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(71) Applicant *(for all designated States except US)*: **ACCESS BUSINESS GROUP INTERNATIONAL LLC** [US/US]; 7575 Fulton Street East, Ada, MI 49355 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BROVELLI, Ernesto, A.** [US/US]; 785 La Loma Lane, Corona, CA 91719 (US). **VALLEJOS, Julio, Andres** [AR/AR]; Bolivar 1334, Corrientes 3400 (AR). **ROH-SCHMIDT, Haeri** [KR/US]; 1090 Dogwood Meadows SE, Ada, MI 49301 (US).

(74) Agent: **AHN, Amy, I.**; 7575 Fulton Street East, Ada, MI 49355 (US).

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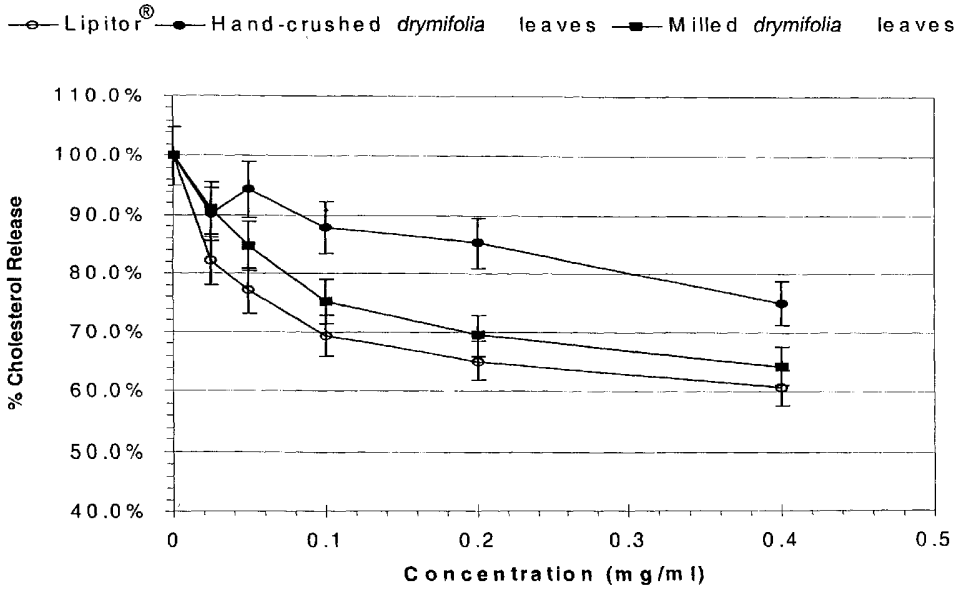
(54) Title: COMPOSITION AND METHOD FOR LOWERING CHOLESTEROL



(57) Abstract: The present invention relates to a composition comprising *Persea american* var. *drymifolia* for lowering cholesterol levels.

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COMPOSITION AND METHOD FOR LOWERING CHOLESTEROL

Technical Field

[001] The present invention relates to compositions and methods for lowering cholesterol. More particularly, the present invention relates compositions and methods having avocado leaf of the species *Persea americana* var. *drymifolia* (hereinafter referred to as "*drymifolia*") for use in lowering cholesterol levels in humans.

Background Art

[002] Cholesterol is a soft, waxy substance found among the lipids in the bloodstream and in cells of the human body. Although cholesterol serves needed bodily functions, too high a level of cholesterol in the blood may be detrimental to a person's health because it increases the risk of cardiovascular disease. High cholesterol generally means that a person's total blood cholesterol level is more than 240 mg/dl or that a person's low density lipoprotein level is more than 160 mg/dl (see, Cleeman, James I., Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), *JAMA* 285(19): 2486-2497 (2001)). Approximately 41.3 million Americans have total blood cholesterol levels of 240mg/dL or higher (see, American Heart Association, Biostatistical Fact Sheet, *www.americanheart.org* (2002)). When blood cholesterol reaches these high levels, it can build up on artery walls because cholesterol does not dissolve in the blood. Rather, cholesterol has to be transported to and from the cells by special carriers called low-density lipoproteins (LDL) and high density proteins (HDL). HDL carries cholesterol away from your arteries and is, thus, considered "good cholesterol." Too much LDL cholesterol can clog the arteries to your heart and is, thus, considered "bad cholesterol." This condition, called atherosclerosis, increases the risk of blood clots, heart attack, and stroke.

[003] Accordingly, there is considerable amount of interest in regulating cholesterol level in the body. There are several classes of available lipid-regulating pharmacological interventions called statins. One common statin is Lipitor®. Although these agents have been proven safe in clinical trials, like any drug, they carry the risk for side-effects. Most notable of the side-effects is myopathy, which becomes evident as muscle pains and weakness. Furthermore, it is important to minimize the potential for

adverse reactions or drug interactions when a patient is undergoing statin therapy (see, Davidson, Michael H., Treatment of the Elderly with 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors: Focus on Drug Interactions, *J Cardiovasc Pharmacol Therapeut*, 6(3): 219-229 (2001)). In its Adult Treatment Panel, the National Cholesterol Education Program recommends the use of plant derived substances in the adjuvant therapies for dyslipidemias (see Cleeman, *supra*). Accordingly, natural alternatives with effective and safe cholesterol reduction are desired.

Disclosure of the Invention

[004] The present invention is a composition containing avocado leaves from the variety *drymifolia* for reducing cholesterol levels. In one embodiment the leaves are dehydrated and milled for consumption as a tea. Surprisingly, the leaves of *drymifolia* showed a comparable cholesterol lowering effect to Lipitor® and a much greater cholesterol lowering effect than the fruit and the leaves of *Persea nubigena* var. *guatamalensis* cv. Nabal and *Persea nubigena* var. *guatamalensis* cv. Haas, which are avocados commonly grown in North America.

[005] These and other aspects and advantages of the present invention will be better understood by reference to the drawings and the detailed description of the preferred embodiment. It is noted that, unless otherwise stated, all percentages given in this specification and the appended claims refer to percentages by weight.

Brief Description of The Drawings

[006] FIG. 1 is a graph displaying dose specific responses of Hepatocyte G2 cells to hand-crushed and milled leaves of *drymifolia* in comparison to Lipitor® in cholesterol release.

[007] FIG. 2 is a graph displaying dose specific responses of Hepatocyte G2 cells to milled leaves of *drymifolia* in comparison to milled leaves of *Persea nubigena* var. *guatamalensis* cv. Nabal and milled leaves of *Persea nubigena* var. *guatamalensis* cv. Haas in cholesterol release.

[008] FIG. 3 is a graph displaying dose specific responses of Hepatocyte G2 cells to milled leaves of *drymifolia* in comparison to the fruit of *Persea nubigena* var. *guatamalensis* cv. Haas in cholesterol release.

Best Mode for Carrying Out the Invention

[009] The present invention comprises avocado leaves from the variety *Persea americana* var. *drymifolia* ("*drymifolia*"). This variety is available in Northern Argentina. The particular substance(s) in the leaves that causes cholesterol lowering is not yet understood. However, it is a commonly understood principle in the herbal industry that active ingredients in fresh plants/herbs generally decompose or diminish in effectiveness as the plant/herb dies or decays. Thus, to preserve the cholesterol reducing capacity of the *drymifolia* leaves for shipment from Argentina to the United States, the fresh *drymifolia* leaves are preferably air dried at average room temperatures of about 60°F to about 80°F. This temperature range is maintained until the leaves are prepared for consumption. Surprisingly, this preconditioning step maintains the efficacy of the *drymifolia* leaves in lowering cholesterol.

[010] Once dehydrated, the leaves are preferably hand-crushed. The hand-crushed leaves showed better cholesterol lowering activity than the fruit and leaves of *Persea nubigena* var. *guatamalensis* cv. Nabal ("Nabal") and the leaves of *Persea nubigena* var. *guatamalensis* cv. Haas ("Haas"). More preferably, dehydrated leaves are milled using a 2 mm screen to mill the leaves to an average particle size of about 28 microns. Even more preferably, the leaves may be milled twice using a 1 mm screen to provide an average particle size of about 9 microns. Most preferably, the dehydrated leaves may be milled using a 1 mm screen, providing an average particle size of about 11 microns. Milling can be performed by a GlenMills hammer mill bench top unit (Mot. KM 80-60, Culatti Typ MFC). Milled *drymifolia* leaves showed greater cholesterol reduction than hand-crushed *drymifolia* leaves. It is contemplated that the milled and dehydrated leaves can be used in the form of tea, capsules, tablets, creams, gels, liquids, and foods. However, for exemplary purposes, preparation and consumption in the form of a tea is described.

[011] The tea is prepared as a 1% extract by preparing 10 mg of hand-crushed *drymifolia* leaves or milled *drymifolia* leaves per 1 ml of water. In one embodiment, 100 mg of *drymifolia* leaves are placed in 10 ml of water to form a tea. The tea is boiled for about 1 minute to about 10 minutes. More preferably, the tea is boiled for about 3 minutes to about 7 minutes and, most preferably, for about 5 minutes. The water can be boiling after or upon immersing the *drymifolia* leaves in the water. It will be appreciated by those of ordinary skill in the art that the boiling times will vary depending upon the volume of the tea.

***Drymifolia* Comparison Studies**

[012] To test the efficacy of the *drymifolia* tea containing milled *drymifolia* leaves having an average particle size of about 11 microns, comparison studies were performed with tea containing hand-crushed *drymifolia* leaves, Lipitor®, Haas fruit, and milled leaves of Haas and Nabal having an average particle size of about 11 microns. Lipitor® is prepared in methanol/buffer to solubilize atorvastatin, the active ingredient in Lipitor® (40 mg active/600 mg tablet). All test materials were applied to serum culture medium at varying doses.

[013] Tea containing *drymifolia* leaves having an average particle size of about 11 microns was added to Dulbecco's Modified Eagle Medium (DMEM, Catalogue # 11965, In Vitrogen Corporation, 1600 Faraday Avenue, P.O. Box 6482, Carlsbad, California 92008) to a final concentration of about 0.1mg/ml to about 0.5 mg/ml *drymifolia* leaves via serial dilution (or 0.01% to 0.05% *drymifolia* leaves). More preferably, the final concentration is about 0.2 mg/ml to about 0.4 mg/ml *drymifolia* leaves and, most preferably, about 0.4 mg/ml *drymifolia* leaves. The culture media provides all necessary nutrients for cell maintenance including cholesterol synthesis.

Components of Culture Medium	**Molarity (mM)**
INORGANIC SALTS:	
Calcium Chloride (CaCl2) (anhyd.)	1.80
Ferric Nitrate (Fe(NO3)3-9H2O)	0.000248
Potassium Chloride (KCl)	5.30
Magnesium Sulfate (MgSO4)	0.813
Sodium Chloride (NaCl)	110.34
Sodium Bicarbonate (NaHCO3)	44.10
Sodium Phosphate (NaH2PO4-H2O)	0.906
OTHER COMPONENTS:	
D-Glucose	25.00
Phenol red	0.0346
AMINO ACIDS:	
L-Arginine-HCl	0.398
L-Cystine 2HCl	0.200
L-Glutamine	4.00
Glycine	0.399
L-Histidine HCl-H2O	0.20
L-Isoleucine	0.802
L-Leucine	0.802
L-Lysine-HCl	0.798
L-Methionine	0.201

L-Phenylalanine	0.400
L-Serine	0.400
L-Threonine	0.078
L-Tryptophan	0.078
L-Tyrosine 2Na 2H20	0.398
L-Valine	0.803
VITAMINS:	
D-Ca pantothenate	0.0083
Choline Chloride	0.0285
Folic Acid	0.00906
i-Inositol	0.04
Niacinamide	0.0328
Pyridoxine HCl	0.0196
Riboflavin	0.00106
Thiamine HCl	0.0118

[014] Amounts of secreted cholesterol and cholesteryl ester were measured from acetate fed hepatocyte culture media using a fluorescent indicator, Amplex Red. As shown in FIG.1, it is apparent that the effect of milled *drymifolia* leaves is comparable to that of Lipitor®. The results also show that milled *drymifolia* leaves with an average particle size of about 11 microns had a more favorable result than that of hand-crushed *drymifolia* leaves when water extracts are made from each. The response of hepatocytes also indicated little or no toxicity associated with the doses treated when cell survival post treatment was assessed via MTT (3-[4,5-dimethylthiazol-2yl]-2,5 --diphenyltetrazolium bromide, Sigma, St. Louis, MO) reduction assay.

[015] FIG. 2 shows the effect of the milled *drymifolia* leaves to milled leaves of Haas and Nabal. FIG. 2 shows that *drymifolia* leaves have a more favorable effect in decreasing cholesterol synthesis/secretion than that of Haas or Nabal.

[016] As shown in FIG. 3, when the cholesterol inhibitory effect of Haas fruit is assessed in comparison to that of the milled *drymifolia* leaves, the milled leaves again exerted much more favorable response than the fruit.

[017] While the above describes what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made thereto without departing from the spirit of the invention. It is intended to claim all such changes and modifications that fall within the true scope of the invention.

What is Claimed:

1. A composition comprising an effective amount of *Persea americana* var. *drymifolia* leaves for lowering cholesterol levels.

2. The composition of claim 1 wherein said composition is in the form of a tea.

3. The composition of claim 1 wherein said composition is a dietary supplement.

4. The composition of claim 1 wherein said composition lowers total blood cholesterol levels by about 10% to about 40%.

5. The composition of claim 1 wherein said *Persea americana* var. *drymifolia* leaves have an average particle size of about 11 microns.

6. The composition of claim 1 wherein said *Persea americana* var. *drymifolia* leaves are present in the amount of about 0.01% to about 0.05%

7. The composition of claim 1 wherein said *Persea americana* var. *drymifolia* leaves are present in the amount of about 0.04%.

8. The tea of claim 7 wherein said *Persea americana* var. *drymifolia* leaves have an average particle size of about 9 microns to about 28 microns.

9. A tea comprising about 0.1 mg/ml to about 0.5 mg/ml of *Persea americana* var. *drymifolia* leaves.

10. A method of lowering cholesterol levels comprising administering to a subject an effective amount of *Persea americana* var. drymifolia leaves.

11. The method of claim 10 wherein said *Persea americana* var. drymifolia leaves lower total blood cholesterol levels by about 10% to about 35%.

12. The method of claim 10 wherein said effective amount of *Persea americana* var. *drymifolia* leaves is about 0.01% to about 0.05%

13. A method of preparing a cholesterol lowering composition comprising the steps of:

(a) dehydrating leaves of *Persea americana* var. drymifolia;

(b) milling the leaves to an average particle size of about 9 microns to about 28 microns; and

(c) heating about 0.1 mg/ml to about 0.5 mg/ml of the milled leaves in boiling water.

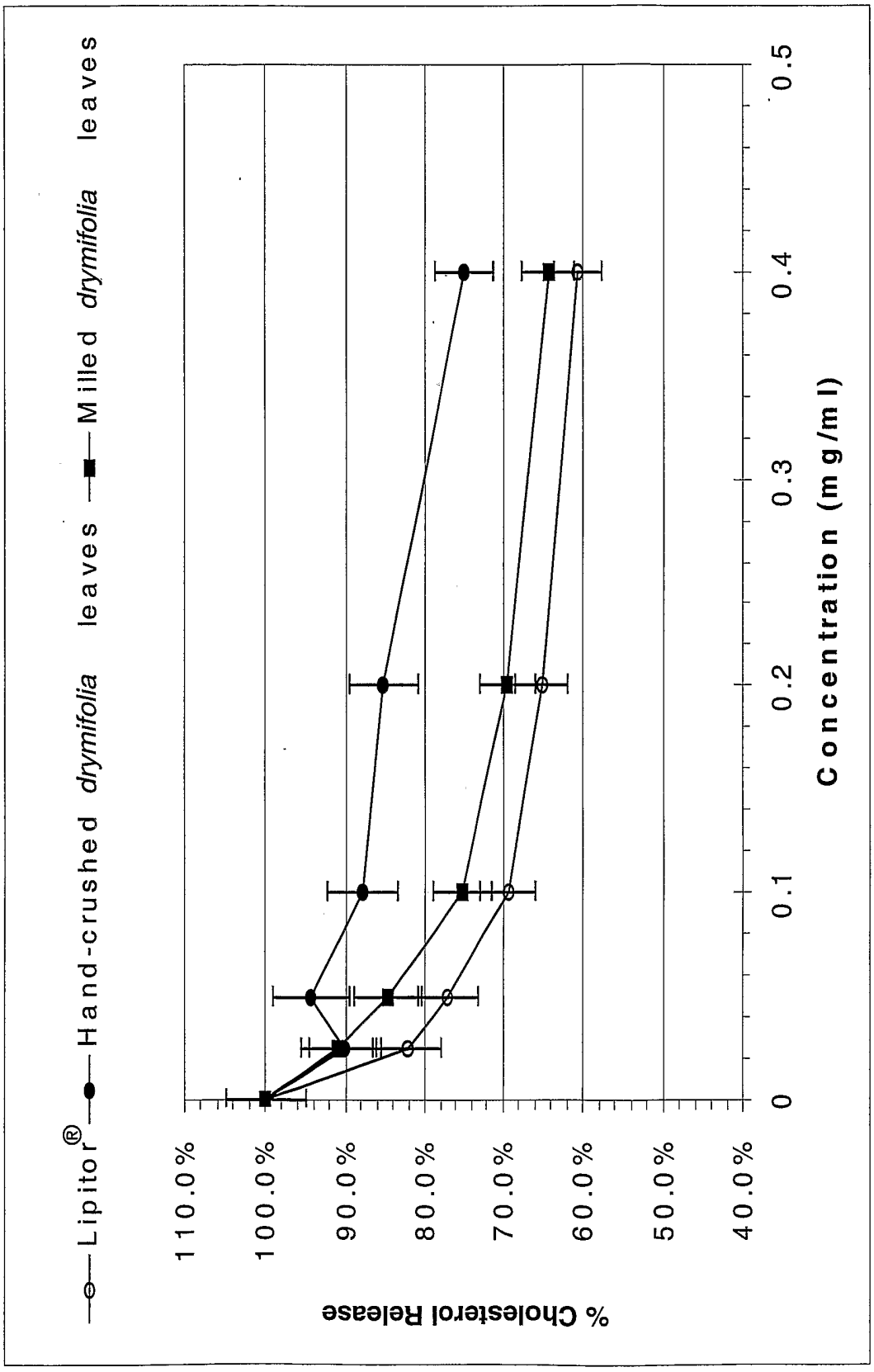
Lipitor®
Hand-crushed drymifolia leaves
Milled drymifolia leaves
% Cholesterol Release
110.0%
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
0
0.1
0.2
0.3
0.4
0.5
Concentration (mg/ml)

Fig. 1

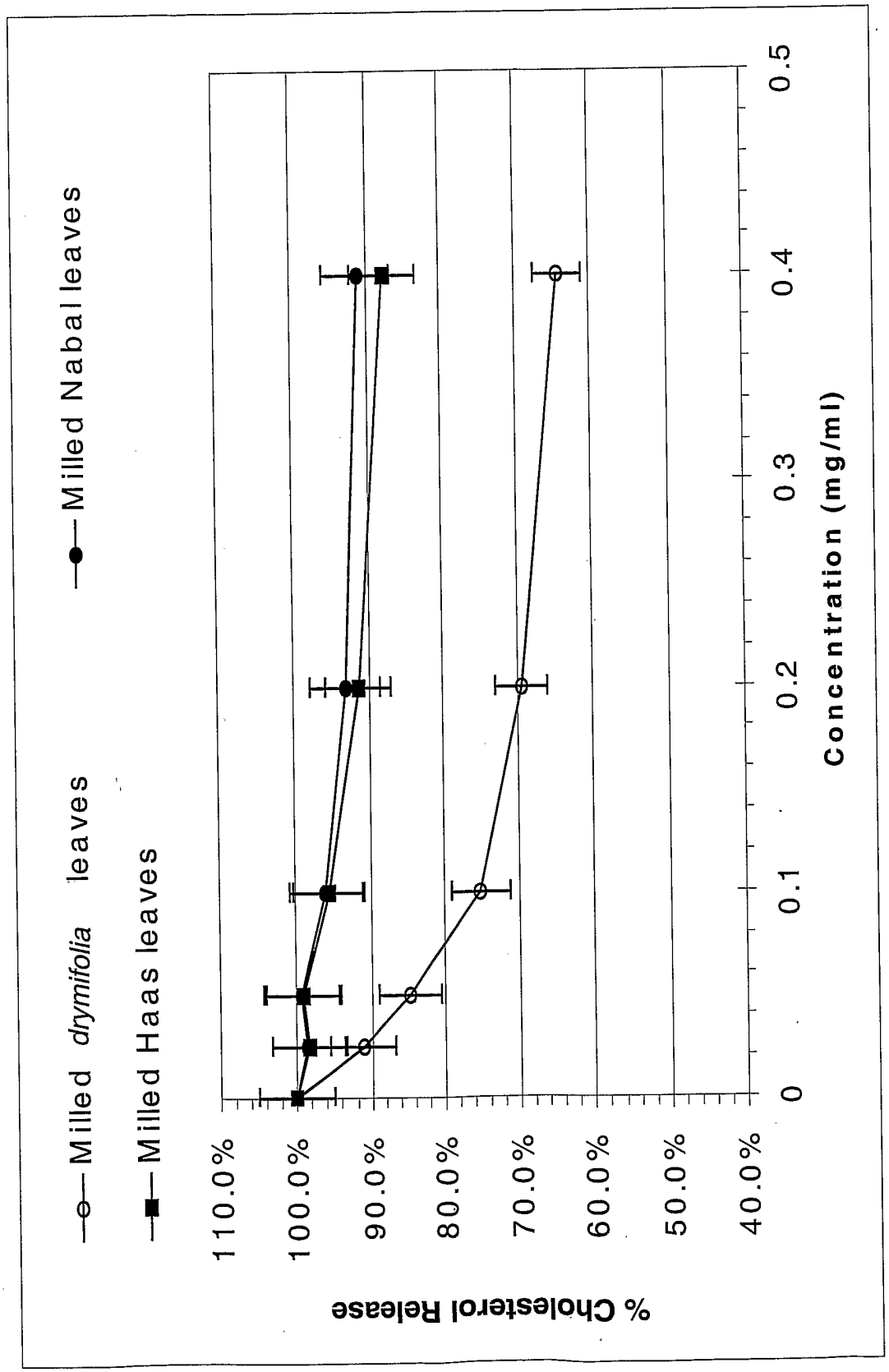
Milled drymifolia leaves
Milled Haas leaves
Milled Naba1 leaves
% Cholesterol Release
110.0%
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
0
0.1
0.2
0.3
0.4
0.5
Concentration (mg/ml)

Fig. 2

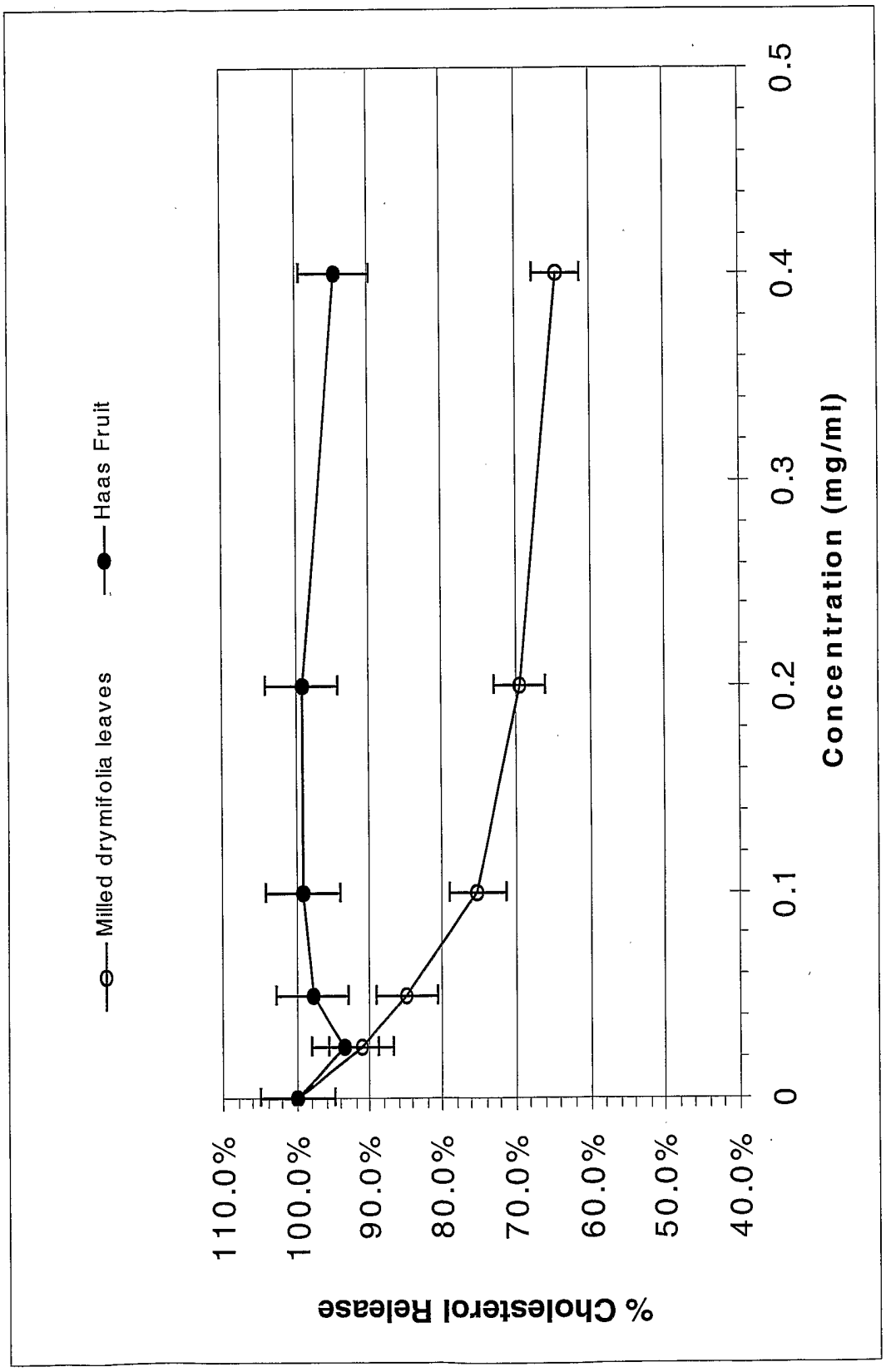
% Cholesterol Release
110.0%
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
0
0.1
0.2
0.3
0.4
0.5
Concentration (mg/ml)
Milled drymifolia leaves
Haas Fruit

Fig. 3