Oligomeric Proanthocyanidin Complexes: History, Structure, and Phytopharmaceutical Applications

Anne Marie Fine, CPA, ND Candidate 2000

Abstract
Considerable recent research has explored therapeutic applications of oligomeric proanthocyanidin complexes (OPCs), naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers, and bark. OPCs are primarily known for their antioxidant activity. However, these compounds have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions. In addition, they have been found to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase. Based on these reported findings, OPCs may be a useful component in the treatment of a number of conditions.
(*Altern Med Rev* 2000;5(2):144-151)

Introduction

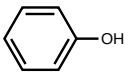
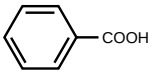
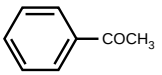
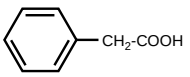
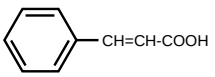
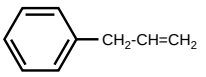
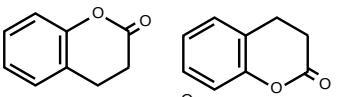
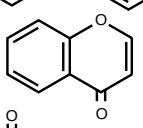
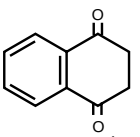
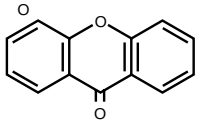
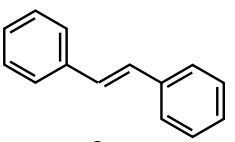
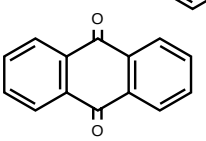
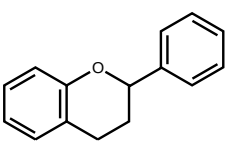
In 1534, a French explorer, Jacques Cartier, led a winter expedition up the St. Lawrence River in New York. The group soon found themselves trapped by ice and forced to survive on salted meat and hard biscuits. The crew began to show signs and symptoms of scurvy, long before anyone knew what caused it. Due to a chance meeting with a Native American, who showed them how to make a tea from the bark and needles of pine trees, the men survived.[1]

Professor Jacques Masquelier, of the University of Bordeaux, France, read the book eventually written by Cartier, became intrigued with the story and postulated that the pine bark must contain vitamin C as well as flavonoids having ascorbate-like effects. Thus began an exhaustive study of these compounds which Masquelier named pycnogenols, a term no longer used in the scientific community today except as a trademark for OPCs derived from French maritime pine bark. Today they are known as oligomeric proanthocyanidin complexes (OPCs) or procyanidolic oligomers (PCOs). Professor Masquelier confirmed the structure, effects, and lack of toxicity of these proanthocyanidins.[1]

Masquelier went on to patent the method of extracting OPCs from pine bark in 1951, and from grape seeds in 1970 (which research has supported as the preferential source).[1]

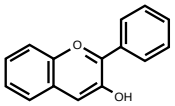
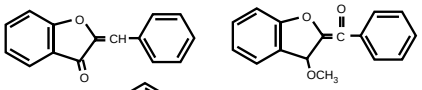
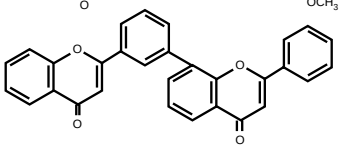
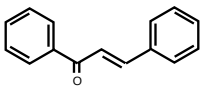
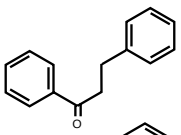
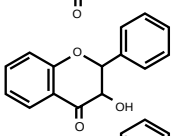
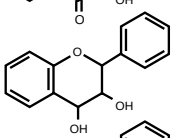
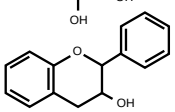
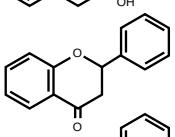
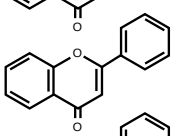
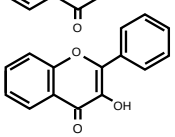
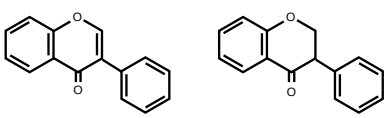
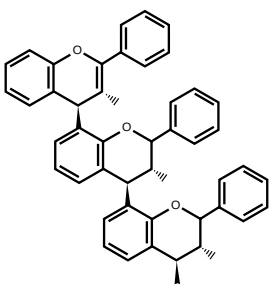
Anne Marie Fine, CPA, ND (Cand. 2000)- Vice President of Finance, International Clinical Research Center, Inc.
Correspondance address: 13910 N. Frank Lloyd Wright Blvd., Ste. 2A #105, Scottsdale, AZ 85260

Table 1: Main Classes of Polyphenolic Compounds

Class	Basic Skeleton	Basic Structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naftoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans, neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)n$	

Adapted from: Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* 1998;56(11):317-333.

Table 2: Classification of Food Flavonoids

Flavonoid	Basic Structure
Anthocyanidin	
Aurones	
Biflavonoids	
Chalcones	
Dihydrochalcones	
Dihydroflavonol	
Flavandiol or leucoanthocyanidin	
Flavanol	
Flavanones	
Flavones	
Flavonols	
Isoflavonoids	
Proanthocyanidins or condensed tannins	

Adapted from: Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* 1998;56(11):317-333

Molecular Structure

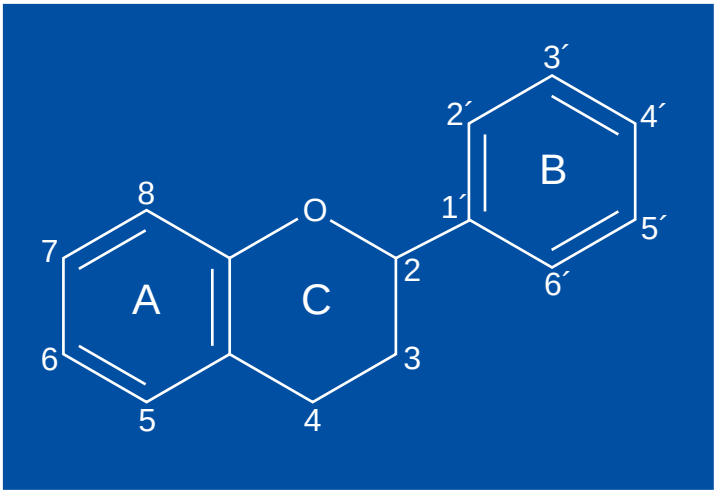
Proanthocyanidins are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers, and bark.[2] Other plant sources of proanthocyanidins include wine, cranberries, and the leaves of bilberry, birch, ginkgo, and hawthorne. Also known as procyanidins, these substances are the main precursors of the blue-violet and red pigments in plants.

These compounds are part of a specific group of polyphenolic compounds – the flavonoids (Table 1).[3] Flavonoids are further categorized by subgroups. Proanthocyanidins belong to the category known as condensed tannins, one of the two main categories of plant tannins (Table 2).[3] Tannins are highly hydroxylated structures that can form insoluble complexes with carbohydrates and protein, a measure of their astringency, based on their ability to cause precipitation of salivary proteins.[3] The polyphenolics, including proanthocyanidins, form a considerable portion of the tannins found in wine, and in particular contribute heavily to the color and flavor of red wines.

Proanthocyanidins are high-molecular-weight polymers comprised of the monomeric unit flavan-3-ol ((+)catechin and (-) epicatechin). Oxidative condensation occurs between carbon C-4 of the heterocycle and carbons C-6 or C-8 of the attached A and B rings (Figure 1).[3] The procyanidins B1-B4, characterized by the C4-C8 linkage, are the most common dimers, occasionally accompanied by corresponding C4-C6 linked isomers (B5-B8) (Figure 2).[4]

At a symposium entitled “Free Radicals in Biotechnology and Medicine” held in London in 1990, it was reported that esterification of (-)-epicatechin and procyanidin B2 by

Figure 1: Basic Structure and Numbering System of Flavonoids



gallic acid increases their free radical scavenging ability. Information was also introduced revealing the dimeric proanthocyanidins having the C4-C8 linkage have greater free radical scavenging activity than the C4-C6 linkage, and that these gallate esters are only found in the grape seed extract form.

Grape seed extract contains OPCs made up of dimers or trimers of (+)-catechin and (-)-epicatechin.[4-6] The procyanidin dimers are comprised of procyanidins B1, B2, B3, B4, B5, B6, B7, and B8. There are six procyanidin trimers which include procyanidin C1 and C2. Furthermore, several gallolyl procyanidins, which are most commonly the gallate esters of the dimeric procyanidins, and some free gallic acid are present.[4,5] Tetramers or greater of these flavonols would be known as polymeric proanthocyanidins and the astringency of the molecule would increase accordingly. Therefore, oligomeric proanthocyanidins are less astringent, bind less strongly to proteins, and are more soluble and mobile in the body.[5]

Biological Properties

The biological properties of flavonoids, including proanthocyanidins, have been extensively reviewed.[2,7-9] In addition to their free radical scavenging and antioxidant activity,[1,8,10,11] proanthocyanidins have been reported to have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions.[2,12] Proanthocyanidins have also been shown to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase.[1,2,12,13]

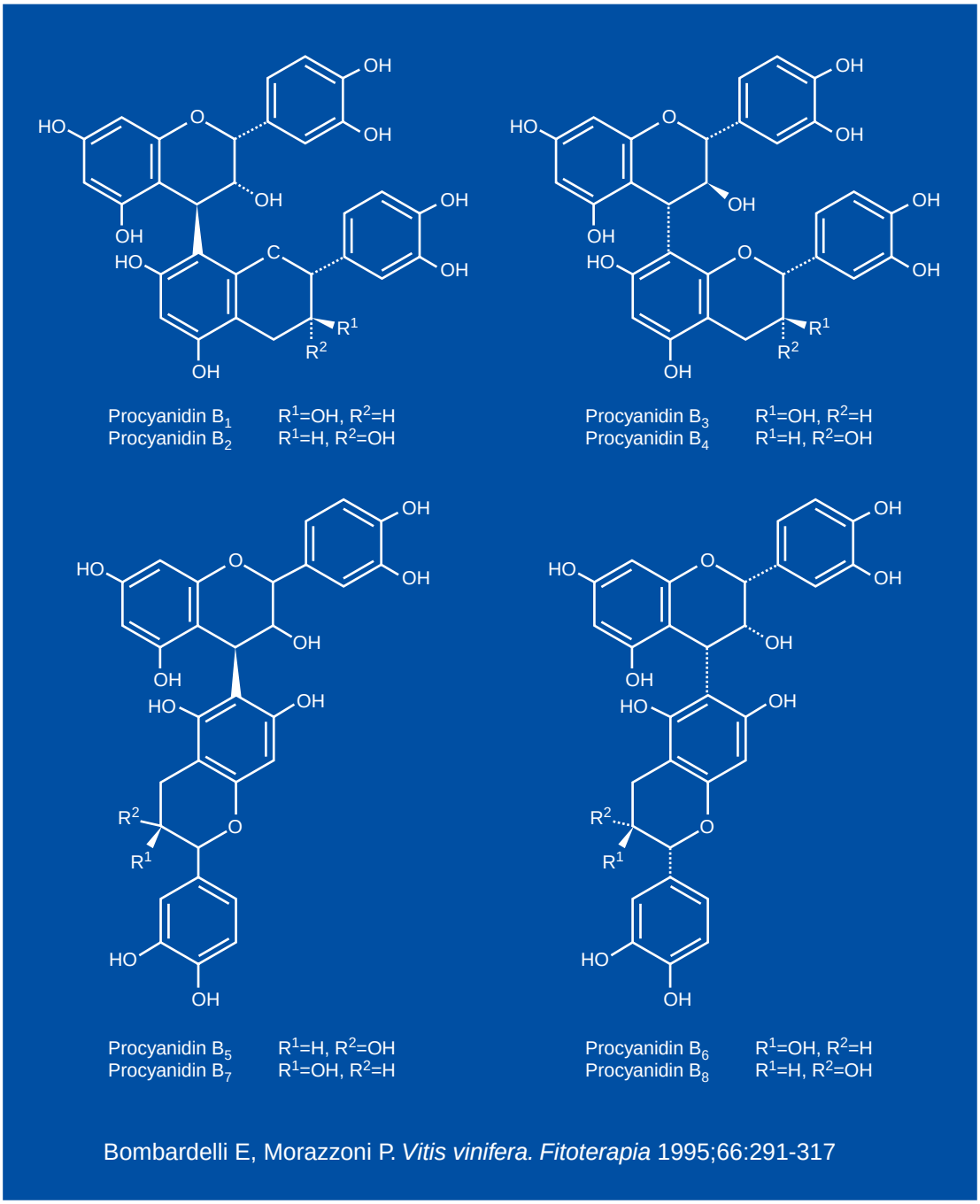
The free radical scavenging abilities of proanthocyanidins have been well documented and command the most attention.[1,2,12,14] *In vivo* studies have shown grape seed proanthocyanidin extract is a better free radical scavenger and inhibitor of oxidative tissue damage than vitamin C, vitamin E succinate, vitamin C and vitamin E succinate combined, and beta carotene.[12] Moreover, *in vitro* experimental results have demonstrated proanthocyanidins have specificity for the hydroxyl radical[1,2] in addition to having the ability to non-competitively inhibit the activity of xanthine oxidase, a major generator of free radicals,[1,9,14] elastase, collagenase, hyaluronidase, and beta-glucuronidase.[9]

OPCs have also demonstrated preferential binding to areas characterized by a high content of glycosaminoglycans (epidermis, capillary wall, gastrointestinal mucosa, etc.). This feature makes them useful for decreasing vascular permeability and enhancing capillary strength, vascular function, and peripheral circulation.[13]

Therapeutic Applications

Free radical damage has been strongly associated with virtually every chronic degenerative disease, including cardiovascular disease, arthritis, and cancer. Free radicals are highly reactive and cause tissue damage by reacting with polyunsaturated fatty acids found in cellular membranes, nucleotides in DNA, and sulfhydryl bonds in proteins. Free radicals may originate endogenously through normal metabolism and exogenously from polluted air, solvent-laden water, pesticide-laced food, or radiation exposure.

Figure 2: Structure of the Main Procyanidin Dimers from *V. vinifera*



Clearly, due to their antioxidant activity, the therapeutic potential of proanthocyanidins is quite broad. In Europe, proanthocyanidins are used mainly for the treatment of vascular disorders such as venous insufficiency, varicose veins, and microvascular problems including capillary fragility and retinopathies.[1] In fact, proanthocyanidins are the active ingredient in a proprietary pharmaceutical product sold in France (Endotelon) used primarily for microcirculatory disorders. The main features of proanthocyanidins that comprise the rationale for use in vascular disorders have been demonstrated experimentally:[1]

- Specificity for the hydroxyl free radical
- Traps lipid peroxides and free radicals
- Markedly delays the onset of lipid peroxidation
- Chelates to free iron molecules, so as to inhibit iron-induced lipid peroxidation
- Non-competitively inhibiting xanthine oxidase, a major generator of free radicals
- Inhibits hyaluronidase, elastase and collagenase, which can degrade connective tissue structures and lead to increased permeability

Proanthocyanidins have garnered recent attention in helping to explain the "French Paradox," the observation in France that high intake of dietary fats does not necessarily lead to high rates of atherosclerosis and coronary heart disease. When this paradox first came to light, research focused on the consumption of alcohol in the form of red wine as the preventive factor, but these results were equivocal.[8] Further research revealed that phenolic substances in red wine, including proanthocyanidins, had potent antioxidant properties, reducing the oxidation of human LDL *in vitro*, as well as inhibiting cyclooxygenase and lipoxygenase of platelets and macrophages *in vivo*, further reducing thrombotic predisposition.[8]

The 1998 Tufts University, "Dietary Antioxidant and Human Health Conference," highlighted the results of new research on proanthocyanidins and has been the most comprehensive scientific review of polyphenols in the United States to date. At this conference, Dr. Morazzoni, scientific director of Indena S.p.A., reviewed epidemiological studies that suggested grape polyphenols, present in red wine among other sources, could prevent the development of chronic vascular disease such as atherosclerosis. His conclusion was, "Grape procyanidins are a viable and clinically-proven, bio-active antioxidant for the prevention of cardiovascular diseases."

Dr. Kendall, from the University of Birmingham in England, also introduced results from his clinical research using standardized grape seed extract. His study demonstrated measurable changes in serum antioxidant activity in the patients receiving the extract, leading him to conclude antioxidants may play a role in sudden death prevention, referring to the fact that for many patients with coronary artery disease, sudden death may be the first and only indication of the disease.[15]

Other noteworthy research in this area, almost exclusively conducted in Europe, includes:

1. Oral administration of procyanidins from grape seed produced a hypocholesterolemic effect in a high-cholesterol animal feed model. Specifically it prevented an increase in total and LDL plasma cholesterol and a decrease in HDL.[16]

2. In a different hypercholesterolemic model, OPCs significantly lowered the amounts of cholesterol bound to aortic elastin compared to controls.[17]

3. Oral administration of OPCs in an experimental model effectively increased natural killer cell cytotoxicity and modulated *ex vivo* levels of interleukin-1, interleukin-6, and interleukin-10 in immune-compromised animals.[18]

4. OPCs from grape seed demonstrated *in vitro* antimutagenic activity.[19]

5. In a double-blind study, 71 patients with peripheral venous insufficiency received 300 mg OPCs from grape seed per day. A significant reduction in functional symptomatology was observed in 75 percent of the treated patients compared to 41 percent of the patients given a placebo.[20]

6. Measurements confirmed that a single administration of 150 mg OPCs increased venous tone in patients with widespread varicose veins.[21]

7. In a double-blind clinical trial, a group of elderly patients with either spontaneous or drug-induced low capillary resistance were treated with 100-150 mg OPCs from grape seed extract per day or placebo. Fifty-three percent of patients in the treated group demonstrated noticeable improvement in capillary resistance after approximately two weeks. All patients in this group reached the maximum attainable result after three weeks.[22]

8. In an open trial, 147 retinopathy patients received 100 mg OPCs from grape seed per day. The OPCs successfully treated exudations secondary to hypoxia of a diabetic, inflammatory, and arteriosclerotic nature.[23]

9. In a placebo-controlled clinical trial of 63 female breast cancer patients, post-surgical edema of the upper extremities was tested using 300 mg per day OPCs in the treated group for six months. At six months the OPC-treated group's functional score was significantly improved. In particular, there was a disappearance of pain in 59 percent of the treated patients compared to 13 percent taking the placebo.[24]

Conclusion

Oligomeric proanthocyanidin complexes are primarily known for their free radical scavenging and antioxidant activity. However, these compounds have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic and vasodilatory actions. In addition, OPCs have been reported to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase. These varied biological activities have resulted in the phytopharmaceutical application of OPCs in reduction of edema, increased peripheral circulation, improvement in vision, treatment of diabetic retinopathy, prevention of cardiovascular disease, treatment of hypercholesterolemia, stabilization of connective tissue tone, reduced adverse allergic and inflammatory responses, and enhanced immune function and wound healing. Additional clinical research is warranted.

References

1. Murray M, Pizzorno J. Procyanidolic oligomers. In: Murray M, Pizzorno J, eds. *The Textbook of Natural Medicine*. 2nd ed. London: Churchill Livingston; 1999:899-902.
2. Bagchi D, Krohn RL, Bagchi M, et al. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract *in vitro*. *Res Commun Mol Pathol Pharmacol* 1997;95:179-189.
3. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev* 1998;56:317-333.
4. Bombardelli E, Morazzoni P. *Vitis vinifera* L. *Fitoterapia* 1995;66:291-317.
5. da Silva J, Rigaud J, Cheynier V, et al. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* 1991;30:1259-1264.
6. Romeyer F, Macheix J, Sapis J. Changes and importance of oligomeric procyanidins during maturation of grape seeds. *Phytochemistry* 1986;25:219-221.
7. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. *Biochem Pharmacol* 1983;32:1141-1148.
8. Frankel EN, Kanner J, German JB, et al. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* 1993;341:454-457.
9. Bombardelli E, Morazzoni P, Carini M, et al. Biological activity of procyanidins from *Vitis vinifera* L. *BioFactors* 1997;6:429-431.
10. Chen ZY, Chan PT, Ho KY, et al. Antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups. *Chem Phys Lipids* 1996;79:157-163.

11. Nuttall SL, Kendall MJ, Bombardelli E, Morazzoni P. An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect. *J Clin Pharm Ther* 1998;23:385-389.
12. Bagchi D, Garg A, Krohn R, et al. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen Pharmacol* 1998;30:771-776.
13. Robert L, Godeau G, Gavignet-Jeannin C, et al. The effect of procyanidolic oligomers on vascular permeability. A study using quantitative morphology. *Pathol Biol (Paris)* 1990;38:608-616. [Article in French]
14. Hatano T, Yasuhara T, Yoshihara R, et al. Effects of interaction of tannins with co-existing substances. VII. Inhibitory effects of tannins and related polyphenols on xanthine oxidase. *Chem Pharm Bull(Tokyo)* 1990;38:1224-1229.
15. Personal communication, Indena SpA., Italy. December 1998.
16. Tebib K, Bessanicon P, Rouanet J. Dietary grape seed tannins affect lipoproteins, lipoproteinlipases, and tissue lipids in rats fed hypercholesterolemic diets. *J Nutr* 1994;124:2451-2457.
17. Wegrowski J, Robert AM, Moczar M. The effect of procyanidolic oligomers on the composition of normal and hypercholesterolemic rabbit aortas. *Biochem Pharmacol* 1984;33:3491-3497.
18. Cheshier JE, Ardestani-Kaboudanian S, Liang B, et al. Immunomodulation by pycnogenol in retrovirus-infected or ethanol-fed mice. *Life Sci* 1996;58:PL 87-96.
19. Liviero L, Puglisi P, Morazzoni P, Bombardelli E. Antimutagenic activity of procyanidins from *Vitis vinifera*. *Fitoterapia* 1993;65:203-209.
20. Thebaut JF, Thebaut P, Vin F. Study of Endotelon in functional manifestations of peripheral venous insufficiency. *Gaz Med France* 1985;92:96-100. [Article in French]
21. Royer RJ, Schmidt CL. Evaluation of venotropic drugs by venous gas plethysmography. A study of procyanidolic oligomers. *Sem Hop* 1981;57:2009-2013. [Article in French]
22. Dartenuc JY, Marache P, Choussat H. Capillary resistance in geriatry. A study of a microangioprotector - Endotelon. *Bord Med* 1980;13:903-907. [Article in French]
23. Verin MMP, Vilda A, Maurin JF. Therapeutic essay. Retinopathy and OPC. *Bord Med* 1978;11:1467-1473. [Article in French]
24. Pecking A, Desprez-Curely JP, Megret G. OPC (Endotelon) in the treatment of post-therapy lymphedemas of the upper extremities. *Int'l d'Antiologie*, Toulouse, France, Oct 4-7, 1989;69-73. [Article in French]