability ↓ RF and PCO_2 ↑ VAR and PO_2 stops ulceration damage
32	30 postphlebotic syndrome	90 3 weeks	↓ circulating endothelial cells
33	19 postphlebotic edema	120 3 weeks	↓ LC/PC and distal edema
34	87 chronic venous insufficiency and postphlebotic syndrome	60-120 2 months	improved microcirculation ↓ RF and PCO_2 ↑ PO_2

LC/PC = lymphatic protein concentration in the interstitial liquid/plasma protein concentration
 PO_2 = transcutaneous oxygen pressure
 PCO_2 = transcutaneous carbon dioxide pressure
 RF = resting blood flow
 VAR = venoarteriolar reflex

a 7-day treatment (30 or 60 mg twice a day), following a randomized cross over design. Plasma concentrations of asiatic acid were measured by HPLC assay method. Asiaticoside is reported to contribute to the plasma levels of asiatic acid because of its *in vivo* conversion into asiatic acid.

In Fig. 3 are reported the plasma concentrations, which appear to be dose-related, both after single and repeated treatments.

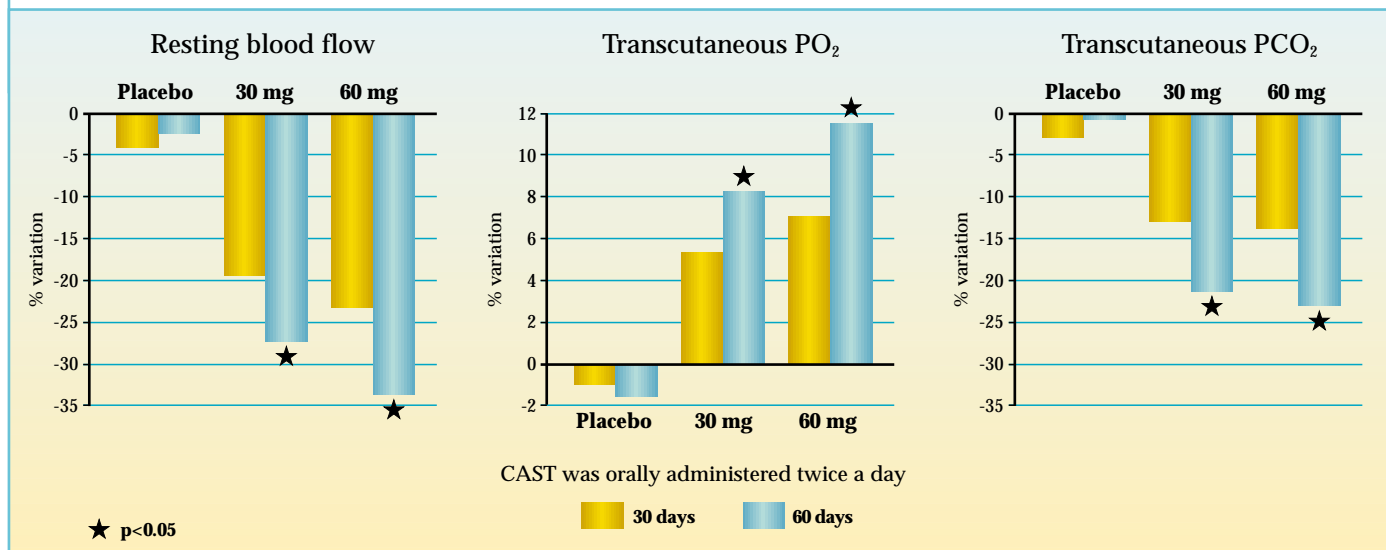
After repeated administrations of CAST peak plasma concentrations (C_{max}), area under curve (AUC_{0-24}) and half-life ($t_{1/2}$) values were significantly higher than those obtained after the corresponding single doses (Table 2).

CLINICAL APPLICATIONS

For a long time preparations of leaves of *C. asiatica* found application in clinical practice for dermatological disorders and in particular for improving the healing process of wounds, burns, skin and vein ulcers³. During the 1980's several clinical studies showed that CAST (60-120 mg daily, p.o., for 30-90 days) was able to improve subjective and objective symptoms associated with primary or secondary (postphlebotic syndrome) chronic venous insufficiency of the lower limbs.^{3,21-27}

More recent clinical studies^{2,28-34} indicate that CAST may positively interfere with the various phases of venous disease: venous wall alterations, change in connective metabolism, endothelial distress, impairment of microcirculation (Table 3). In patients suffering from chronic venous insufficiency, metabolic

Fig. 4 CAST: effects on microcirculatory parameters in patients with venous insufficiency.



alterations in the vascular and perivascular connective tissue, such as reduction of the collagen and elastic components, increase in some lysosomal enzymes in vascular wall and in serum, have been described.² CAST has been reported to have a stimulating activity on collagen synthesis *in vitro* and to reduce serum lysosomal enzymes in patients with varicose veins.²⁸ In patients with chronic venous insufficiency CAST has proved to reduce the high number of circulating endothelial cells in the peripheral blood - sign of a state of distress of intima.³² CAST has been shown to hinder the alterations of microcirculatory system,

evaluated by using a Laser-Doppler flowmetry and by transcutaneous oxygen tension (PO₂) and carbon dioxide tension (PCO₂) in patients with chronic venous insufficiency and in patients with varicose ulcers.^{24,25} This finding has recently been confirmed in a double-blind study performed on 90 patients with primary or secondary chronic venous insufficiency.³⁴ CAST (30 or 60 mg, twice a day, p.o.) improved microcirculatory parameters such as perimalleolar skin flow at rest (RF) and transcutaneous PO₂ and PCO₂ (Fig. 4). Furthermore CAST, in patients suffering from chronic venous insufficiency associated with venous hypertension as well as in patients with diabetic

microangiopathy, reduced capillary permeability and improved microcirculatory parameters and ankle edema.²⁹⁻³¹ In addition CAST has shown to influence the lymphatic function in patients with lymphatic and postphlebotic edema.³³ In all the trials so far cited CAST proved to be effective in relieving the symptomatology associated with chronic venous insufficiency such as phlebodynia, tiredness, itching in the legs, and night cramps, which disappeared in a high percentage of patients. This multi-action extract is endowed with a good tolerability, no severe adverse effects were observed during the trials.²

CONCLUSIVE REMARKS

The clinical studies so far reported proved that CAST is a venoactive compound which may act on different phases of the chronic venous insufficiency and slow down the development of the degenerative process of the venous system associated with this illness.

Different mechanisms of action seem to be involved in its therapeutic activity:

- a modulating activity on the vascular and perivascular connective tissue, which results in an improvement of tone and elasticity of vascular wall²
- a protective effect at endothelial level^{18,32}
- a protective activity on microcirculation, with reduction of the abnormally increased capillary permeability²⁹⁻³¹
- a positive effect on the lymphatic drainage function³³
- an antiaggregating activity and a profibrinolytic effect, that could hinder the negative effects of venous stasis associated with chronic venous insufficiency and varicosities^{18,35}

REFERENCES

1. Del Guercio R., Piovella C., Manuale di Microcircolazione per la Clinica, Edizioni Minerva Medica, Torino, 1995.
2. Cesarone M.R., Laurora G., De Sanctis M.T., Belcaro G., *Min. Cardioangiol.* 40, 137, 1992.
3. Kartnig T., Herbs, Spices and Medicinal Plants, vol. 3, L.E. Cracker, J.E. Simon (Eds.), Oryx Press, Arizona, USA, 1998, pp. 145-173.
4. Pedretti M., *Erboristeria Domani* 6, 45, 1987.
5. Pedretti M., *Erboristeria Domani* 7-8, 21, 1987.
6. Selected Medicinal Plants of India, compiled by Bharatiya Vidya Bhavan's Swami Prakashananda Ayurveda Research Centre, Chemexcil, Bombay, 1992, pp. 83-86.
7. Tang W., Eisenbrand G., Chinese Drugs of Plant Origin, Springer-Verlag, Berlin, 1992, pp. 273-276.
8. Rosen H., Blumenthal A., McCallum J., *Proc. Soc. Exp. Biol. Med.* 125, 279, 1967.
9. Tsurumi K., Hiramatsu Y., Hayashi M., Fujimura H., *Oyo Yakuri* 7, 833, 1973.
10. Poizot A., Dumez D., *C.R. Acad. Sci. Paris* 286, 789, 1978.
11. Maquart F.X., Bellon G., Brieu M.A., Borel J.P., Abstract First United Kingdom Meeting, Union Internationale de Phlébologie, London 16-20 September 1985.
12. Del Vecchio A., Senni I., Cossu G., Molinaro M., *Il Farmaco (Ed. Prat.)* 39, 355, 1984.
13. Maquart F.X., Bellon G., Gillery P., Randoux A., Borel J.P., *Sem. Hôp. Paris* 65, 1571, 1989.
14. Bonté F., Dumas M., Chaudagne C., Meybeck A., *Planta Med.* 60, 133, 1994.
15. Bonté F., Dumas M., Chaudagne C., Meybeck A., *Ann. pharmaceutiques françaises* 53, 38, 1995.
16. Tenni R., Zanaboni G., De Agostini M.P., Rossi A., Bendotti C., Cetta G., *Ital. J. Biochem.* 37, 69, 1988.
17. Suguna L., Sivakumar P., Chandrakasan G., *Indian J. Exp. Biol.* 34, 1208, 1996.
18. Fowst C., Cro L., Marozzi A., Marelli C., Tantalò V., Pogliani E.M., *Giorn. It. Angiol.* 7, 145, 1987.
19. A textbook of Natural Medicine, J.E. Pizzorno, M.T. Murray (Eds.), Bastyr University Publications, Bothell, Washington, 1996, pp V: Centel-1-5.
20. Grimaldi R., De Ponti F., D'Angelo L., Caravaggi M., Guidi G., Lecchini S., Frigo G.M., Crema A., *J. Ethnopharmacol.* 28, 235, 1990.
21. Allegra C., Pollari G., Criscuolo A., Bonifacio M., Tabassi D., *Clin. Terap.* 99, 507, 1981.
22. Allegra C., *Clin. Terap.* 110, 555, 1984.
23. Cospite M., Ferrara F., Milio G., Amato C., Lo Presti T., Ballo M., Meli F., Raimondi F., *Giorn. It. Angiol.* 4, 200, 1984.
24. Allegra C., Antonini V., Carlizza A., Tonelli V., *Min. Angiol.* 12, 107, 1987.
25. Belcaro G., *Phlebology* 2, 189, 1987.
26. Belcaro G., Laurora G., Cesarone M.R., Errichi B.M., Grimaldi R., Guidi G., *Curr. Ther. Res.* 46, 1015, 1989.
27. Pointel J.P., Boccalon H., Cloarec M., Ledevhat C., Joubert M., *Angiology* 38, 46, 1987.
28. Arpaia M.R., Ferrone R., Amitrano M., Nappo C., Leonardo G., Del Guercio R., *Int. J. Clin. Pharm. Res.* 10, 229, 1990.
29. Belcaro G., Rulo A., Grimaldi R., *Angiology* 41, 12, 1990.
30. Belcaro G., Grimaldi R., Guidi G., *Angiology* 41, 533, 1990.
31. Belcaro G., Grimaldi R., Guidi G., Laurora G., Cesarone M.R., Steigerwalt R., Pomante P., *Curr. Ther. Res.* 47, 421, 1990.
32. Montecchio G.P., Samaden A., Carbone S., Vigotti M., Siragusa S., Piovella F., *Haematologica* 76, 256, 1991.
33. Cesarone M.R., Laurora G., Pomante P., Belcaro G., Grimaldi R., Marelli C., *Min. Cardioangiol.* 39, 475, 1991.
34. Cesarone M.R., Laurora G., De Sanctis M.T., Incandela L., Grimaldi R., Marelli C., Belcaro G., *Min. Cardioangiol.* 42, 299, 1994.
35. Salvatore M., Cro L., Casaroli I., Marelli C., Pogliani E.M., *Giorn. It. Angiol.* 7, 45, 1987.