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**UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY**

ARCHER DANIELS MIDLAND COMPANY,

Plaintiff,

v.

SABINSA CORPORATION,

Defendant.

Civil Action No.:

COMPLAINT AND JURY DEMAND

Plaintiff, Archer Daniels Midland Company, through its undersigned attorneys, by way of Complaint against Defendant Sabinsa Corporation, hereby states as follows:

NATURE OF THIS ACTION

1. This is a civil action for patent infringement seeking damages and injunctive relief and is brought pursuant to the Patent Laws of the United States, Title 35 of the United States Code.
2. Plaintiff Archer Daniels Midland Company ("ADM") is a corporation organized and existing under the laws of the state of Delaware and has executive offices at 4666 Faries Parkway, Decatur, Illinois 62526. ADM has undertaken considerable research and development efforts to create novel soy-based products, processes and applications. Preliminary independent studies have shown isoflavones extracted from soy, either alone or in conjunction with other phytochemicals, provide various health benefits. For example, some studies have shown that soy

based isoflavones promote cardiovascular health by keeping arteries healthy, help maintain bone health in post menopausal women, and reduce menopausal symptoms such as hot flashes. ADM has an extensive portfolio of patents, both in the U.S. and throughout the world, and is committed to preserving the integrity of its proprietary interests in the composition, production and application of soy-based products.

3. Upon information and belief, Novogen Research Pty Ltd. ("NOVOGEN") is a proprietary limited company organized and existing under the laws of Australia, and having its principal place of business at 140 Wicks Road, North Ryde NSW 2113, Australia. ADM is the exclusive licensee of NOVOGEN's patents concerning soy based isoflavone products, compositions and applications.

4. Upon information and belief, Defendant Sabinsa Corporation ("SABINSA") is a corporation organized and existing under the laws of the state of New Jersey, having a principal place of business at 70 Ethel Road West, Suite 6, Piscataway, New Jersey 08854. Upon information and belief, SABINSA is infringing upon and has knowingly aided and abetted the infringement of ADM's patent rights by, *inter alia*, making, using, offering to sell, selling, and/or importing its GENIMAX® soy based isoflavone products without permission from ADM.

JURISDICTION AND VENUE

5. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331 and 1338(a).

6. This Court has personal jurisdiction over SABINSA because, *inter alia*, SABINSA transacts business in and resides in the State of New Jersey.

7. Venue is proper in this judicial district pursuant to 28 U.S.C. §§ 1391 and 1400(b).

COUNT I
INFRINGEMENT OF U.S. PATENT NO. 6,261,565

8. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 7 as if fully set forth herein.

9. On July 17, 2001, the United States Patent and Trademark Office duly and legally issued to Mark Empie and Eric Gugger, as inventors, United States Patent No. 6,261,565 entitled "Method of Preparing and Using Isoflavones" ("the '565 patent"). Mark Empie and Eric Gugger assigned the entire right, title and interest in the '565 patent to ADM. A copy of the '565 patent is attached as Exhibit 1.

10. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '565 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

11. Upon information and belief, SABINSA will continue to infringe the '565 patent unless and until it is enjoined by this Court.

12. Upon information and belief, SABINSA's infringement of the '565 patent is taking place with knowledge of the '565 patent and is willful. By continuing to commit acts of infringement with full knowledge of the '565 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

13. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '565 patent. ADM will suffer further irreparable injury, for which ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '565 patent.

COUNT II
INFRINGEMENT OF U.S. PATENT NO. 6,391,308

14. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 13 as if fully set forth herein.

15. On May 21, 2002, the United States Patent and Trademark Office duly and legally issued to Mark Empie and Eric Gugger, as inventors, United States Patent No. 6,391,308 entitled "Method of Preparing and Using Isoflavones for the Treatment of Blood Related Illnesses" ("the '308 patent"). Mark Empie and Eric Gugger assigned the entire right, title and interest in the '308 patent to ADM. A copy of the '308 patent is attached as Exhibit 2.

16. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '308 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

17. Upon information and belief, SABINSA will continue to infringe the '308 patent unless and until it is enjoined by this Court.

18. Upon information and belief, SABINSA's infringement of the '308 patent is taking place with knowledge of the '308 patent and is willful. By continuing to commit acts of infringement with full knowledge of the '308 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

19. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '308 patent. ADM will suffer further irreparable injury, for which ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '308 patent.

COUNT III
INFRINGEMENT OF U.S. PATENT NO. 6,391,309

20. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 19 as if fully set forth herein.

21. On May 21, 2002, the United States Patent and Trademark Office duly and legally issued to Mark Empie and Eric Gugger, as inventors, United States Patent No. 6,391,309 entitled "Method of Preparing and Using Isoflavones for the Treatment of Female Symptoms" ("the '309 patent"). Mark Empie and Eric Gugger assigned the entire right, title and interest in the '309 patent to ADM. A copy of the '309 patent is attached as Exhibit 3.

22. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '309 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

23. Upon information and belief, SABINSA will continue to infringe the '309 patent unless and until it is enjoined by this Court.

24. Upon information and belief, SABINSA's infringement of the '309 patent is taking place with knowledge of the '309 patent and is willful. By continuing to commit acts of infringement with full knowledge of the '309 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

25. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '309 patent. ADM will suffer further irreparable injury, for which ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '309 patent.

COUNT IV
INFRINGEMENT OF U.S. PATENT NO. 6,518,319

26. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 25 as if fully set forth herein.

27. On February 11, 2003, the United States Patent and Trademark Office duly and legally issued to Mark Empie and Eric Gugger, as inventors, United States Patent No. 6,518,319 entitled "Method of Preparing and Using Compositions Extracted from Vegetable Matter for the Treatment of Female Symptoms" ("the '319 patent"). Mark Empie and Eric Gugger assigned the entire right, title and interest in the '319 patent to ADM. A copy of the '319 patent is attached as Exhibit 4.

28. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '319 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

29. Upon information and belief, SABINSA will continue to infringe the '319 patent unless and until it is enjoined by this Court.

30. Upon information and belief, SABINSA's infringement of the '319 patent is taking place with knowledge of the '319 patent and is willful. By continuing to commit acts of infringement with full knowledge of the '319 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

31. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '319 patent. ADM will suffer further irreparable injury, for which

ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '319 patent.

COUNT V
INFRINGEMENT OF U.S. PATENT NO. 6,562,380

32. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 31 as if fully set forth herein.

33. On May 13, 2003, the United States Patent and Trademark Office duly and legally issued to Graham Edmund Kelly, as inventor, United States Patent No. 6,562,380 entitled "Methods for Treating or Reducing Predisposition to Breast Cancer, Pre-Menstrual Syndrome or Symptoms Associated with Menopause by Administration of Phyto-Estrogen" ("the '380 patent"). Upon information and belief, Graham Edmund Kelly assigned the entire right, title and interest in the '380 patent to NOVOGEN. A copy of the '380 patent is attached as Exhibit 5.

34. ADM is the exclusive licensee of soy-derived inventions claimed in the '380 patent.

35. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '380 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

36. Upon information and belief, SABINSA will continue to infringe the '380 patent unless and until it is enjoined by this Court.

37. Upon information and belief, SABINSA's infringement of the '380 patent is taking place with knowledge of the '380 patent and is willful. By continuing to commit acts of infringement with full knowledge of the '380 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

38. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '380 patent. ADM will suffer further irreparable injury, for which ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '380 patent.

COUNT VI
INFRINGEMENT OF U.S. PATENT NO. 6,642,212

39. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 38 as if fully set forth herein.

40. On November 4, 2003, the United States Patent and Trademark Office duly and legally issued to Graham Edmund Kelly, as inventor, United States Patent No. 6,642,212 entitled "Health Supplements Containing Phyto-Oestrogens, Analogues or Metabolites Thereof" ("the '212 patent"). Upon information and belief, Graham Edmund Kelly assigned the entire right, title and interest in the '212 patent to NOVOGEN. A copy of the '212 patent is attached as Exhibit 6.

41. ADM is the exclusive licensee of soy-derived inventions claimed in the '212 patent.

42. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '212 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

43. Upon information and belief, SABINSA will continue to infringe the '212 patent unless and until it is enjoined by this Court.

44. Upon information and belief, SABINSA's infringement of the '212 patent is taking place with knowledge of the '212 patent and is willful. By continuing to commit acts of

infringement with full knowledge of the '212 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

45. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '212 patent. ADM will suffer further irreparable injury, for which ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '212 patent.

COUNT VII
INFRINGEMENT OF U.S. PATENT NO. 6,987,098

46. Plaintiff ADM reaffirms and incorporates by reference each and every allegation contained in paragraphs 1 through 45 as if fully set forth herein.

47. On January 17, 2006, the United States Patent and Trademark Office duly and legally issued to Graham Edmund Kelly, as inventor, United States Patent No. 6,987,098 entitled "Health Supplement" ("the '098 patent"). Upon information and belief, Graham Edmund Kelly assigned the entire right, title and interest in the '098 patent to NOVOGEN. A copy of the '098 patent is attached as Exhibit 7.

48. ADM is the exclusive licensee of soy-derived inventions claimed in the '098 patent.

49. Upon information and belief, SABINSA has and continues to directly infringe, contributorily infringe, actively induce the infringement of, and/or knowingly aid and abet the infringement of the '098 patent by making, using, offering to sell, selling and/or importing products embodying the patented invention in the United States.

50. Upon information and belief, SABINSA will continue to infringe the '098 patent unless and until it is enjoined by this Court.

51. Upon information and belief, SABINSA's infringement of the '098 patent is taking

place with knowledge of the '098 patent and is willful. By continuing to commit acts of infringement with full knowledge of the '098 patent, SABINSA has failed to meet the required standard of care to avoid a finding of willful infringement.

52. SABINSA has caused and will continue to cause ADM irreparable injury and damage by infringing the '098 patent. ADM will suffer further irreparable injury, for which ADM has no adequate remedy at law, unless and until SABINSA is enjoined from infringing the '098 patent.

PRAYER FOR RELIEF

WHEREFORE, Plaintiff prays that this Court:

A. Enter judgment that Defendant has infringed United States Patent Nos. 6,261,565, 6,391,308, 6,391,309, 6,518,319, 6,562,380, 6,642,212 and 6,987,098.

B. Enter an order preliminarily and permanently enjoining Defendant, its officers, agents, servants, employees, attorneys, and all persons acting in concert or participation with it, from infringing United States Patent Nos. 6,261,565, 6,391,308, 6,391,309, 6,518,319, 6,562,380, 6,642,212 and 6,987,098.

C. Award ADM its damages resulting from Defendant's patent infringement pursuant to 35 U.S.C. § 284.

E. Find that Defendant's infringement has been willful and increase the damages awarded to ADM to three times the amount assessed, pursuant to 35 U.S.C. § 284.

F. Find this to be an exceptional case and award ADM its attorneys' fees and costs, pursuant to 35 U.S.C. § 285.

G. Award ADM such other and further relief as this Court deems just and appropriate.

JURY TRIAL DEMAND

Plaintiff ADM hereby demands a trial by jury on all issues appropriately tried by a jury.

Dated: March 23, 2006

Respectfully submitted,

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CERTIFICATION

I hereby certify that the matter in controversy is not the subject of any other court, arbitration or administrative proceeding.

/s/ Anthony P. La Rocco (APL 5986)

Dated: March 23, 2006

EXHIBIT 1



US006261565B1

(12) United States Patent
Empie et al.

(10) Patent No.: US 6,261,565 B1
(45) Date of Patent: Jul. 17, 2001

- (54) **METHOD OF PREPARING AND USING ISOFLAVONES**
- (75) **Inventors:** Mark Empie, Forsyth; Eric Gugger, Latham, both of IL (US)
- (73) **Assignee:** Archer Daniels Midland Company, Decatur, IL (US)
- (*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.
- (21) **Appl. No.:** 09/162,038
- (22) **Filed:** Sep. 28, 1998

Related U.S. Application Data

- (65) **Continuation-in-part of application No. 09/035,588, filed on Mar. 5, 1998, now Pat. No. 6,033,714, which is a continuation-in-part of application No. 08/868,629, filed on Jun. 4, 1997, now Pat. No. 5,792,503, which is a division of application No. 08/614,545, filed on Mar. 13, 1996, now Pat. No. 5,702,752**
- (60) **Provisional application No. 60/060,549, filed on Oct. 2, 1997.**
- (51) **Int. Cl.⁷** A01N 65/00
- (52) **U.S. Cl.** 424/195.1; 514/783; 514/26; 514/25; 514/27; 514/568; 514/717; 514/726
- (58) **Field of Search** 424/195.1; 514/783; 514/26, 25, 27, 568, 717, 726

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English translation of relevant material re Patent Appln. Laid Open Nos. (1) Hei 02-261365; (2) Hei 01-312965; (3) Hei 04-152845; (4) Hei 08-73369; (5) Hei 07-147903; (6) Hei 04-506402; (7) Hei 10-179100; and (8) Hei 11-12172.

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Primary Examiner—Ralph Oitomet

Assistant Examiner—D Khare

(74) Attorney, Agent, or Firm—Laff, Whitesel & Saret, Ltd., J. Warren Whitesel

(57) ABSTRACT

The invention provides for a refinement of phytochemicals in order to tailor the refined end product to particular human dietary needs. More particularly, a composition is prepared by extracting phytochemicals from plant matter. This composition is enriched preferably in two or more isoflavones, lignans, saponins, catechins and phenolic acids. Soy is the preferred source of these chemicals; however, other plants may also be used, such as red clover, kudzu, flax, and cocoa. The composition is a dietary supplement for treatment of various cancers, pre-and-post-menstrual syndromes, and various other disorders.

54 Claims, No Drawings

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METHOD OF PREPARING AND USING ISOFLAVONES

This is a formal application that replaces provisional application Ser. No. 60/060,549 filed Oct. 2, 1997. This is a continuation-in-part of application Ser. No. 09/035,588, filed Mar. 5, 1998, now U.S. Pat. No. 6,033,714, a continuation-in-part of Ser. No. 08/868,629, filed Jun. 4, 1997, now U.S. Pat. No. 5,792,503, which in turn is a division of Ser. No. 08/614,545, filed Mar. 13, 1996, now U.S. Pat. No. 5,702,752.

This invention relates to compositions extracted from vegetable matter and more particularly to phytochemicals, including saponogenins and saponins, lignans, phenolic acids, catechins and isoflavones, and especially those extracted from a family of plants including soy, flax, tea, and cocoa and methods of using these compositions as nutritional supplements or food additives.

BACKGROUND

As used herein, the term "isoflavone" includes malonyl, acetyl, glucoside, and aglycone forms of the isoflavones.

The invention relates to a process for refining phytochemicals in order to produce a combination with isoflavones which are tailored to the needs of specific human illnesses.

Currently, there is almost an epidemic of cancer, at least some of which is thought to be either caused or exacerbated by foods having a hormonal supplement derived from an animal origin. This thought is especially true for breast and prostate cancer. Other forms of cancers which are of special concern are skin cancer, colon cancer, urinary cancer, bladder and the like.

It is thought that many of these cancers, especially breast and prostate cancers, are either preventable or treatable by a use of a phytochemicals, especially isoflavones, as a source of supplemental hormones. However, it is also thought that there are superior results when a plurality of such phytochemicals are consumed in combinations.

In addition to cancer, there are many other illnesses which may be treated by ingesting certain phytochemicals. Exemplary of these illnesses are: blood related illnesses such as excessive levels of cholesterol, coronary disease, abnormal blood lipid profiles and vascular effects; female symptoms; neurological symptoms such as migraine headaches, immunological symptoms, inflammations, dementia and alcoholism. However, it is also thought that there are superior results when a plurality of such phytochemicals are consumed in combinations.

A proper diet contains the desired phytochemicals. However, a trouble is that many people do not have or do not like the proper kind of diet which provides the desirable effects. The problem is to furnish the necessary food values in some other form. Hence, there is a need for a process to refine phytochemicals in a manner which tailors the combination of isoflavones and phytochemicals to specific needs of particular illnesses.

Plant materials are known to contain a number of classes of organic low molecular weight compounds which exert bioactivity in various animals. Historically, these compounds have been considered to be somewhat non-nutritive, however, recent scientific evidence now suggests these compounds may play an important role in the maintenance of health, in chemoprevention, and in the mitigation of certain conditions or diseases associated with the circulation of sex hormones, including sleep disorders and vaginal dryness.

Edible plants normally contained in the diet, or materials used as herbal remedies/dietary supplements, may contain

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collections of structurally related compounds. These related substances are often unique in their amounts and distribution when compared among various plant sources. The most notable groups of compounds exhibiting bioactivity are known as flavonoids, isoflavones, saponins, lignans, alkaloids, catechins and phenolic acids.

Epidemiology studies relating diet to disease suggest that dietary components may predispose populations to reduced risk of certain diseases. Far eastern populations consuming soy have reduced rates of breast, prostate and colon cancers and coronary heart disease, while populations in Finland have reduced rates of prostate cancer. Researchers are just now studying the specific compounds in the diet to understand the basis for the epidemiological observations.

Among the various plants consumed in the diet, several are rich sources of phytochemicals. Soy products contain high amounts of isoflavones and saponins. Unrefined diet grains include plants such as wheat, psyllium, rice, flax and oats that contain lignans. Cocoa contains catechins and phenolic acids. Certain non-dietary plants are also sources of the same chemical molecules, such as lignans and isoflavones in kudzu root or red clovers. Isoflavones and lignans act as weak estrogenic substances. Tea plants are also a rich source of phytochemicals, including catechins and phenolic acids.

Isoflavones can be used alone to treat or prevent breast cancer, prostate cancer, skin cancer, and colon cancer or as mechanism inhibitors. Isoflavones alone may also reduce or prevent various symptoms related to the onset and duration of menopause, including hot flashes and osteoporosis. Isoflavones alone may also be effective in certain cardiovascular applications, including heart disease, reducing cholesterol-lipid levels, modulating angiogenesis, and other vascular effects. Moreover, isoflavones alone have been implicated in reducing headaches, dementia, inflammation, and alcohol abuse, as well as immunomodulation.

Lignans alone have been implicated in preventing or treating breast cancer, prostate cancer and colon cancer as well as reducing hot flashes, preventing osteoporosis and showing antiviral potential. Lignans also have antimicrobial and fungicidal activity. A plant lignan, the catecholic nordihydro-guaiaretic acid, was a potent antioxidant once used by the food industry.

Saponins alone have been implicated in preventing or treating skin cancer, colon cancer, reducing serum cholesterol, and in immunomodulation and antiviral activity. Saponins also exhibit antioxidant effects and act as free radical scavengers.

Phenolic acids have shown antioxidant activity.

People who eat a high soy diet show reduction of many of these above-discussed symptoms. This suggests that ingesting a combination of these phytochemicals in a ratio such as that found in soy may result in an additive or synergistic effect. However, a high soy diet has some undesirable effects, including flatulence, undesirable taste, and hesitancy among Western consumers to change their lifestyle to incorporate soy in their diets, even for such benefits.

Isoflavones, which are heterocyclic phenols, are understood to include the soy compounds genistin, daidzin and glycitein, as well as biochanin A, equol, formononetin, and o-desmethylandolensin and natural derivatives thereof. These compounds and their aglycone or de-methylated aglycone forms, such as genistein and daidzein, are believed to have similar activities once they are ingested. They are sometimes referred to as phytoestrogens.

Lignans are defined to be compounds possessing a 2,3-dibenzylbutane structure. They include matairesinol, secoisolariciresinol, lariciresinol, isolariciresinol, nordihydroguaiaretic acid, pinocresinol, divil, other compounds

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which may be precursors of enterolactone and enterodiol and modifications thereof, including diglycosides.

Phenolic acids include p-hydrobenzoic acid, protocatechuic acid, and vanillic acid. Other phenolic acids are chlorogenic acid, caffeic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, coumaric acid, cinnamic acid, genistic acid, salicylic acid, hydroxy benzoic acid and hydroxy phenyl acetic acids and derivatives. This list of phenolic acids should be understood to include the various isomers and derivatives found in the natural vegetable source.

Catechins, or flavan-3-ols, include epigallocatechin, catechin, epicatechin and galocatechin.

Sapogenins are C-27 sterols in which the side chain has undergone metabolic changes to produce a spiroketal. Sapogenins occur naturally as saponins, which are 3-O-glycosides of the parent steroid or terpenes. Digitonin from *Digitalis* is a saponin. Saponins include glucosides of sapogenin such as triterpenoides or steroids and saccharides such as glucose, arabinose, galactose or glucuronic acid. Typical examples of leguminous saponins are glycyrrhizin (glycyrrhetic acid+glucuronic acid) contained in *Glycyrrhiza glabra*, soysapogenin contained in soybean and alkalifasapogenin contained in *Medicago sativa*. Saponins also include chemical entities identified as triterpene phenols such as tomatin, soysapogenols A, B, C, D, E and F, glucoside fraction 3 and 4, medicagenic acid, hederagenin, glycyrrhizin diglucoside, quillaja saponin, lucernic acid and zahnic acid. The natural modifications of these compounds found in the vegetable source are also included in this identification.

A need exists for an improved composition consisting substantially of isoflavones, lignans, saponogenins, saponins, and/or phenolic acids which will produce improved results over any of these taken alone. Furthermore, a need exists for a composition in which the beneficial phytochemicals are enriched as compared to their original source. This permits individuals to conveniently consume such phytochemicals as a nutritional supplement or as a food additive.

SUMMARY OF THE INVENTION

An object of this invention is to provide a convenient way for individuals to consume isoflavones, lignans, saponins, catechins and/or phenolic acids, either as a nutritional supplement or as an ingredient in a more traditional type of food.

Another object of this invention is to provide an optimized extract composition of phytochemicals which is in sufficient concentration to be delivered in an easy to consume dosage such as a pill, tablet, capsule, liquid or ingredient in a food including health bars.

Yet another object of this invention is to prepare the phytochemical extract to be delivered as a topical application in a cream or lotion. In this form, the isoflavones, lignans, saponins, catechins and/or phenolic acids are dispersed and suspended in a suitable liquid or gel matrix to render a stable cream or lotion as the delivery vehicle.

A further object of this invention is to provide an extract concentrate which is closely similar in chemical composition to the chemical entities found in the natural plant source.

In keeping with this aspect of the invention, the isoflavones, lignans, saponins, catechins and/or phenolic acids are extracted from a suitable vegetable source to render a composition which is substantially more concentrated than the original material and by more than 5 times in one or more of the desired bioactive components.

This extract may be used alone or combined with one or more other plant extracts to produce the optimized compo-

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sition. Further, this extract composition may be formulated with one or more other dietary nutrients, such as vitamins, minerals, amino acids, etc., to provide a nutritional supplement further optimized for a desired health effect. All these ingredients may be combined with necessary binders, excipients, preservatives, colors and the like known to those in the industry in order to produce a suitable tablet, capsule, pill, liquid, cream, powder or food ingredient including health bars.

These phytochemicals may be packaged and provided in final form by means known to the supplements and food ingredient industries. The materials are intended to provide health and well-being benefits.

DETAILED DESCRIPTION OF THE INVENTION

The improved composition is obtained by fractionating a plant source high in isoflavones, lignans and other phytochemicals such as defatted soybean flakes, soy molasses, soy whey, red clover, alfalfa, flax, cocoa, tea, or kudzu root. These may be fractionated alone or in combination with these other plants known to be high in the various isoflavones, lignans, saponins, catechins and phenolic acids. The fractionation results in substantially removing water, carbohydrates, proteins, and lipids from the source material. The fractionation method may be preferably that disclosed in U.S. Pat. Nos. 5,702,752; 6,017,555; 6,033,714; or U.S. Pat. No. 4,428,576, or an extraction using ethyl acetate or n-butanol may be used. U.S. Pat. Nos. 5,702,752; 6,017,555; 6,033,714 are assigned to the assignee of this invention.

Other extraction processes, which may be used alone or in combination, include differential solubility, distillation, solvent extraction, adsorptive means, differential molecular filtration and precipitation.

The preferred composition is an improvement over known commercial materials regarding the amount of phytochemicals per gram of substance and the amounts of different phytochemicals present which affect physiologic function.

These natural substances have been consumed in food sources for long periods of time and more closely relate to the substances consumed which provide the basis for the epidemiological evidence for health benefits. Additional benefits may be derived from improved physical properties relative to phytochemicals chemically modified from their original food source form.

The resulting composition is expected to comprise in a preferred form: between 5% and 95% isoflavones, between 0% and 70% lignans, and between 2% and 70% saponins and sapogenins. In a more preferred form, the composition will be extracted from soy. In another preferred form, the composition will contain a ratio of (saponins plus sapogenins) to isoflavones from 1:100 to 100:1, with the isoflavones consisting predominantly of naturally occurring derivatives of genistein and/or its precursor biochanin A and daidzein and/or its precursor formononetin, with a ratio of the genistein derivatives to daidzein derivatives from 100:1 to 1:100. Preferably, the isoflavones are predominantly glycosylated derivatives.

The composition's ratios may be readily varied by changing the plant source or by combining several plant sources for extraction. Thus, as further study shows which phytochemical combinations are more efficacious for certain health effects, the particular composition will also vary.

It is known that isoflavones, lignans, and saponins can be used advantageously to treat or prevent various cancers, including breast cancer, prostate cancer, skin cancer, and colon cancer.

It is believed that the improved composition will provide increased benefits in the form of chemoprevention. Recent experiments appear to confirm this belief.

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EXAMPLE 1

An initial series of animal studies was made to investigate the effects of dietary soy products on the growth of s.c. (SUBCUTANEOUS) implanted LNCaP in male SCID mice. A high isoflavone-containing soy protein isolate (SPI) (2.0 mg isoflavones/g SPI) is provided by Protein Technology International (St. Louis, Mo.). A soy phytochemicals extract, soy phytochemicals concentrate (SPC) which contains 28.5% total soy isoflavones and a diverse amount of other soy phytochemicals, is provided by Archer Daniels Midland Company (Decatur, Ill.). These materials were used to prepare six experimental diets. Table 1 shows ingredients of the diets.

Eight-week-old male SCID mice were s.c. injected on the right flank with 2×10^6 LNCaP cells from hosts, randomized into six groups (n=10) and assigned to one of the experimental diets. Food intake, body weight, and tumor volume were measured. At the termination of the experiment, blood samples were collected and serum separated for PSA analysis. An aliquot of tumor tissues was formalin-fixed, paraffin-embedded, and cut into 4 μ m sections for in situ histochemical detection of apoptotic cells, and immunohistochemical analyses of angiogenesis and proliferation. Another aliquot was prepared for cell lysates for western blot to determine the expression of apoptosis-related gene products.

Table 2 summarizes the effects of treatment on food intake, body weight, isoflavone intake and tumor volume. Soy products did not significantly alter food intake or body weight. Compared to casein-fed controls, tumor volumes from mice treated with SPI (20%), SPC (1.0%), and SPI and SPC (1.0%) were reduced by 12%, 28% (P<0.04), or 40% (P<0.005), respectively. Factorial analysis indicated that there was no significant effect of protein source on tumor growth. Linear regression analysis indicated that tumor volumes were inversely correlated to total dietary isoflavones (Tumor volume (cm³)=0.0008+2.121xIsoflavones (mg), R²=0.76, p<0.03).

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TABLE 1

	Ingredients of experimental diets (grams)					
	Diet 1 Casein	Diet 2 SPI	Diet 3 Casein/ LSPC	Diet 4 SPI/ LSPC	Diet 5 Casein/ HSPC	Diet 6 SPI/ HSPC
SPI	0	200	0	200	0	200
Casein	200	0	200	0	200	0
DL-methionine	3	3	3	3	3	3
Corn starch	150	150	150	150	150	150
Sucrose	500	500	500	500	500	500
Collhose, BW200	50	50	50	50	50	50
Corn oil	50	50	50	50	50	50
Mineral Mix, S10001 ¹	35	35	35	35	35	35
Vitamin Mix, V10001 ¹	10	10	10	10	10	10
Choline	2	2	2	2	2	2
Biotin	2	2	2	2	2	2
Soy phytochemicals	0	0	2	2	10	10
Treat (g)	1000	1000	1002	1002	1010	1010
(Isoflavones, mg/kg diet)	0	245	341	586	705	950

¹AJN formulation with minor modification by Dr. E. A. Ulfano, Research Diets, Inc.

TABLE 2

Final body weight, total food intake, total isoflavone intake, and tumor volume				
Treatment	Body weight	Food intake grams/m	Total isoflavone	Tumor volume (cm ³)
Casein	22.4 ± 0.5 ¹	46.6 ± 3.1	0.00 ± 0.00	2.32 ± 0.31 ²
SPI	23.1 ± 0.7	46.2 ± 2.8	17.00 ± 6.37	2.06 ± 0.32
Casein/LSPC	21.4 ± 0.7	41.2 ± 3.4	14.03 ± 7.4	1.88 ± 0.35
SPI/LSPC	22.6 ± 0.6	50.1 ± 4.7	29.36 ± 7.6	1.66 ± 0.29 ³
Casein/HSPC	22.2 ± 0.7	44.8 ± 6.1	76.38 ± 10.40	1.64 ± 0.22 ³
SPI/HSPC	22.0 ± 0.6	47.5 ± 1.7	92.53 ± 3.22	1.39 ± 0.30 ^{3*}

¹Values are means ± SE. There are no significant differences of food intake or body weight among treatment groups.

²Compared with control group, SPI/LSPC, casein/HSPC, and SPI/HSPC had significantly smaller tumor volumes (*p<0.04; **p<0.005).

Table 3 shows the effects of SPC at 1.0% of the diet on apoptosis, proliferation, and angiogenesis of tumors from a pilot study. It indicates that dietary supplementation of soy phytochemicals inhibits the growth of LNCaP tumor in vivo by enhancing apoptosis and inhibiting proliferation of tumor cells. Its inhibitory effect on tumor angiogenesis is not significant which may be due to small sample size (n=2).

Results from in vitro study showed that genistein and soy phytochemical concentrate inhibited secretion of PSA by LNCaP cells into media. PSA concentrations were reduced 68% and 74% by 25 and 50 μ M of genistein treatment respectively, and 31% and 42% by 25 and 50 μ M of soy phytochemical concentrate treatment respectively.

TABLE 3

Effects of treatment on apoptotic index (AI, % TUNEL), proliferation index (PI, % PCNA staining) and angiogenesis (microvessel density)			
Treatment	AI (% TUNEL)	PI (% PCNA)	Microvessel Density
Control (n = 2)	6.07 ± 0.88	60.1 ± 1.1	12.5 ± 3.8
Casein/HSPC (n = 2)	10.75 ± 0.54	51.7 ± 1.3	9.7 ± 0.7
F value	<0.02	<0.01	>0.05

Values are means ± SE.

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In summary, preliminary results indicate that soy products inhibit the *s.c.* growth of LNCaP tumor in SCID mice, possibly via induction of apoptosis, and inhibition of angiogenesis and proliferation.

Isoflavones or lignans can alleviate menopausal-related symptoms such as hot flashes and osteoporosis as well as alleviate symptoms associated with menstruation. This is further believed to be due to their estrogenic activity. It is believed that the improved composition described here will alleviate these symptoms even more effectively.

Also, isoflavones positively affect various cardiovascular-related conditions, including heart disease, cholesterol (saponins also positively affect cholesterol), angiogenesis and other vascular effects. It is believed that the improved composition will produce results for these cardiovascular conditions at least as beneficial as those hitherto known and at a reduced cost.

As explained earlier, isoflavones, lignans, and saponins are known to individually positively affect various neurological and immunological symptoms. It is believed that the improved composition will result in alleviating neurological and immunological symptoms at least as well as those compounds hitherto known and at a reduced cost. Moreover, it would be expected that some synergism would arise out of the combination described herein.

The improved composition may be administered orally, parenterally, for instance, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation or by application of an aerosol spray to mucous membranes, or to the skin by an ointment or a cream.

Administering the improved composition may be done with any suitable carrier, in solid or liquid dosage form such as tablets, capsules, powders, soft gels, solutions, suspensions, emulsions, ointments, or creams. The improved composition may also be administered as a food supplement or as a food ingredient.

The amount of the improved composition administered will vary depending on the person, the mode of administration, and the desired result. An effective amount is expected to be 10 mg to 2000 mg/per dose.

EXAMPLE 2

Tablet Manufacture

The composition provided for in this patent may be used to prepare tablets or other dosage forms. An example of a capsule preparation is provided in Example 1. The higher the concentration of the active component, the easier it is to form a tablet or emulsion. This leads to an added ability to incorporate other dietary nutrients. An example would be to prepare a phytochemical tablet which incorporates calcium and vitamin E as a supplement to maintain bone health and/or reduce post menopausal symptoms such as hot flashes. In an example of this embodiment, a 600 mg dry compression tablet was prepared containing a total of 125

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mg of isoflavones concentrate (50 mg isoflavone compound). Included in the tablet formulation was a source of calcium and magnesium.

Dry compression tablets were produced by first blending the following ingredients: 4 kg of the improved composition (39.83% isoflavones), 1.91 kg sorbitol, 0.095 kg magnesium stearate, and 13.11 kg dicalcium phosphate in a 120 quart capacity Hobart mixer. This blend of ingredients was then dry compressed at 1 ton pressure with a Stokes BE2 simple press into tablets having a total weight of 600 mg containing 125.53 mg of the improved composition and therefore 50 mg of total isoflavones.

Alternatively, a phytochemical concentrate may be provided in a single dosage form, a skin cream or as a food ingredient added to conventional food in amounts from 10 mg to 2000 mg/per dose, the purpose of which is to exert a positive effect on health and well being. These benefits include: cancer prevention, estrogen and sex hormone related maladies, inhibition of the pituitary-thyroid-gonadotrophic axis, alcohol dependency reduction, modulation of the cardiovascular, immune and nervous systems, antiviral effects and analgesic effects.

EXAMPLE 3

Two-piece gelatin capsules were produced by filling the receiving end of the empty size "0" capsules with 0.106 g of the improved composition (44.35% isoflavones) and closed with the capping end, providing a capsule containing 47.2 mg of total isoflavones.

EXAMPLE 4

A comparison between various sources of phytochemical preparations is given in Table 4. It is readily seen that the phytochemical components of the composition of the "Isoflavone Concentrate" of this invention is substantially higher than the corresponding amounts in the natural vegetable materials. Notably, the amount of glycone isoflavones and saponins are over 100 times more concentrated compared to the food source and over twenty times more concentrated compared to the germ of the plant which naturally concentrates these phytochemicals. Comparison of the "Isoflavone Concentrate" of this invention to other concentrates shows that the isoflavone fraction predominates in these latter products, reducing the amount of other healthful phytochemicals. Additionally, the extraction methods of these other products employ techniques which modify the components, particularly the isoflavones, so that they are not identical to the substances found in the natural vegetable material (U.S. Pat. No. 5,637,562).

One version of the improved composition was compared to other previously described compositions. The results are shown in Table 4.

TABLE 4

Product Example	Comparative Products to the Invention					
	Isoflavone Glycosides in Product (mg/g)	Isoflavone Aglycones in Product (mg/g)	Genistein/ Daidzein Ratio	Lignans (mg/g)	Saponins (mg/g)	Phenolic Acids (mg/g)
Improved composition	401.0	3.37	1.06 to 1	0.2	460.7	25.47
Soybean	1,748-2,776 ^a	0.044 ^a -0.075	1.59-2.7	NA	0.9-3.2 ^b	2.870 ^c
Soy Fiber (defatted)	1.969 ^a	0.045 ^a	3.58	0.0013		
Soy germ	24.32 ^a	0.05 ^a		NA	16.7-1.58 ^b	NA

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TABLE 4-continued

Comparative Products to the Invention						
Product Example	Isoflavone Glycosides in Product (mg/g)	Isoflavone Aglycones in Product (mg/g)	Genistein/Daidzein Ratio	Lignans (mg/g)	Saponins (mg/g)	Phenolic Acids (mg/g)
Product ^a patent (FTI)	NA	2.5-6.5 ^a	0.5-3.5	NA	NA	NA
Product ^b patent (FTI)	NA	5.1-14.7 ^b	0.633-3.48	NA	NA	NA
Product ^c patent (FTI)	NA	1.7-3.5 ^c	0.66-2.86	NA	NA	NA
Product ^d FTI	NA	970	12.8	NA	NA	NA
Product ^e FTI	NA	640	2.0	NA	NA	NA
Soy Molasses (dried)	27.6	0.1	1.37	NA	NA	5.788
Novogen ^f	0.0	550	1-1.7 to 1	NA	NA	NA

^aWang H. and Murphy R. A., J. Agric. Food Chem 1994, 42, 1665-1673.^bAnderson R. L. and Wolf W. J., J. Nutr 125: 5818-5825, 1995^cSeo A. and Mori C. V., J. Agric. Food Chem 1984, 32, 530-533.^dSoy Life™ promotional literature^eWO 95/10530, PCT/US94/0697^fWO 95/16512, PCT/US94/0699^gWO 95/09929, PCT/US94/0696^hFTI paperⁱNovogen promotional literature

EXAMPLE 5

The improved composition, containing the glycoside forms of isoflavones, has as one aspect an improved solubility at body temperature over the previously described compositions containing the aglycoside forms.

Separate solutions (0.02% in distilled water) were made for genistein, genistin, daidzein, daidzin, and isoflavone concentrate in volumetric flasks. Samples were then placed in a 37° C. water bath for 17 hours, followed by rapid filtration through a 0.2 micron syringe-type filter to remove particulates. Filtered samples were then analyzed for isoflavone concentration by HPLC. Results are tabulated as shown in Table 5.

TABLE 5

Differential Solubility of Isoflavone Glycosides vs. Aglycones				
Isoflavone sample	Genistein (ppm)	Genistin (ppm)	Daidzein (ppm)	Daidzin (ppm)
Genistein	7.42			
Genistin		33.89		
Daidzein			3.64	
Daidzin				48.51
Isoflavone Concentrate	0.492	30.075	0.672	37.69

The glycoside forms, genistin and daidzin, are at least 4.57 and 13.32 fold higher in concentration at 37° C. than their corresponding aglycone forms, respectively.

The modifications made to the isoflavones are to remove the carbohydrate attached to the isoflavone moiety. This modification renders the isoflavone less soluble in water. Maintenance of the natural modification, glycosylation, enhances solubility. This fact is shown in the comparative solubility chart of Table 5. This chart shows that the genistin isoflavone is 4.6 times higher and the daidzin isoflavone is 13.3 times higher than the corresponding non-glycosylated form. Higher solubility can lead to better bioavailability to

intestinal organisms. The glycosylation does not inhibit absorption in the gut because the intestinal microflora convert the glycone form to the aglycone form before absorption occurs.

EXAMPLE 6

Extraction of Lignans from Flax

Lignans can be readily extracted from flax using this following method.

978 g of defatted flax meal (F1) was extracted with 2000 g of 85% ethanol at 40° C. for 10 minutes, forming a slurry. The resulting slurry was filtered and extraction was repeated twice with a total of 6000 g of ethanol.

The ethanolic fraction was then evaporated under vacuum at 70° C., resulting in an aqueous fraction of 1186 g. The aqueous fraction was combined with 1000 g of water and mixed.

The mixed sample was then ultra-filtered through a 5000 molecular weight cutoff membrane, resulting in a 767 g permeate fraction and a retentate fraction of 1283 g.

The retentate fraction was freeze-dried, resulting in a 27.84 g sample (F2).

The 767 g permeate fraction at 50° C. was fed to a 35 ml bed volume, XAD-4 resin column at a rate of 10 ml/min.

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The column effluent was collected and dried, resulting in a 14.8 g sample (F3). XAD-4 is a trademark for an absorbent resin, available from Rohm & Haas.

The column was then eluted with four bed volumes (140 ml) of 70% ethanol at 50° C. The eluent sample was evaporated under vacuum at 70° C. and dried, resulting in a 1.79 g sample (F4). The four fractions were then analyzed for their lignan content, measured as the concentration by weight of secoisolariciresinol. As Table 6 shows, this extraction method enriches lignan concentration.

TABLE 6

LIGNAN CONCENTRATIONS AS SECOISOLARICRESINOL				
FRACTION	F1	F2	F3	F4
SECO. CONC. (mg/g)	2.3	1.9	4.8	13.4
PHENOLIC ACID				

While the present invention has been disclosed in terms of the preferred embodiment in order to facilitate a better understanding of the invention, it should be appreciated that the invention can be embodied in various ways without departing from the principles of the invention. Therefore, the invention should be understood to include all possible embodiments, modifications, and equivalents to the described embodiment which do not depart from the principles of the inventions as set out in the appended claims.

What is claimed is:

1. A composition from a plant matter in which the composition is enriched in at least a first phytochemical and a second phytochemical selected from the group consisting of isoflavones, lignans, saponins, catechins and phenolic acids, said second phytochemical being a member of the group which is different from said first phytochemical.
2. The composition of claim 1 which essentially consists of at least 70% by weight phytochemicals selected from the group comprising isoflavones, lignans, saponins, catechins and phenolic acids.
3. The composition of claim 1 in which at least one of the selected phytochemicals comprises at least 10% by weight of the composition.
4. The composition of claim 1 which essentially consists of at least 80% by weight phytochemicals selected from the group comprising isoflavones, lignans, saponins, catechins and phenolic acids.
5. The composition of claim 1 which essentially consists of at least 90% by weight phytochemicals selected from the group comprising isoflavones, lignans, saponins, catechins and phenolic acids.
6. The composition of claim 1 in which the ratio by weight of isoflavones to lignans is selected from the range of about 1000:1 to about 1:50.
7. The composition of claim 1 in which the ratio by weight of isoflavones to saponins is selected from the range of about 1:10 to about 10:1.
8. The composition of claim 1 in which the ratio by weight of isoflavones to phenolic acids is selected from the range of about 100 to 1 to about 1 to 100.
9. The composition of claim 1 in which the ratio by weight of lignans to saponins is selected from the range of out 100 to 1 to about 1 to 100.
10. The composition of claim 1 in which the ratio by weight of lignans to phenolic acids is selected from the range of about 100 to 1 to about 1 to 100.
11. The composition of claim 1 in which the ratio by weight of saponins to phenolic acids is selected from the range of about 100 to 1 to about 1 to 100.
12. The composition of claim 1 in which the ratio of catechins to phenolic acid is selected from a range by weight of about 100 to 1 to about 1 to 100.

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13. The composition of claim 1 in which the isoflavones are present in an amount from approximately 5% to approximately 90% by weight.

14. The composition of claim 1 in which the lignans are present in an amount from about 1% to about 70% by weight.

15. The composition of claim 1 in which the saponins are present in an amount from about 5% to about 70% by weight.

16. The composition of claim 1 in which the phenolic acids are present in an amount from about 1% to about 70% by weight.

17. The composition of claim 1 in which the isoflavones are selected from the group consisting essentially of genistein, daidzein, glycitein, biochanin A, formononetin, and natural modifications thereof.

18. The composition of claim 1 in which the lignans are selected from the group of compounds possessing a 2,3-dibenzylbutane structure and consisting essentially of matairesinol, secoisolariciresinol, lariciresinol, isolariciresinol, nordihydroguaiaretic acid, pinoresinol, olivil, and precursors of enterolactone and enterodiol and natural modifications thereof.

19. The composition of claim 1 in which the saponins are selected from the group consisting essentially of icomatine, soyasapogenols A, B, C, D, E and F, soyasapoin, alfalfasapoin, ginsenoside fraction 3 and 4, medicagenic acid, hederagenin, glycyrrhizin, digitonin, quillaaja saponin, lucrenic acid, zahnic acid, and natural modifications of these compounds.

20. The composition of claim 1 in which the phenolic acids are selected from the group consisting essentially of chlorogenic acid, caffeic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, vanillic acid, coumaric acid, cinnamic acid, genistic acid, salicylic acid, hydroxy benzoic acid and hydroxy phenyl acetic acids and derivatives thereof.

21. The composition of claim 1 in which catechins are selected from the group consisting essentially of catechin, epicatechin, gallocatechin, and epigallocatechin.

22. The composition of claim 1 in which the plant matter is selected from one or more of the group consisting essentially of soy, red clover, kudzu, flax, alfalfa, tea, and cocoa.

23. The composition of claim 1 in which the plant matter is soy.

24. The composition of claim 23, in which the soy is selected from the group consisting of soybean, soy foods, soy molasses, soy whey, soy protein, and soy flour.

25. A product for oral delivery comprising a composition extracted from plant matter which is enriched in at least a first phytochemical and a second phytochemical selected from the group consisting of isoflavones, lignans, saponins, catechins and phenolic acids, said second phytochemical being a member of the group which is different from said first phytochemical.

26. The product of claim 25 wherein the form of the product is selected from the group consisting of tablets, capsules, pills, concentrates, powders, liquids, and added food ingredients.

27. The product of claim 26 comprising tablets comprising

- a. the plant matter composition; and
- b. a filler selected from the group consisting of sorbitol, lactose, cellulose and dicalcium phosphate.

28. The product of claim 27 additionally comprising a dietary supplemental nutrient selected from the group consisting of vitamins and minerals.

29. The oral delivery product of claim 27 wherein the product comprises between about 15% and about 25% by weight of the composition and between about 65% and about 85% by weight of the filler.

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- 30. The product of claim 28 wherein the product comprises
 - a. between about 15% and about 25% by weight of the composition;
 - b. between about 60% and about 84% by weight of the filler; and
 - c. between about 1% and about 25% by weight of the dietary supplemental nutrient.
- 31. The oral delivery product of claim 26 comprising capsules including
 - a. a predetermined dosage of the plant matter composition; and
 - b. a gelatin capsule.
- 32. The oral delivery product of claim 26 wherein the plant matter composition is extracted from plants selected from the group consisting of soy, red clover, kudzu, flax, alfalfa, tea, and cocoa.
- 33. The oral delivery product of claim 25 wherein the product comprises between about 10 milligrams and about 2000 milligrams of the plant matter composition.
- 34. The composition of claim 1 in which the selected phytochemicals are substantially in a native form.
- 35. The composition of claim 1 in which the isoflavones are substantially in a glycosylated form.
- 36. The composition of claim 1 which is in a form suitable for administering as a food supplement.
- 37. The composition of claim 1 which is in a form suitable for administering as a dietary supplement.
- 38. The composition of claim 1 in which the plant matter is tea.
- 39. The composition of claim 1 in which the plant matter is cocoa.
- 40. The composition of claim 1 in which the plant matter is flax.
- 41. The composition of claim 40 which consists of at least about 1% by weight lignans.
- 42. The composition of claim 40 which consists of at least about 50% by weight lignans.
- 43. A composition made by the process comprising the steps of:
 - a. extracting a defatted material from a group of vegetable matter consisting of protein, meal, whey, molasses, solubles and germs in a solution including an alcoholic solvent to produce a slurry;
 - b. filtering the slurry of step (a) to produce an alcoholic fraction;

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- c. evaporating said alcoholic fraction of step (b) to produce an aqueous fraction;
- d. ultrafiltering said aqueous fraction of step (c);
- e. feeding a permeate of step (d) through a resin column; and
- f. collecting an effluent from said column after said wash.
- 44. The composition of claim 43 and the further step of preparing said effluent of step (f) into a form which is suitable for administering orally, said form being taken from a group consisting of a concentrate, dried powder, capsule, pellet, and pill.
- 45. The composition of claim 41 wherein said dried powder is a bulk volume of material for further manufacture to provide individual dose sizes for said oral administration.
- 46. The composition of claim 43 wherein said vegetable matter is selected from a group consisting of soy, red clover, kudzu, flax, alfalfa, tea, and cocoa.
- 47. The composition of claim 43 wherein said vegetable matter is soy.
- 48. The composition of claim 43 wherein step (c) includes a step of diluting said aqueous fraction.
- 49. The composition of claim 43 and the added step of fractionating said effluent to select at least one of the group consisting essentially of isoflavones, lignans, saponins, catechins, and phenolic acid.
- 50. The composition of claim 43 and the added step of fractionating said effluent to select isoflavones.
- 51. The composition of claim 43 wherein the solution of step (a) is about 70% ethanol and the extraction is carried out at about 40° C.
- 52. The composition of claim 43 where the evaporation of step (c) is carried out under vacuum at about 70° C.
- 53. The composition of claim 2 in a form suitable for administering as a medication to treat an illness selected from a group consisting of breast cancer, colon cancer, bladder cancer, prostate cancer, urinary cancer, migraine headaches, dementia, alcohol dependency, reduction of bloodstream cholesterol, coronary heart disease, modulation of blood lipid profile, hot flashes, osteoporosis, sleep disorders, vaginal dryness, and premenstrual syndrome.
- 54. The product of claim 28 wherein the dietary supplemental nutrient is selected from the group consisting of dicalcium phosphate, magnesium stearate, calcium citrate, and calcium malate.

* * * * *

EXHIBIT 2



(12) United States Patent
Empie et al.

(10) Patent No.: **US 6,391,308 B1**
 (45) Date of Patent: ***May 21, 2002**

- (54) **METHOD OF PREPARING AND USING ISOFLAVONES FOR THE TREATMENT OF BLOOD RELATED ILLNESSES**
- (75) Inventors: Mark Empie, Forsyth; Eric Gugger, Latham, both of IL (US)
- (73) Assignee: Archer Daniels Midland Company, Decatur, IL (US)
- (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

- (21) Appl. No.: 09/615,239
 (22) Filed: Jul. 13, 2000

Related U.S. Application Data

- (82) Division of application No. 09/162,038, filed on Sep. 28, 1998, now Pat. No. 6,261,565, which is a continuation-in-part of application No. 09/035,588, filed on Mar. 5, 1998, now Pat. No. 6,033,714, which is a continuation-in-part of application No. 08/868,629, filed on Jun. 4, 1997, now Pat. No. 5,792,503, which is a division of application No. 08/614,545, filed on Mar. 13, 1996, now Pat. No. 5,702,752.
- (60) Provisional application No. 60/060,549, filed on Oct. 2, 1997.
- (51) Int. Cl.⁷ A01N 65/00
 (52) U.S. Cl. 424/195.1; 514/783; 514/26; 514/25; 514/27; 514/568; 514/717; 514/726; 514/824
 (58) Field of Search 424/195.1; 514/783; 514/26, 25, 27, 568, 717, 720, 824

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(57) ABSTRACT

A composition is prepared by extracting phytochemicals from plant matter and is administered to provide treatment for cardiovascular medical conditions, such as: excessive bloodstream cholesterol, the risk of heart disease, abnormal blood lipid profiles, and abnormal vascular effects. This composition is enriched preferably with two or more fractions of plant matter, namely: isoflavones, lignans, saponins, saponinins, catechins and phenolic acids. The isoflavones are selected from a group including malonyl, acetyl, glucoside and aglycone. Soy is the preferred source of these chemicals; however, other plants may also be used, such as wheat, psyllium, rice, oats, red clover, kudzu, alfalfa, flax, and cocoa. The composition is a dietary supplement in a concentrated form, preferably in an easy to consume form, for treatment of various cardiovascular conditions and various other related disorders.

16 Claims, No Drawings

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METHOD OF PREPARING AND USING ISOFLAVONES FOR THE TREATMENT OF BLOOD RELATED ILLNESSES

This is a division of Ser. No. 09/162,038, filed Sep. 28, 1998 (a formal application which replaced provisional application Ser. No. 60/060,549 filed Oct. 2, 1997) now U.S. Pat. No. 6,261,565, which in turn, is a continuation-in-part of Ser. No. 09/035,588, filed Mar. 5, 1998, now U.S. Pat. No. 6,033,714, which in turn, is a continuation-in-part of Ser. No. 08/868,629, filed Jun. 4, 1997, now U.S. Pat. No. 5,792,503, which in turn, is a division of Ser. No. 08/614,545, filed Mar. 13, 1996, now U.S. Pat. No. 5,702,752.

This invention relates to compositions extracted from vegetable matter and more particularly to phytochemicals, including saponinogenins and saponins, catechins, lignans, phenolic acids, and isoflavones, and especially those extracted from a family of plants including soy, flax, tea, and cocoa and methods of using these compositions as nutritional supplements or food additives.

BACKGROUND

As used herein, the term "isoflavone" includes malonyl, acetyl, glucoside, and aglycone forms of the isoflavones.

Currently there is great interest in treating blood related illnesses such as excessive levels of cholesterol, coronary disease, abnormal blood lipid profiles and vascular effects. It is thought that at least some of these illnesses may be either caused or exacerbated by foods, especially those of an animal origin. Therefore, it is thought that many of these illnesses are either preventable or treatable by a use of phytochemicals, especially isoflavones, as a source of supplemental hormones.

It is also thought that there are superior results when a plurality of such phytochemicals are consumed in combinations which are tailored to particular symptoms. A proper diet should contain the desired phytochemicals. However, a trouble is that many people do not have or do not like the proper kind of diet which provides the desirable effects. Hence, the problem is to furnish the necessary food values in some other form. Therefore, there is a need for a refining process which will enable a selection of specific phytochemical food values in combinations that are desirable for specific blood related illnesses.

Plant materials are known to contain a number of classes of organic low molecular weight compounds which exert bioactivity in various animals. Historically, these compounds have been considered to be somewhat non-nutritive, however, recent scientific evidence now suggests these compounds may play an important role in the maintenance of health, in chemoprevention, and in the mitigation of certain conditions or diseases associated with the circulation of sex hormones, including sleep disorders and vaginal dryness.

Edible plants normally contained in the diet, or materials used as herbal remedies/dietary supplements, may contain collections of structurally related compounds. These related substances are often unique in their amounts and distribution when compared among various plant sources. The most notable groups of compounds exhibiting bioactivity are known as flavonoids, isoflavones, saponins, lignans, alkaloids, catechins and phenolic acids.

Epidemiology studies relating diet to disease suggest that dietary components may predispose populations to reduced risk of certain diseases. For eastern populations consuming soy have reduced rates of breast, prostate and colon cancers

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and coronary heart disease, while populations in Finland have reduced rates of prostate cancer. Researchers are just now studying the specific compounds in the diet to understand the basis for the epidemiological observations.

Among the various plants consumed in the diet, several are rich sources of phytochemicals. Soy products contain high amounts of isoflavones and saponins. Unrefined diet grains include plants such as wheat, psyllium, rice, flax and oats that contain lignans. Cocoa contains catechins and phenolic acids. Certain non-dietary plants are also sources of the same chemical molecules, such as lignans and isoflavones in kudzu root or red clovers. Isoflavones and lignans act as weak estrogenic substances. Tea plants are also a rich source of phytochemicals, including catechins and phenolic acids.

Isoflavones can be used alone to treat or prevent breast cancer, prostate cancer, skin cancer, and colon cancer or as mechanism inhibitors. Isoflavones alone may also reduce or prevent various symptoms related to the onset and duration of menopause, including hot flashes and osteoporosis. Isoflavones alone may also be effective in certain cardiovascular applications, including heart disease, reducing cholesterol-lipid levels, modulating angiogenesis, and other vascular effects. Moreover, isoflavones alone have been implicated in reducing headaches, dementia, inflammation, and alcohol abuse, as well as immunomodulation.

Lignans alone have been implicated in preventing or treating breast cancer, prostate cancer and colon cancer as well as reducing hot flashes, preventing osteoporosis and showing antiviral potential. Lignans also have antimutagenic and fungicidal activity. A plant lignan, the catecholic nordihydro-guaiaretic acid, was a potent antioxidant once used by the food industry.

Saponins alone have been implicated in preventing or treating skin cancer, colon cancer, reducing serum cholesterol, and to immunomodulation and antiviral activity. Saponins also exhibit antioxidant effects and act as free radical scavengers.

Phenolic acids have shown antioxidant activity.

People who eat a high soy diet show reduction of many of these above-discussed symptoms. This suggests that ingesting a combination of these phytochemicals in a ratio such as that found in soy may result in an additive or synergistic effect. However, a high soy diet has some undesirable effects, including flatulence, undesirable taste, and hesitancy among Western consumers to change their lifestyle to incorporate soy in their diets, even for such benefits.

Isoflavones, which are heterocyclic phenols, are understood to include the soy compounds genistin, daidzin and glycitin, as well as biochanin A, equol, formononetin, and o-desmethylnangolensin and natural derivatives thereof. These compounds and their aglycone or de-methylated aglycone forms, such as genistein and daidzein, are believed to have similar activities once they are ingested. They are sometimes referred to as phytoestrogens.

Lignans are defined to be compounds possessing a 2,3-dibenzylbutane structure. They include matricresinol, secoisolariciresinol, lariciresinol, isolariciresinol, nordihydroguaiaretic acid, pinoresinol, olivil, other compounds which may be precursors of enterolactone and enterodiol and modifications thereof, including diglycosides.

Phenolic acids include p-hydrobenzoic acid, protocatechuic acid, and vanillic acid. Other phenolic acids are chlorogenic acid, caffeic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, coumaric acid, cinnamic acid, gentisic acid, salicylic acid, hydroxy benzoic acid and

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hydroxy phenyl acetic acids and derivatives. This list of phenolic acids should be understood to include the various isomers and derivatives found in the natural vegetable sources.

Catechins, or flavan-3-ols, include epigallocatechin, catechin, epicatechin and galocatechin.

Saponogenins are C-27 sterols in which the side chain has undergone metabolic changes to produce a spiroketal. Saponogenins occur naturally as saponins, which are 3-O-glycosides of the parent sterol or triterpene. Diglucoside from *Digitalis* is a saponin. Saponins include glucosides of saponogenin such as triterpenoids or steroids and saccharides such as glucose, arabinose, galactose or glucuronic acid. Typical examples of leguminous saponins are glycyrrhizin (glycyrrhetic acid-glucuronic acid) contained in *Glycyrrhiza glabra*, soysaponin contained in soybean and alfalfa-saponin contained in *Medicago sativa*. Saponins also include chemical entities identified as triterpene phenols such as tomatin, soysapogenols A, B, C, D, E and F, ginsenoside fraction 3 and 4, medicagenic acid, hederagenin, glycyrrhizin diglucoside, quillaja saponin, licoric acid and zahnic acid. The natural modifications of these compounds found in the vegetable source are also included in this identification.

A need exists for an improved composition consisting substantially of isoflavones, lignans, saponogenins, saponins, and/or phenolic acids which will produce improved results over any of these taken alone. Furthermore, a need exists for a composition in which the beneficial phytochemicals are enriched as compared to their original source. This permits individuals to conveniently consume such phytochemicals as a nutritional supplement or as a food additive.

SUMMARY OF THE INVENTION

An object of this invention is to provide a convenient way for individuals to consume isoflavones, lignans, saponins, catechins and/or phenolic acids, either as a nutritional supplement or as an ingredient in a more traditional type of food.

An other object of this invention is to provide an optimized extract composition of phytochemicals which is in sufficient concentration to be delivered in an easy to consume dosage such as a pill, tablet, capsule, liquid or ingredient in a food including health bars.

Yet another object of this invention is to prepare the phytochemical extract to be delivered as a topical application in a cream or lotion. In this form, the isoflavones, lignans, saponins, catechins and/or phenolic acids are dispersed and suspended in a suitable liquid or gel matrix to render a stable cream or lotion as the delivery vehicle.

A further object of this invention is to provide an extract concentrate which is closely similar in chemical composition to the chemical entities found in the natural plant source.

In keeping with this aspect of the invention, the isoflavones, lignans, saponins, catechins and/or phenolic acids are extracted from a suitable vegetable source to render a composition which is substantially more concentrated than the original material and by more than 5 times in one or more of the desired bioactive components.

This extract may be used alone or combined with one or more other plant extracts to produce the optimized composition. Further, this extract composition may be formulated with one or more other dietary nutrients, such as vitamins,

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minerals, amino acids, etc., to provide a nutritional supplement further optimized for a desired health effect. All these ingredients may be combined with necessary binders, excipients, preservatives, colors and the like known to those in the industry in order to produce a suitable tablet, capsule, pill, liquid, cream, powder or food ingredient including health bars.

These phytochemicals may be packaged and provided in final form by means known to the supplements and food ingredient industries. The materials are intended to provide health and well-being benefits.

DETAILED DESCRIPTION OF THE INVENTION

The improved composition is obtained by fractionating a plant source high in isoflavones, lignans and other phytochemicals such as defatted soybean flakes, soy molasses, soy whey, red clover, alfalfa, flax, cocoa, tea, or kudzu root. These may be fractionated alone or in combination with these other plants known to be high in the various isoflavones, lignans, saponins, catechins and phenolic acids. The fractionation results in substantially removing water, carbohydrates, proteins, and lipids from the source material. The fractionation method may be preferably that disclosed in U.S. Pat. No. 5,702,752 or U.S. Pat. No. 4,428,876, or an extraction using ethyl acetate or n-butanol may be used. U.S. Pat. Nos. 5,702,752, 6,017,556, 6,033,714 are assigned to the assignee of this invention.

Other extraction processes, which may be used alone or in combination, include differential solubility, distillation, solvent extraction, adsorptive means, differential molecular filtration and precipitation.

The preferred composition is an improvement over known commercial materials regarding the amount of phytochemicals per gram of substance and the amounts of different phytochemicals present which affect physiologic function.

These natural substances have been consumed in food sources for long periods of time and more closely relate to the substances consumed which provide the basis for the epidemiological evidence for health benefits. Additional benefits may be derived from improved physical properties relative to phytochemicals chemically modified from their original food source form.

The resulting composition is expected to comprise in a preferred form: between 5% and 95% isoflavones, between 0% and 70% lignans, and between 2% and 70% saponins and saponogenins. In a more preferred form, the composition will be extracted from soy. In another preferred form, the composition will contain a ratio of (saponins plus saponogenins) to isoflavones from 1:100 to 100:1, with the isoflavones consisting predominantly of naturally occurring derivatives of genistein and/or its precursor biochanin A and daidzein and/or its precursor formononetin, with a ratio of the genistein derivatives to daidzein derivatives from 100:1 to 1:100. Preferably, the isoflavones are predominantly glycosylated derivatives.

The composition's ratios may be readily varied by changing the plant source or by combining several plant sources for extraction. Thus, as further study shows which phytochemical combinations are more efficacious for certain health effects, the particular composition will also vary.

It is known that isoflavones, lignans, and saponins can be used advantageously to treat or prevent various cancers, including breast cancer, prostate cancer, skin cancer, and colon cancer.

It is believed that the improved composition will provide increased benefits in the form of chemoprevention. Recent experiments appear to confirm this belief.

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EXAMPLE 1

An initial series of animal studies was made to investigate the effects of dietary soy products on the growth of s.c. (SUBCUTANEOUS) implanted LNCaP in male SCID mice. A high isoflavone-containing soy protein isolate (SPI) (2.0 mg isoflavones/g SPI) is provided by Protein Technology International (St. Louis, Mo.). A soy phytochemicals extract, soy phytochemicals concentrate (SPC) which contains 28.5% total soy isoflavones and a diverse amount of other soy phytochemicals, is provided by Archer Daniels Midland Company (Decatur, Ill.). These materials were used to prepare six experimental diets. Table 1 shows ingredients of the diets.

Eight-week-old male SCID mice were s.c. injected on the right flank with 2×10^6 LNCaP cells from hosts, randomized into six groups (n=10) and assigned to one of the experimental diets. Food intake, body weight, and tumor volume were measured. At the termination of the experiment, blood samples were collected and serum separated for PSA analysis. An aliquot of tumor tissues was formalin-fixed, paraffin-embedded, and cut into 4 μ m sections for in situ histochemical detection of apoptotic cells, and immunohistochemical analyses of angiogenesis and proliferation. Another aliquot was prepared for cell lysates for western blot to determine the expression of apoptosis-related gene products.

Table 2 summarizes the effects of treatment of food intake, body weight, isoflavone intake and tumor volume. Soy products did not significantly alter food intake or body weight. Compared to casein-fed controls, tumor volumes from mice treated with SPI (20%), SPC (1.0%), and SPI and SPC (1.0%) were reduced by 12%, 28% (P<0.04), or 40% (P<0.005), respectively. Factorial analysis indicated that there was no significant effect of protein source on tumor growth. Linear regression analysis indicated that tumor volumes were inversely correlated to total dietary isoflavones (Tumor volume (cm³) = -0.008 + 2.121 x Isoflavones (mg), R² = 0.76, p < 0.03).

Table 3 shows the effects of SPC at 1.0% of the diet on apoptosis, proliferation, and angiogenesis of tumors from a pilot study. It indicates that dietary supplementation of soy phytochemicals inhibits the growth of LNCaP tumor in vivo by enhancing apoptosis and inhibiting proliferation of tumor cells. Its inhibitory effect on tumor angiogenesis is not significant which may be due to small sample size (n=2).

Results from in vitro study showed that genistein and soy phytochemical concentrate inhibited secretion of PSA by LNCaP cells into media. PSA concentrations were reduced 68% and 74% by 25 and 50 μ M of genistein treatment respectively, and 31% and 42% by 25 and 50 μ M of soy phytochemical concentrate treatment respectively.

TABLE 1

	uz,8/25 Ingredients of experimental diets (grams)					
	Diet 1 casein	Diet 2 SPI	Diet 3 Casein/ LSPC	Diet 4 SPI/ LSPC	Diet 5 Casein/ HSPC	Diet 6 SPI/ HSPC
SPI	0	200	0	200	0	200
Casein	200	0	200	0	200	0
DL-methionine	3	3	3	3	3	3
Core starch	150	150	150	150	150	150
Sucrose	500	500	500	500	500	500
Cellulose, BW200	50	50	50	50	50	50
Corn oil	50	50	50	50	50	50
Mineral Mix,	35	35	35	35	35	35

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TABLE 1-continued

	uz,8/25 Ingredients of experimental diets (grams)					
	Diet 1 casein	Diet 2 SPI	Diet 3 Casein/ LSPC	Diet 4 SPI/ LSPC	Diet 5 Casein/ HSPC	Diet 6 SPI/ HSPC
S10001 ² Vitamin Mix, V10001 ²	10	10	10	10	10	10
Choline Bitartrate Soy	2	2	2	2	2	2
	0	0	2	2	10	10
phytochemicals Total (g) (isoflavones, mg/g diet)	1000 0	1000 245	1002 341	1002 386	1010 705	1010 930

²AIN formulation with minor modification by DR. E. A. Ulfman, Research Diets, Inc.

TABLE 2

Treatment	Final body weight, total food intake, (total isoflavone intake, and tumor volume)			
	Body weight, grams/m	Food intake, grams/m	Total isoflavone	Tumor volume (cm ³)
Casein	22.4 ± 0.5 ¹	46.6 ± 3.1	0.00 ± 0.00	2.32 ± 0.31 ²
SPI	23.1 ± 0.7	46.2 ± 2.8	17.00 ± 5.37	2.06 ± 0.32
Casein/LSPC	21.4 ± 0.7	41.2 ± 3.4	14.03 ± .14	1.88 ± 0.35
SPI/LSPC	22.6 ± 0.6	50.1 ± 4.7	29.26 ± 2.76	1.66 ± 0.29*
Casein/HSPC	22.2 ± 0.7	44.8 ± 6.1	76.38 ± 10.49	1.04 ± 0.23*
SPI/HSPC	22.0 ± 0.6	47.5 ± 1.7	92.53 ± 7.82	1.39 ± 0.30**

¹Values are mean ± S.E. There are no significant differences of food intake or body weight among treatment groups.

²Compared with control group, SPI/LSPC, casein/HSPC, and SPI/HSPC had significantly small tumor volumes

*p > 0.04,
**p > 0.005

TABLE 3

Treatment	Effects of treatment on apoptotic index (AI, % TUNEL), proliferation index (PI, % PCNA Staining) and angiogenesis (microvessel density)		
	AI (% TUNEL)	PI (% PCNA)	Microvessel Density
Control (n = 2)	6.07 ± 0.88	60.1 ± 1.1	12.5 ± 3.8
Casein/HSPC (n = 2)	10.75 ± 0.54	51.7 ± 1.3	9.7 ± 0.7
P value	<0.02	<0.01	>0.05

Values are mean ± S.E.

In summary, preliminary results indicate that soy products inhibit the s.c. growth of LNCaP tumor in SCID mice, possibly via induction of apoptosis, and inhibition of angiogenesis and proliferation.

Isoflavones or lignans can alleviate menopausal-related symptoms such as hot flashes and osteoporosis as well as alleviate symptoms associated with menstruation. This is further believed to be due to their estrogenic activity. It is believed that the improved composition described here will alleviate these symptoms even more effectively.

Also, isoflavones positively affect various cardiovascular-related conditions, including heart disease, cholesterol (saponins also positively effect cholesterol), angiogenesis and other vascular effects. It is believed that the improved composition will produce results for these cardiovascular

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conditions at least as beneficial as those hitherto known and at a reduced cost.

As explained earlier, isoflavones, lignans, and saponins are known to individually positively affect various neurological and immunological symptoms. It is believed that the improved composition will result in alleviating neurological and immunological symptoms at least as well as those compounds hitherto known and at a reduced cost. Moreover, it would be expected that some synergism would arise out of the combination described herein.

The improved composition may be administered orally, parenterally, for instance, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation or by application of an aerosol spray to mucous membranes, or to the skin by an ointment or a cream.

Administering the improved composition may be done with any suitable carrier, in solid or liquid dosage form such as tablets, capsules, powders, soft gels, solutions, suspensions, emulsions, ointments, or creams. The improved composition may also be administered as a food supplement or as a food ingredient.

The amount of the improved composition administered will vary depending on the person, the mode of administration, and the desired result. An effective amount is expected to be 10 mg to 2000 mg/per dose.

EXAMPLE 2

Tablet Manufacture

The composition provided for in this patent may be used to prepare tablets or other dosage forms. An example of a capsule preparation is provided in Example 2. The higher the concentration of the active component, the easier it is to form a tablet or emulsion. This leads to an added ability to incorporate other dietary nutrients. An example would be to prepare a phytochemical tablet which incorporates calcium and vitamin E as a supplement to maintain bone health and/or reduce post menopausal symptoms such as hot flashes. In an example of this embodiment, a 600 mg dry compression tablet was prepared containing a total of 125 mg of isoflavones concentrate (50 mg isoflavones compound). Included in the tablet formulation was a source of calcium and magnesium.

Dry compression tablets were produced by first blending the following ingredients: 4 kg of the improved composition (39.83% isoflavones), 1.91 kg sorbitol, 0.095 kb magnesium

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stearate, and 13.11 kg dicalcium phosphate in a 120 quart capacity Hobart mixer. This blend of ingredients was then dry compressed at 1 ton pressure with a Stokes BB2 simple press into tablets having a total weight of 600 mg containing 125.53 mg of the improved composition and therefore 50 mg of total isoflavones.

Alternatively, a phytochemical concentrate may be provided in a single dosage form, a skin cream or as a food ingredient added to conventional food in amounts from 10 mg to 2000 mg/per dose, the purpose of which is to exert a positive effect on health and well being. These benefits include: cancer prevention, estrogen and sex hormone related maladies, inhibition of the pituitary-thyroid-gonadotrophic axis, alcohol dependency reduction, modulation of the cardiovascular, immune and nervous systems, antiviral effects and analgesic effects.

EXAMPLE 3

Two-piece gelatin capsules were produced by filling the receiving end of the empty size "0" capsules with 0.106 g of the improved composition (44.35% isoflavones) and closed with the capping end, providing a capsule containing 47.2 mg of total isoflavones.

EXAMPLE 4

A comparison between various sources of phytochemical preparations is given in Table 4. It is readily seen that the phytochemical components of the compositions of the "Isoflavone Concentrate" of this invention is substantially higher than the corresponding amounts in the natural vegetable materials. Notably, the amount of glycone isoflavones and saponins are over 100 times more concentrated compared to the food source and over twenty times more concentrated compared to the germ of the plant which naturally concentrates these phytochemicals. Comparison of the "Isoflavone Concentrate" of this invention to other concentrates shows that the isoflavone fraction predominates in these latter products, reducing the amount of other healthful phytochemicals. Additionally, the extraction methods of these other products employ techniques which modify the components, particularly the isoflavones, so that they are not identical to the substances found in the natural vegetable material (U.S. Pat. No. 5,637,562).

One version of the improved composition was compared to other previously described compositions. The results are shown in Table 4.

TABLE 4

Comparative Products to the Invention

Product Example	Isoflavone Glycosides in Product (mg/g)	Isoflavone Aglycones in Product (mg/g)	Genistein/ Daidzein Ratio	Lignans (mg/g)	Saponins (mg/g)	Flavonoid Acids (mg/g)
Improved composition	401.0	3.37	1.06 to 1	0.2	460.7	25.47
Soybean	1.798-2.776*	0.044*-0.075*	1.59-2.7	NA	0.9-3.2*	
Soy Flour (defatted)	1.969*	0.045*	3.58	0.0013		2.870*
Soy germ Product ²	24.32*	0.85*		NA	16.7-1.98*	NA
Product ³ patent (PTI)	NA	2.5-6.5*	0.5-3.5	NA	NA	NA
Product ⁴ patent (PTI)	NA	3.1-14.7*	0.433-3.08	NA	NA	NA
Product ⁵ patent (PTI)	NA	1.7-3.5*	0.66-2.85	NA	NA	NA

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TABLE 4-continued

Competitive Products to the Invention						
Product Example	Isoflavone Glycoside in Product (mg/g)	Isoflavone Aglycone in Product (mg/g)	Genistein/Daidzin Ratio	Lignans (mg/g)	Saponins (mg/g)	Phenolic Acids (mg/g)
PTI product ^b	NA	970	12.8	NA	NA	NA
PTI product ^b	NA	640	2.0	NA	NA	NA
Soy Molasses (dried)	27.6	0.1	1.37	NA	NA	5,788
Novogen ^d	0.0	550	1-1.7 to 1	NA	NA	NA

^aWang H. and Murphy P. A., *J. Agric. Food Chem.* 1994, 42, 1666-1673.^bAnderson R. L. and Wolk W. J., *J. Nutr.* 125:581S-582S, 1995^cSee A. and Morr C. W., *J. Agric. Food Chem.* 1984, 32, 530-533.^dSoy Life™ promotional literature^eWO 95/16530, PCT/US94/0697^fWO 95/10512, PCT/US94/0699^gWO 95/10525, PCT/US94/0696^hNCI paperⁱNovogen promotional literature

EXAMPLE 5

The improved composition, containing the glycoside forms of isoflavones, has as one aspect an improved solubility at body temperature over the previously described compositions containing the aglycone forms.

Separate solutions (0.02% in distilled water) were made for genistein, genistin, daidzein, daidzin, and isoflavone concentrate in volumetric flasks. Samples were then placed in a 37° C. water bath for 17 hours, followed by rapid filtration through a 0.2 micron syringe-type filter to remove particulates. Filtered samples were then analyzed for isoflavone concentration by HPLC. Results are tabulated as shown in Table 5.

TABLE 5

Differential Solubility of Isoflavone Glycoside vs. Aglycone				
Isoflavone sample	Genistein (ppm)	Genistin (ppm)	Daidzein (ppm)	Daidzin (ppm)
Genistein	7.42			
Genistin		33.89		
Daidzein			3.64	
Daidzin				49.51
Isoflavone Concentrate	0.492	30.075	0.672	37.69

The glycoside forms, genistin, and daidzin, are at least 4.57 and 13.32 fold higher in concentration at 37° C. than their corresponding aglycone forms, respectively.

The modifications made to the isoflavones are to remove the carbohydrate attached to the isoflavone moiety. This modification renders the isoflavone less soluble in water. Maintenance of the natural modification, glycosylation, enhances solubility. This fact is shown in the comparative solubility chart of Table 5. This chart shows that the genistin isoflavone is 4.6 times higher than and the daidzin isoflavone is 13.3 times higher than the corresponding non-glycosylated form. Higher solubility can lead to better bioavailability to intestinal organisms. The glycosylation does not inhibit absorption in the gut because the intestinal microflora convert the glycone form to the aglycone form before absorption occurs.

EXAMPLE 6

Extraction of Lignans from Flax

Lignans can be readily extracted from flax using the following method.

978 g of defatted flax meal (F1) was extracted with 2000 g of 85% ethanol at 40° C. for 10 minutes, forming a slurry. The resulting slurry was filtered and extraction was repeated twice with a total of 6000 g of ethanol.

The ethanolic fraction was then evaporated under vacuum at 70° C., resulting in an aqueous fraction of 1186 g. The aqueous fraction was combined with 1000 g of water and mixed.

The mixed sample was then ultra-filtered through a 5000 molecular weight cutoff membrane, resulting in a 767 g permeate fraction and a retentate fraction of 1283 g.

The retentate fraction was freeze-dried, resulting in a 27.84 g sample (F2).

The 767 g permeate fraction at 50° C. was fed to a 35 ml bed volume, XAD-4 resin column at a rate of 10 ml/min. The column effluent was collected and dried, resulting in a 14.8 g sample (F3). XAD-4 is a trademark for an absorbent resin, available from Rohm & Haas.

The column was then eluted with four bed volumes (140 ml) of 70% ethanol at 50° C. The eluted sample was evaporated under vacuum at 70° C. and dried, resulting in a 1.79 g sample (F4). The four fractions were then analyzed for their lignan content, measured as the concentration by weight of secoisolaricresinol. As Table 6 shows, this extraction method enriches lignan concentration.

TABLE 6

LIGNAN CONCENTRATIONS AS SECOISOLARICRESINOL				
FRACTION	F1	F2	F3	F4
SECO. CONC. (mg/g)	2.3	1.9	4.8	13.4
PHENOLIC ACID				

While the present invention has been disclosed in terms of the preferred embodiment in order to facilitate a better understanding of the invention, it should be appreciated that

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the invention can be embodied in various ways without departing from the principles of the invention. Therefore, the invention should be understood to include all possible embodiments, modifications, and equivalents to the described embodiment which do not depart from the principles of the invention as set out in the appended claims.

What is claimed is:

1. A composition for treatment of cardiovascular conditions, said composition being made from a plant matter in which the composition is a medicament enriched in at least two of the phytochemicals selected from the group consisting of isoflavones, lignans, saponins, saponinins, catechins and phenolic acids, said medicament having a therapeutic treatment amount of phytochemicals selected on a basis of a therapeutic treatment for a cardiovascular condition.

2. The composition of claim 1 in which the ratio of isoflavones to saponins is selected from the range of about 1:10 to about 10:1.

3. The composition of claim 1 in which the isoflavones are present in an amount from approximately 5% to approximately 90% by weight.

4. The composition of claim 1 in which said medicament is in a form of a product for oral delivery, said product form being selected from a group consisting of a concentrate, liquid, soft gel, dried powder, capsule, pellet, pill, suspensions, emulsions, and a food supplement including health bars.

5. The composition of claim 4 wherein the product comprises between about 15% and about 25% by weight of the medicament and between about 65% and about 85% by weight of a filler.

6. The composition of claim 4 wherein the product comprises:

- a. between about 15% and about 25% by weight of the medicament;
- b. between about 60% and about 84% by weight of a filler; and

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c. between about 1% and about 25% by weight of a dietary supplemental nutrient.

7. The composition of claim 1 in which the lignans are present in an amount from about 1% to about 70% by weight.

8. The composition of claim 1 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for a coronary heart disease.

9. The composition of claim 1 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for modulating a cardiovascular development.

10. The composition of claim 1 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for modulating blood lipid profiles.

11. The composition of claim 1 wherein said therapeutic amount of said medicament is selected on a basis of a treatment of vascular conditions.

12. The composition of claim 1 wherein said isoflavones are selected from a group consisting of malonyl, acetyl, glucoside, and aglycone.

13. The composition of claim 1 wherein said composition is a food in a form of a concentrated, easy to consume dosage.

14. A method of treating a human subject for a cardiovascular condition, said method comprising the step of administering to the human subject a therapeutically effective amount of a composition for treating said condition, said composition being extracted from plant matter which is enriched in at least two phytochemical fractions.

15. The method of claim 14 wherein said phytochemical fractions are selected from the group of isoflavones, lignans, saponins, saponinins, catechins, and phenolic acids.

16. The method of claim 15 wherein said isoflavones are selected from a group consisting of malonyl, acetyl, glucoside, and aglycone and said composition is in a form selected from a group consisting of a concentrate, liquid, soft gel, dried powder, capsule, pill, suspension, emulsions, and food supplements including health bars.

* * * * *

EXHIBIT 3



US006391309B1

(12) **United States Patent**
Empie et al.

(10) Patent No.: **US 6,391,309 B1**
 (45) Date of Patent: ***May 21, 2002**

(54) **METHOD OF PREPARING AND USING ISOFLAVONES FOR THE TREATMENT OF FEMALE SYMPTOMS**

WO WO 95/10512 4/1995
 WO WO 97/07811 3/1997
 WO WO 97/32593 9/1997

(75) Inventors: Mark Empie, Forsyth; Eric Gugger, Latham, both of IL (US)

(73) Assignee: Archer Daniels Midland Company, Decatur, IL (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 09/615,240

(22) Filed: Jul. 13, 2000

Related U.S. Application Data

(62) Division of application No. 09/162,038, filed on Sep. 28, 1998, now Pat. No. 6,261,565, which is a continuation-in-part of application No. 09/035,588, filed on Mar. 5, 1998, now Pat. No. 6,033,714, which is a continuation-in-part of application No. 08/868,629, filed on Jun. 4, 1997, now Pat. No. 5,792,503, which is a division of application No. 08/614,545, filed on Mar. 13, 1996, now Pat. No. 5,702,752
 (60) Provisional application No. 60/060,549, filed on Oct. 2, 1997.

(51) Int. Cl.⁷ A01N 65/00
 (52) U.S. Cl. 424/195.1; 514/783; 514/226; 514/25; 514/27; 514/568; 514/717; 514/726; 514/874; 514/899; 514/923
 (58) Field of Search 424/195.1; 514/783, 514/26, 25, 27, 568, 717, 726, 874, 899, 923

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(57) **ABSTRACT**

A composition is prepared by extracting phytochemicals from plant matter to form a dietary supplement for treatment of female symptoms. This composition is enriched preferably with two or more fractions of plant matter, namely: isoflavones, lignans, saponins, saponinins, catechins and phenolic acids. Soy is the preferred source of these chemicals; however, other plants may also be used, such as wheat, psyllium, rice, oats, red clover, kudzu, alfalfa, flax, and cocoa. The isoflavone may be any in a group including malonyl, acetyl, glucoside, and aglycone. The composition is in a concentrated form to be delivered in an easy to consume dosage, such as a pill, tablet, liquid, capsule, or a food supplement including health bars. Most commonly, these female symptoms are hot flashes, osteoporosis, sleep disorders, menopausal symptoms, vaginal dryness, premenstrual syndrome, or other problems relating to a reduction of sex hormones and to menstruation.

15 Claims, No Drawings

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METHOD OF PREPARING AND USING ISOFLAVONES FOR THE TREATMENT OF FEMALE SYMPTOMS

This is a division of Ser. No. 09/162,038, filed Sep. 28, 1998 (a formal application which replaced provisional application Ser. No. 60/069,549 filed Oct. 2, 1997) now U.S. Pat. No. 6,261,565, which in turn, is a continuation-in-part of Ser. No. 09/035,588, filed Mar. 5, 1998, now U.S. Pat. No. 6,033,714, which in turn, is a continuation-in-part of Ser. No. 08/868,629, filed Jun. 4, 1997, now Pat. No. 5,792,503, which in turn, is a division of Ser. No. 08/614,545, filed Mar. 13, 1996, now Pat. No. 5,702,752.

This invention relates to compositions extracted from vegetable matter and more particularly to phytochemicals including saponogenins and saponins, catechins, lignans, phenolic acids, and isoflavones, and especially those extracted from a family of plants including soy, flax, tea and cocoa and methods of using these compositions as nutritional supplements or food additives.

BACKGROUND

As used herein, the term "isoflavone" includes malonyl, acetyl, glucoside, and aglycone forms of the isoflavones.

Currently, western women suffer symptoms, especially in mid-life, which Asian women do not suffer. It is thought that diet accounts for these differences. It is further thought that many of these symptoms are either preventable or treatable by a use of phytochemicals, especially isoflavones, as a source of supplemental hormones, and particularly if such use begins before a woman's puberty. Men also benefit from such phytochemical treatments which address prostate cancer; however, the benefits do not appear to be so dependent upon when the use of isoflavones begins.

However, it is also thought that there are superior results when a plurality of such phytochemicals are consumed in combinations which are selected for particular symptoms. While a proper diet should contain the desired phytochemicals, a trouble is that many people do not have or do not like the proper kind of diet which provides the desirable effects. This is particularly true of the pre-pubescent years when the consumption of isoflavones is thought to have the most beneficial effect upon reducing the symptoms that females encounter later in life. Hence, the problem is to furnish the necessary food values in some other form which is more acceptable to the personal taste of people in the western world. Therefore, there is a need for a refining process which will enable a selection of specific isoflavones in the proper combinations.

Plant materials are known to contain a number of classes of organic low molecular weight compounds which exert bioactivity in various animals. Historically, these compounds have been considered to be somewhat non-nutritive however, recent scientific evidence now suggests these compounds may play an important role in the maintenance of health, in chemoprevention, and in the mitigation of certain conditions or diseases associated with the circulation of sex hormones, including sleep disorders and vaginal dryness.

Edible plants normally contained in the diet, or materials used as herbal remedies/dietary supplements, may contain collections of structurally related compounds. These related substances are often unique in their amounts and distribution when compared among various plant sources. The most notable groups of compounds exhibiting bioactivity are known as flavonoids, isoflavones, saponins, lignans, alkaloids, catechins and phenolic acids.

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Epidemiology studies relating diet to disease suggest that dietary components may predispose populations to reduced risk of certain diseases. Far eastern populations consuming soy have reduced rates of breast, prostate and colon cancers and coronary heart disease, while populations in Finland have reduced rates of prostate cancer. Researchers are just now studying the specific compounds in the diet to understand the basis for the epidemiological observations.

Among the various plants consumed in the diet, several are rich sources of phytochemicals. Soy products contain high amounts of isoflavones and saponins. Unrefined diet grains include plants such as wheat, psyllium, rice, flax and oats that contain lignans. Cocoa contains catechins and phenolic acids. Certain non-dietary plants are also sources of the same chemical molecules, such as lignans and isoflavones in kudzu root or red clovers. Isoflavones and lignans act as weak estrogenic substances. Tea plants are also a rich source of phytochemicals, including catechins and phenolic acids.

Isoflavones can be used alone to treat or prevent breast cancer, prostate cancer, skin cancer, and colon cancer or as mechanism inhibitors. Isoflavones alone may also reduce or prevent various symptoms related to the onset and duration of menopause, including hot flashes and osteoporosis. Isoflavones alone may also be effective in certain cardiovascular applications, including heart disease, reducing cholesterol-lipid levels, modulating angiogenesis, and other vascular effects. Moreover, isoflavones alone have been implicated in reducing headaches dementia, inflammation, and alcohol abuse, as well as immunomodulation.

Lignans alone have been implicated in preventing or treating breast cancer, prostate cancer and colon cancer as well as reducing hot flashes, preventing osteoporosis and showing antiviral potential. Lignans also have antimutagenic and fungicidal activity. A plant lignan (the catecholic nordihydro-guaiaretic acid, was a potent antioxidant once used by the food industry.

Saponins alone have been implicated in preventing or treating skin cancer, colon cancer, reducing serum cholesterol, and in immunomodulation and antiviral activity. Saponins also exhibit antioxidant effects and act as free radical scavengers.

Phenolic acids have shown antioxidant activity.

People who eat a high soy diet show reduction of many of these above-discussed symptoms. This suggests that ingesting a combination of these phytochemicals in a ratio such as that found in soy may result in an additive or synergistic effect. However, a high soy diet has some undesirable effects, including flatulences, undesirable taste, and hesitancy among Western consumers to change their lifestyle to incorporate soy in their diets, even for such benefits.

Isoflavones, which are heterocyclic phenols, are understood to include the soy compounds genistin, daidzin and glycitein, as well as biochanin A, equol, formononetin, and o-desmethylandrogenin and natural derivatives thereof. These compounds and their aglycone or de-methylated aglycone forms, such as genistein and daidzein, are believed to have similar activities once they are ingested. They are sometimes referred to as phyto-estrogens.

Lignans are defined to be compounds possessing a 2,3-dibenzylbutane structure. They include matairesinol, secoisolariciresinol, lariciresinol, isolariciresinol, nordihydroguaiaretic acid, pinoresinol, olivil, other compounds which may be precursors of enterolactone and enterodiol and modifications thereof, including diglucosides.

Phenolic acids include p-hydrobenzoic acid, protocatechuic acid and vanillic acid. Other phenolic acids are

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chlorogenic acid, caffeic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, coumaric acid, cinnamic acid, gentisic acid, salicylic acid, hydroxy benzoic acid and hydroxy phenyl acetic acids and derivatives. This list of phenolic acids should be understood to include the various isomers and derivatives found in the natural vegetable source.

Catechins, or flavan-3-ols, include epigallocatechin catechin, epicatechin and galocatechin.

Saponogenins are C-27 sterols in which the side chain has undergone metabolic changes to produce a spiroketal. Saponogenins occur naturally as saponins, which are 3-O-glycosides of the parent steroid or triterpene. Digitonin from *Digitalis* is a saponin. Saponins include glucosides of saponogenin such as triterpenoids or steroids and saccharides such as glucose, arabinose, galactose or glucuronic acid. Typical examples of leguminous saponins are glycyrrhizin (glycyrrhetic acid-glucuronic acid) contained in *Glycyrrhiza glabra*, soyasaponin contained in soybean and alfalfa-saponin contained in *Medicago sativa*. Saponins also include chemical entities identified as triterpene phenols such as tomatin, soyasapogenols A, B, C, D, E and F, ginsenoside fraction 3 and 4, medicagenic acid, hederagenin, glycyrrhizin digitonin, quillaja saponin, lucernic acid and zsaic acid. The natural modifications of these compounds found in the vegetable source are also included in this identification.

A need exists for an improved composition consisting substantially of isoflavones, lignans, saponogenins, saponins, and/or phenolic acids which will produce improved results over any of these taken alone. Furthermore, a need exists for a composition in which the beneficial phytochemicals are enriched as compared to their original source. This permits individuals to conveniently consume such phytochemicals as a nutritional supplement or as a food additive.

SUMMARY OF THE INVENTION

An object of this invention is to provide a convenient way for individuals to consume isoflavones, lignans, saponins, catechins and/or phenolic acids, either as a nutritional supplement or as an ingredient in a more traditional type of food.

Another object of this invention is to provide an optimized extract composition of phytochemicals which is in sufficient concentration to be delivered in an easy to consume dosage such as a pill, tablet, capsule, liquid or ingredient in a food including health bars.

Yet another object of this invention is to prepare the phytochemical extract to be delivered as a topical application in a cream or lotion. In this form, the isoflavones, lignans, saponins catechins and/or phenolic acids are dispersed and suspended in a suitable liquid or gel matrix to render a stable cream or lotion as the delivery vehicle.

A further object of this invention is to provide an extract concentrate which is closely similar in chemical composition to the chemical entities found in the natural plant source.

In keeping with this aspect of the invention, the isoflavones, lignans, saponins, catechins and/or phenolic acids are extracted from a suitable vegetable source to render a composition which is substantially more concentrated than the original material and by more than 5 times in one or more of the desired bioactive components.

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This extract may be used alone or combined with one or more other plant extracts to produce the optimized composition. Further, this extract composition may be formulated with one or more other dietary nutrients, such as vitamins, minerals, amino acids etc., to provide a nutritional supplement further optimized for a desired health effect. All these ingredients may be combined with necessary binders excipients, preservatives, colors and the like known to those in the industry in order to produce a suitable tablet, capsule, pill, liquid, cream, powder or food ingredient.

These phytochemicals may be packaged and provided in final form by means known to the supplements and food ingredient industries. The materials are intended to provide health and well-being benefits.

DETAILED DESCRIPTION OF THE INVENTION

The improved composition is obtained by fractionating a plant source high in isoflavones, lignans and other phytochemicals such as defatted soybean flakes, soy molasses, soy whey, red clover, alfalfa, flax, cocoa, tea, or kudzu root. These may be fractionated alone or in combination with these other plants known to be high in the various isoflavones, lignans, saponins, catechins and phenolic acids. The fractionation results in substantially removing water, carbohydrates, proteins and lipids from the source material. The fractionation method may be preferably that disclosed in U.S. Pat. Nos. 5,702,752; 6,017,555; 6,033,714; or U.S. Pat. No. 4,428,876, or an extraction using ethyl acetate or n-butanol may be used U.S. Pat. Nos. 5,702,752; 6,017,555; 6,033,714 are assigned to the assignee of this invention.

Other extraction processes, which may be used alone or in combination, include differential solubility, distillation, solvent extraction, adsorptive means, differential molecular filtration and precipitation.

The preferred composition is an improvement over known commercial materials regarding the amount of phytochemicals per gram of substance and the amounts of different phytochemicals present which affect physiologic function.

These natural substances have been consumed in food sources for long periods of time and more closely relate to the substances consumed which provide the basis for the epidemiological evidence for health benefits. Additional benefits may be derived from improved physical properties relative to phytochemicals chemically modified from their original food source form.

The resulting composition is expected to comprise in a preferred form: between 5% and 95% isoflavones, between 0% and 70% lignans, and between 2% and 70% saponins and saponogenins. In a more preferred form, the composition will be extracted from soy. In another preferred form, the composition will contain a ratio of (saponins plus saponogenins) to isoflavones from 1:100 to 100:1, with the isoflavones consisting predominantly of naturally occurring derivatives of genistein and/or its precursor biochanin A and daidzein and/or its precursor formononetin, with a ratio of the genistein derivatives to daidzein derivatives from 100:1 to 1:100. Preferably, the isoflavones are predominantly glycosylated derivatives.

The composition's ratios may be readily varied by changing the plant source or by combining several plant sources for extraction. Thus, as further study shows which phytochemical combinations are more efficacious for certain health effects, the particular composition will also vary.

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It is known that isoflavones, lignans, and saponins can be used advantageously to treat or prevent various cancers, including breast cancer, prostate cancer, skin cancer, and colon cancer.

It is believed that the improved composition will provide increased benefits in the form of chemoprevention. Recent experiments appear to confirm this belief.

EXAMPLE 1

An initial series of animal studies was made to investigate the effects of dietary soy products on the growth of s.c. (SUBCUTANEOUS) implanted LNCaP in male SCID mice. A high isoflavone-containing soy protein isolate (SPI) (2.0 mg isoflavones/g SPI) is provided by Protein Technology International (St. Louis, Mo.) A soy phytochemicals extract soy phytochemicals concentrate (SPC) which contains 28.5% total soy isoflavones and a diverse amount of other soy phytochemicals, is provided by Archer Daniels Midland Company (Decatur, IL). These materials were used to prepare six experimental diets. Table 1 shows ingredients of the diets.

Eight-week-old male SCID mice were s.c. injected on the right flank with 2×10^6 LNCaP cells from hosts, randomized into six groups ($n=10$) and assigned to one of the experimental diets. Food intake, body weight, and tumor volume were measured. At the termination of the experiment, blood samples were collected and serum separated for PSA analysis. An aliquot of tumor tissues was formalin-fixed, paraffin-embedded, and cut into $4 \mu\text{m}$ sections for in situ histochemi-

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cal detection of apoptotic cells, and immunohistochemical analyses of angiogenesis and proliferation. Another aliquot was prepared for cell lysate for western blot to determine the expression of apoptosis-related gene products.

Table 2 summarizes the effects of treatment on food intake, body weight, isoflavone intake and tumor volume. Soy products did not significantly alter food intake or body weight. Compared to casein-fed controls, tumor volumes from mice treated with SPI (20%), SPC (1.0%), and SPI and SPC (1.0%) were reduced by 12%, 28% ($P < 0.04$), or 40% ($P < 0.005$), respectively. Factorial analysis indicated that there was no significant effect of protein source on tumor growth. Linear regression analysis indicated that tumor volumes were inversely correlated to total dietary isoflavones (Tumor volume (cm^3) = $-0.008 + 3.121 \times \text{isoflavones}$ (mg), $R^2 = 0.76$, $p < 0.03$).

Table 3 shows the effects of SPC at 1.0% of the diet on apoptosis, proliferation, and angiogenesis of tumors from a pilot study. It indicates that dietary supplementation of soy phytochemicals inhibits the growth of LNCaP tumor *in vivo* by enhancing apoptosis and inhibiting proliferation of tumor cells. Its inhibitory effect on tumor angiogenesis is not significant which may be due to small sample size ($n=2$).

Results from *in vitro* study showed that genistein and soy phytochemical concentrate inhibited secretion of PSA by LNCaP cells into media. PSA concentrations were reduced 68% and 74% by 25 and 50 μM of genistein treatment respectively, and 31% and 42% by 25 and 50 μM of soy phytochemical concentrate treatment respectively.

TABLE 1

	Ingredients of experimental diets (grams)					
	Diet 1 Casein	Diet 2 SPI	Diet 3 Casein/LSPC	Diet 4 SPI/LSPC	Diet 5 Casein/HSPC	Diet 6 SPI/HSPC
SPI	0	200	0	200	0	200
Casein	200	0	200	0	200	0
DL-methionine	3	3	3	3	3	3
Corn starch	150	150	150	150	150	150
Sucrose	500	500	500	500	500	500
Cellulose, BW200	50	50	50	50	50	50
Corn oil	50	50	50	50	50	50
Mineral Mix, S10001 ¹	35	35	35	35	35	35
Vitamin Mix, V10001 ¹	10	10	10	10	10	10
Choline Bitartrate	2	2	2	2	2	2
Soy phytochemicals	0	0	2	2	10	10
Total (g)	1000	1000	1003	1002	1010	1010
(Isoflavones, mg/kg diet)	0	245	341	586	705	950

¹AIN formulation with minor modification by Dr. E. A. Ulmann, Research Diets, Inc.

TABLE 2

Treatment	Final body weight, total food intake, total isoflavone intake, and tumor volume			
	Body weight	Food intake g/m/m	Total isoflavone	Tumor volume (cm^3)
Casein	22.4 ± 0.5^1	46.6 ± 3.1	0.00 ± 0.00	2.32 ± 0.31^2
SPI	23.1 ± 0.7	46.2 ± 2.8	17.00 ± 6.97	2.06 ± 0.32
Casein/LSPC	21.4 ± 0.7	41.2 ± 3.4	$14.03 \pm .14$	1.86 ± 0.35
SPI/LSPC	22.6 ± 0.6	50.1 ± 4.7	29.36 ± 2.76	$1.65 \pm 0.29^*$
Casein/HSPC	22.2 ± 0.7	44.8 ± 6.1	76.38 ± 10.40	$1.64 \pm 0.22^*$
SPI/HSPC	23.0 ± 0.6	47.5 ± 1.7	92.53 ± 3.22	$1.39 \pm 0.30^{**}$

¹Values are means \pm SE. There are no significant differences of food intake or body weight among treatment groups.

²Compared with control group, SPI/LSPC, casein/HSPC, and SPI/HSPC had significantly smaller tumor volumes.

* $p < 0.05$.

** $p < 0.005$.

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TABLE 3

Effects of treatment on apoptotic index (AI, % TUNEL), proliferation index (PI, % PCNA staining) and angiogenesis (microvessel density)

Treatment	AI (% TUNEL)	PI (% PCNA)	Microvessel Density
Control (n = 2)	6.97 ± 0.88	69.1 ± 1.1	12.5 ± 3.8
Casein/MSFC (n = 2)	10.75 ± 0.94	51.7 ± 1.3	9.7 ± 0.7
P value	<0.02	<0.01	>0.05

Values are means ± SE.

In summary, preliminary results indicate that soy products inhibit the *s.c.* growth of LNCaP tumor in SCID mice, possibly via induction of apoptosis, and inhibition of angiogenesis and proliferation.

Isoflavones or lignans can alleviate menopausal-related symptoms such as hot flashes and osteoporosis as well as alleviate symptoms associated with menstruation. This is further believed to be due to their estrogenic activity. It is believed that the improved composition described here will alleviate these symptoms even more effectively.

Also, isoflavones positively affect various cardiovascular-related conditions, including heart disease, cholesterol (saponins also positively affect cholesterol), angiogenesis and other vascular effects. It is believed that the improved composition will produce results for these cardiovascular conditions at least as beneficial as those hitherto known and at a reduced cost.

As explained earlier, isoflavones, lignans, and saponins are known to individually positively affect various neurological and immunological symptoms. It is believed that the improved composition will result in alleviating neurological and immunological symptoms at least as well as those compounds hitherto known and at a reduced cost. Moreover, it would be expected that some synergism would arise out of the combination described herein.

The improved composition may be administered orally, parenterally, for instance, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation or by application of an aerosol spray to mucous membranes, or to the skin by an ointment or a cream.

Administering the improved composition may be done with any suitable carrier, in solid or liquid dosage form such as tablets, capsules, powders, soft gels, solutions, suspensions, emulsions, ointments, or creams. The improved composition may also be administered as a food supplement or as a food ingredient.

The amount of the improved composition administered will vary depending on the person, the mode of administration, and the desired result. An effective amount is expected to be 10 mg to 2000 mg/per dose.

EXAMPLE 2

Tablet Manufacture

The composition provided for in this patent may be used to prepare tablets or other dosage forms. An example of a capsule preparation is provided in Example 2. The higher the concentration of the active component, the easier it is to form a tablet or emulsion. This leads to an added ability to incorporate other dietary nutrients. An example would be to

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prepare a phytochemical tablet which incorporates calcium and vitamin E as a supplement to maintain bone health and/or reduce post menopausal symptoms such as hot flashes. In an example of this embodiment, a 600 mg dry compression tablet was prepared containing a total of 125 mg of isoflavones concentrate (50 mg isoflavone compound). Included in the tablet formulation was a source of calcium and magnesium.

Dry compression tablets were produced by first blending the following ingredients: 4 kg of the improved composition (39.83% isoflavones), 1.91 kg sorbitol, 0.095 kg magnesium stearate, and 13.11 kg dicalcium phosphata in a 120 quart capacity Hobart mixer. This blend of ingredients was then dry compressed at 1 ton pressure with a Stokes BB2 simple press into tablets having a total weight of 600 mg containing 125.53 mg of the improved composition and therefore 50 mg of total isoflavones.

Alternatively, a phytochemical concentrate may be provided in a single dosage form, a skin cream or as a food ingredient added to conventional food in amounts from 10 mg to 2000 mg/per dose, the purpose of which is to exert a positive effect on health and well being. These benefits include: cancer prevention, estrogen and sex hormone related maladies inhibition of the pituitary-thyroid-gonadotrophic axis, alcohol dependency reduction, modulation of the cardiovascular, immune and nervous systems, antiviral effects and analgesic effects.

EXAMPLE 3

Two-piece gelatin capsules were produced by filling the receiving end of the empty size "0" capsules with 0.106 g of the improved composition (44.35% isoflavones) and closed with the capping end, providing a capsule containing 47.2 mg of total isoflavones.

EXAMPLE 4

A comparison between various sources of phytochemical preparations is given in Table 4. It is readily seen that the phytochemical components of the composition of the "Isoflavone Concentrate" of this invention is substantially higher than the corresponding amounts in the natural vegetable materials. Notably, the amount of glycone isoflavones and saponins are over 100 times more concentrated compared to the food source and over twenty times more concentrated compared to the germ of the plant which naturally concentrates these phytochemicals. Comparison of the "Isoflavone Concentrate" of this invention to other concentrates shows that the isoflavone fraction predominates in these latter products, reducing the amount of other healthful phytochemicals. Additionally, the extraction methods of these other products employ techniques which modify the components, particularly the isoflavones, so that they are not identical to the substances found in the natural vegetable material (U.S. Pat. No. 5,637,562).

One version of the improved composition was compared to other previously described compositions. The results are shown in Table 4

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TABLE 4

Comparative Products to the Invention						
Product Example	Isoflavone Glycosides in Product (mg/g)	Isoflavone Aglycones in Product (mg/g)	Genistin/Daidzin Ratio	Lignans (mg/g)	Saponins (mg/g)	Phenolic Acids (mg/g)
Improved composition	407.0	3.37	1.06 to 1	0.2	400.7	25.47
Soybean Soy Flour (defatted)	1.748-2.776 ^a	0.004 ^a -0.075 ^a	1.59-2.7	NA	0.9-3.2 ^b	2.870 ^c
Soy germ Product ^d	24.32 ^d	0.85 ^d	2.5-6.5 ^e	NA	16.7-192 ^b	NA
Product ^f patent (PTT)	NA	5.1-14.7 ^f	0.433-3.48	NA	NA	NA
Product ^g patent (PTT)	NA	1.7-3.5 ^g	0.66-2.86	NA	NA	NA
Product ^h patent (PTT)	NA	970	12.8	NA	NA	NA
Product ⁱ patent (PTT)	NA	640	2.0	NA	NA	NA
Soy Molasses (dried)	27.6	0.1	1.37	NA	NA	5.738
Novogen ¹	0.0	350	1-1.7 to 1	NA	NA	NA

^aWang H. and Murphy P. A., J. Agric. Food Chem 1994, 42, 1666-1673.^bAnderson R. L. and Wolf W. J., J. Nutr 123:581S-586S, 1995^cSee A. and Morr C. V., J. Agric. Food Chem 1984, 32, 530-533.^dSoy Life™ promotional literature^eWO 95/10530, PCT/US94/10697^fWO 95/10532, PCT/US94/10699^gWO 95/10532, PCT/US94/10696^hNCI paperⁱNovogen promotional literature

EXAMPLE 5

The improved composition, containing the glycoside forms of isoflavones, has as one aspect an improved solubility at body temperature over the previously described compositions containing the aglycoside forms.

Separate solutions (0.02% in distilled water) were made for genistein, genistin, daidzein, daidzin and isoflavone concentrate in volumetric flasks. Samples were then placed in a 37° C. water bath for 17 hours, followed by rapid filtration through a 0.2 micron syringe-type filter to remove particulates. Filtered samples were then analyzed for isoflavone concentration by HPLC. Results are tabulated as shown in Table 5.

TABLE 5

Differential Solubility of Isoflavone Glycosides vs. Aglycones				
Isoflavone sample	Genistein (ppm)	Genistin (ppm)	Daidzein (ppm)	Daidzin (ppm)
Genistein	7.42			
Genistin		33.89		
Daidzein			3.64	
Daidzin				48.51
Isoflavone Concentrate	0.492	30.075	0.672	37.09

The glycoside forms, genistin and daidzin, are at least 4.57 and 13.32 fold higher in concentration at 37° C. than their corresponding aglycone forms, respectively.

The modifications made to the isoflavones are to remove the carbohydrate attached to the isoflavone moiety. This modification renders the isoflavone less soluble in water. Maintenance of the natural modification, glycosylation, enhances solubility. This fact is shown in the comparative

solubility chart of Table 5. This chart shows that the genistin isoflavone is 4.6 times higher and the daidzin isoflavone is 13.3 times higher than the corresponding non-glycosylated form. Higher solubility can lead to better bioavailability to intestinal organisms. The glycosylation does not inhibit absorption in the gut because the intestinal microflora convert the glycone form to the aglycone form before absorption occurs.

EXAMPLE 6

Extraction of Lignans From Flax

Lignans can be readily extracted from flax using this following method.

978 g of defatted flax meal (F1) was extracted with 2000 g of 85% ethanol at 40° C. for 10 minutes, forming a slurry. The resulting slurry was filtered and extraction was repeated twice with a total of 6000 g of ethanol.

The ethanolic fraction was then evaporated under vacuum at 70° C., resulting in an aqueous fraction of 1186 g. The aqueous fraction was combined with 1,000 g of water and mixed.

The mixed sample was then ultra-filtered through a 5000 molecular weight cutoff membrane, resulting in a 767 g permeate fraction and a retentate fraction of 1283 g.

The retentate fraction was freeze-dried, resulting in a 27.84 g sample (F2).

The 767 g permeate fraction at 30° C. was fed to a 35 ml bed volume, XAD-4 resin column at a rate of 10 ml/min. The column effluent was collected and dried, resulting in a 14.8 g sample (F3) XAD-4 is a trademark for an absorbent resin available from Rohm & Haas.

The column was then eluted with four bed volumes (140 ml) of 70% ethanol at 50° C. The eluent sample was evaporated under vacuum at 70° C. and dried, resulting in a

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1.79 g sample (F4). The four fractions were then analyzed for their lignan content, measured as the concentration by weight of secoisolariciresinol. As Table 6 shows, this extraction method enriches lignan concentration.

TABLE 6

LIGNAN CONCENTRATIONS AS SECOISOLARICIRESINOL				
FRACTION	F1	F2	F3	F4
SECO. CONC. (mg/g)	2.3	1.9	4.8	13.4
TRABVOLIC ACID				

While the present invention has been disclosed in terms of the preferred embodiment in order to facilitate a better understanding of the invention, it should be appreciated that the invention can be embodied in various ways without departing from the principles of the invention. Therefore, the invention should be understood to include all possible embodiments, modifications and equivalents to the described embodiment which do not depart from the principles of the inventions as set out in the appended claims.

What is claimed is:

1. A composition for treatment of medical conditions resulting from circulation of female sex hormones, said composition being made from a plant matter in which the composition is a medicament enriched in at least two of the phytochemicals selected from the group consisting of isoflavones, lignans, saponins, saponinins, catechins and phenolic acids, said medicament having an effective amount of phytochemicals selected on a basis of a therapeutic treatment for female disorders selected from a group consisting of hot flashes, osteoporosis, sleep disorders, menopausal symptoms, vaginal dryness, premenstrual syndrome, and menstrual problems.

2. The composition of claim 1 in which the ratio of isoflavones to saponins is selected from the range of about 1:10 to about 10:1.

3. The composition of claim 1 in which the isoflavones are present in an amount from approximately 5% to approximately 90% by weight.

4. The composition of claim 1 in which said medicament is in a form of a product for oral delivery, said product form being selected from a group consisting of a concentrate, liquid, dried powder, soft gels, solutions, suspensions,

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emulsions, ointments, capsule, pellet, pill, and a food supplement including health bars.

5. The composition of claim 4 wherein the product comprises between about 15% and about 25% by weight of the medicament and between about 65% and about 85% by weight of a filler.

6. The composition of claim 4 wherein the product comprises:

- a. between about 15% and about 25% by weight of the medicament;
- b. between about 60% and about 84% by weight of a filler; and
- c. between about 1% and about 25% by weight of a dietary supplemental nutrient.

7. The composition of claim 1 in which the lignans are present in an amount from about 1% to about 70% by weight.

8. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for problems related to hot flashes.

9. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for menstrual problems.

10. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for menopausal symptoms.

11. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for osteoporosis.

12. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for vaginal dryness.

13. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for sleep disorders.

14. The composition of claim 7 wherein said therapeutic amount of said medicament is selected on a basis of a treatment for premenstrual syndrome.

15. The composition of claim 1 wherein said isoflavone is selected from a group consisting of malonyl, acetyl, glucoside, and aglycone.

* * * * *

EXHIBIT 4



US006518319B1

(12) **United States Patent**
Empie et al.

(10) **Patent No.:** US 6,518,319 B1
(45) **Date of Patent:** Feb. 11, 2003

(54) **METHOD OF PREPARING AND USING COMPOSITIONS EXTRACTED FROM VEGETABLE MATTER FOR THE TREATMENT OF FEMALE SYMPTOMS**

WO WO 95/03816 2/1995
WO WO 95/10512 4/1995
WO WO 97/07811 3/1997
WO WO 97/32593 9/1997
WO WO 99/58124 * 11/1999

(75) **Inventors:** Mark Empie, Forsyth, IL (US); Eric Guggen, Lubam, IL (US)

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(73) **Assignee:** Archer Daniels Midland Company, Decatur, IL (US)

Naik et al., "An in vitro and in vivo study of antitumor effects of Genistein on hormone refractory prostate cancer." *Anticancer Research*, 14:2617-2620 (1994).*

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

Messina et al., "Soy intake and cancer risk: A review of the in vitro and in vivo data." *Nutrition and cancer*, 21(2), 113-31, 1994.*

(21) **Appl. No.:** 10/136,150

Barnes et al. "Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer." *J. Cellular Biochem, Supp.* 22:181-187, 1995.*

(22) **Filed:** May 1, 2002

Coward et al. "Genistein, Daidzein, and their B-glycoside conjugates: Antitumor isoflavones in soybean foods from American and Asian diets." *J. Agric. Food Chem.*, 41, 1962-1967, 1993.*

Related U.S. Application Data

(62) Division of application No. 09/615,240, filed on Jul. 13, 2000, now Pat. No. 6,391,309, which is a division of application No. 09/162,038, filed on Sep. 28, 1998, now Pat. No. 6,261,565, which is a continuation-in-part of application No. 09/035,588, filed on Mar. 5, 1998, now Pat. No. 6,033,714, which is a continuation-in-part of application No. 08/868,829, filed on Jun. 4, 1997, now Pat. No. 5,792,503, which is a division of application No. 08/614,545, filed on Mar. 13, 1996, now Pat. No. 5,702,752.

Keung et al. "Therapeutic lessons from traditional oriental medicine to contemporary occidental pharmacology." *Abstract; EXS*, 71, 371-81, 1994.*

(50) Provisional application No. 60/060,549, filed on Oct. 2, 1997.

Article: No. XP-002096529 "Saponins as Anticarcinogens", "The Journal of Nutrition", by Rao, A. V. and Sung, M. K. 1995.

(51) **Int. Cl. 7** A01N 65/00
(52) **U.S. Cl.** 514/783; 514/25; 514/26; 514/27; 514/568; 514/717; 514/726; 514/874; 514/899; 514/823; 424/195.1

English translation of relevant material re Patent Appln. Laid Open Nos. (1) Hei 02-261365; (2) Hei 01-312965; (3) Hei 04-132845; (4) Hei 08-73369; (5) Hei 07-147903; (6) Hei 04-506402; (7) Hei 10-179100; and (8) Hei 11-12172. Article: No. XP-002096530 "Dietary Soybean Protein Prevents Bone Loss in an Ovariectomized Rat Model of Osteoporosis", "The Journal of Nutrition", Arjmandi, H. H. et al. 1996.

(58) **Field of Search** 514/783, 26, 25, 514/27, 568, 717, 726, 874, 899, 923; 424/195.1

European Patent Office, Patent Abstract of Japan Publication No. 07304655 dated Nov. 21, 1995 for JP 59085265.

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Primary Examiner—Samuel Harris
Assistant Examiner—Devesh Khare
(74) **Attorney, Agent, or Firm**—Michael Best & Friedrich, LLC

(57) **ABSTRACT**

A composition is prepared by extracting phytochemicals from plant matter. This composition is enriched preferably in isoflavones, lignans, saponins, catechins and phenolic acids. Soy is the preferred source of these chemicals; however, other plants may also be used, such as red clover, kudzu, flax, and cocoa. The composition is a dietary supplement for treatment of various cancers, pre- and post-menstrual syndromes, and various other disorders.

41 Claims, No Drawings

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**METHOD OF PREPARING AND USING
COMPOSITIONS EXTRACTED FROM
VEGETABLE MATTER FOR THE
TREATMENT OF FEMALE SYMPTOMS**

This is a division of Ser. No. 09/615,240, filed Jul. 13, 2000 now U.S. Pat. No. 6,391,309, which, in turn, is a division of Ser. No. 09/162,038, filed Sep. 28, 1998 (a formal application which replaced provisional application Ser. No. 60/060,549 filed Oct. 2, 1997), now U.S. Pat. No. 6,261,565, which, in turn, is a continuation-in-part of Ser. No. 09/035,588 filed Mar. 5, 1998, now U.S. Pat. No. 6,033,714, which, in turn, is a continuation-in-part of Ser. No. 08/868,629, filed Jun. 4, 1997, now U.S. Pat. No. 5,792,503, which, in turn, is a division of Ser. No. 08/614, 545, filed Mar. 13, 1996, now U.S. Pat. No. 5,702,752.

This invention relates to compositions extracted from vegetable matter and more particularly to phytochemicals, including saponogenins and saponins, catechins, lignans, phenolic acids, catechins and isoflavones, and especially those extracted from a family of plants including soy, flax, tea, and cocoa and methods of using these compositions as nutritional supplements or food additives.

BACKGROUND

Plant materials are known to contain a number of classes of organic low molecular weight compounds which exert bioactivity in various animals. Historically, these compounds have been considered to be somewhat non-nutritive; however, recent scientific evidence now suggests these compounds may play an important role in the maintenance of health, in chemoprevention, and in the mitigation of certain conditions or diseases associated with the circulation of sex hormones, including sleep disorders and vaginal dryness.

Edible plants normally contained in the diet, or materials used as herbal remedies/dietary supplements, may contain collections of structurally related compounds. These related substances are often unique in their amounts and distribution when compared among various plant sources. The most notable groups of compounds exhibiting bioactivity are known as flavonoids, isoflavones, saponins, lignans, alkaloids, catechins and phenolic acids.

Epidemiology studies relating diet to disease suggest that dietary components may predispose populations to reduced risk of certain diseases. Far eastern populations consuming soy have reduced rates of breast, prostate and colon cancers and coronary heart disease, while populations in Finland have reduced rates of prostate cancer. Researchers are just now studying the specific compounds in the diet to understand the basis for the epidemiological observations.

Among the various plants consumed in the diet, several are rich sources of phytochemicals. Soy products contain high amounts of isoflavones and saponins. Unrefined diet grains include plants such as wheat, psyllium, rice, flax and oats that contain lignans. Cocoa contains catechins and phenolic acids. Certain non-dietary plants are also sources of the same chemical molecules, such as lignans and isoflavones in kudzu root or red clovers. Isoflavones and lignans act as weak estrogenic substances. Tea plants are also a rich source of phytochemicals, including catechins and phenolic acids.

Isoflavones can be used alone to treat or prevent breast cancer, prostate cancer, skin cancer, and colon cancer or as mechanism inhibitors. Isoflavones alone may also reduce or prevent various symptoms related to the onset and duration

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of menopause, including hot flashes and osteoporosis. Isoflavones alone may also be effective in certain cardiovascular applications, including heart disease, reducing cholesterol-lipid levels, modulating angiogenesis, and other vascular effects. Moreover, isoflavones alone have been implicated in reducing headaches, dementia, inflammation, and alcohol abuse, as well as immunomodulation.

Lignans alone have been implicated in preventing or treating breast cancer, prostate cancer and colon cancer as well as reducing hot flashes, preventing osteoporosis and showing antiviral potential. Lignans also have antimutagenic and fungicidal activity. A plant lignan, the catecholic nordihydro-gallic acid, was a potent antioxidant once used by the food industry.

Saponins alone have been implicated in preventing or treating skin cancer, colon cancer, reducing serum cholesterol, and in immunomodulation and antiviral activity. Saponins also exhibit antioxidant effects and act as free radical scavengers.

Phenolic acids have shown antioxidant activity.

People who eat a high soy diet show reduction of many of these above-discussed symptoms. This suggests that ingesting a combination of these phytochemicals in a ratio such as that found in soy may result in an additive or synergistic effect. However, a high soy diet has some undesirable effects, including flatulence, undesirable taste, and hesitancy among Western consumers to change their lifestyle to incorporate soy in their diets, even for such benefits.

Isoflavones, which are heterocyclic phenols, are understood to include the soy compounds genistin, daidzin and glycitein, as well as biochanin A, equol, formononetin, and o-desmethylangolensin and natural derivatives thereof. These compounds and their aglycone or de-methylated aglycone forms, such as genistein and daidzein, are believed to have similar activities once they are ingested. They are sometimes referred to as phyto-estrogens.

Lignans are defined to be compounds possessing a 2,3-dibenzylbutane structure. They include matairesinol, secoisolariciresinol, lariciresinol, isolariciresinol, nordihydrogallic acid, pinoresinol, olivil, other compounds which may be precursors of enterolactone and enterodiol and modifications thereof, including diglycosides.

Phenolic acids include p-hydrobenzoic acid, protocatechuic acid, and vanillic acid. Other phenolic acids are chlorogenic acid, caffeic acid, ferulic acid, gallic acid, sinapic acid, syringic acid, coumaric acid, cinnamic acid, gentisic acid, salicylic acid, hydroxy benzoic acid and hydroxy phenyl acetic acids and derivatives. This list of phenolic acids should be understood to include the various isomers and derivatives found in the natural vegetable source.

Catechins, or flavan-3-ols, include epigallocatechin, catechin, epicatechin and gallocatechin.

Saponogenins are C-27 sterols in which the side chain has undergone metabolic changes to produce a spiroketal. Saponogenins occur naturally as saponins, which are 3-O-glycosides of the parent steroid or triterpenes. Digitalin from *Digitalis* is a saponin. Saponins include glycosides of saponin such as triterpenoids or steroids and saccharides such as glucose, arabinose, galactose or glucuronic acid. Typical examples of leguminous saponins are glycyrrhizin (glycyrrhetic acid+glucuronic acid) contained in *Glycyrrhiza glabra*, soyasaponin contained in soybean and afflasaponin contained in *Medicago sativa*. Saponins also include chemical entities identified as triterpene phenols such as tomatine, soyasapogenols A, B, C, D, E and F,

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ginsengoside fraction 3 and 4, medicagenic acid, hederagenin, glycyrrhizin diglucuronide, quillaja saponin, luteoic acid and zahaic acid. The natural modifications of these compounds found in the vegetable source are also included in this identification.

A need exists for an improved composition consisting substantially of isoflavones, lignans, saponogenins, saponins, and/or phenolic acids which will produce improved results over any of these taken alone. Furthermore, a need exists for a composition in which the beneficial phytochemicals are enriched as compared to their original source. This permits individuals to conveniently consume such phytochemicals as a nutritional supplement or as a food additive.

SUMMARY OF THE INVENTION

An object of this invention is to provide a convenient way for individuals to consume isoflavones, lignans, saponins, catechins and/or phenolic acids, either as a nutritional supplement or as an ingredient in a more traditional type of food.

Another object of this invention is to provide an optimized extract composition of phytochemicals which is in sufficient concentration to be delivered in an easy to consume dosage such as a pill, tablet, capsule, liquid or ingredient in a food.

Yet another object of this invention is to prepare the phytochemical extract to be delivered as a topical application in a cream or lotion. In this form, the isoflavones, lignans, saponins, catechins and/or phenolic acids are dispersed and suspended in a suitable liquid or gel matrix to render a stable cream or lotion as the delivery vehicle.

A further object of this invention is to provide an extract concentrate which is closely similar in chemical composition to the chemical entities found in the natural plant source.

In keeping with this aspect of the invention, the isoflavones, lignans, saponins, catechins and/or phenolic acids are extracted from a suitable vegetable source to render a composition which is substantially more concentrated than the original material and by more than 5 times in one or more of the desired bioactive components.

This extract may be used alone or combined with one or more other plant extracts to produce the optimized composition. Further, this extract composition may be formulated with one or more other dietary nutrients, such as vitamins, minerals, amino acids, etc., to provide a nutritional supplement further optimized for a desired health effect. All these ingredients may be combined with necessary binders, excipients, preservatives, colors and the like known to those in the industry in order to produce a suitable tablet, capsule, pill, liquid, cream, powder or food ingredient.

These phytochemicals may be packaged and provided in final form by means known to the supplement and food ingredient industries. The materials are intended to provide health and well-being benefits.

DETAILED DESCRIPTION OF THE INVENTION

The improved composition is obtained by fractionating a plant source high in isoflavones, lignans and other phytochemicals such as defatted soybean flakes, soy molasses, soy whey, red clover, alfalfa, flax, cocoa, tea, or kudzu root. These may be fractionated alone or in combination with these other plants known to be high in the various

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isoflavones, lignans, saponins, catechins and phenolic acids. The fractionation results in substantially removing water, carbohydrates, proteins, and lipids from the source material. The fractionation method may be preferably that disclosed in co-pending U.S. Pat. No. 5,702,752 or U.S. Pat. No. 4,428,876, or an extraction using ethyl acetate or n-butanol may be used. U.S. Pat. No. 5,702,752 is assigned to the assignee of this invention.

Other extraction processes, which may be used alone or in combination, include differential solubility, distillation, solvent extraction, adsorptive means, differential molecular filtration and precipitation.

The preferred composition is an improvement over known commercial materials regarding the amount of phytochemicals per gram of substance and the amounts of different phytochemicals present which affect physiologic function.

These natural substances have been consumed in food sources for long periods of time and more closely relate to the substances consumed which provide the basis for the epidemiological evidence for health benefits. Additional benefits may be derived from improved physical properties relative to phytochemicals chemically modified from their original food source form.

The resulting composition is expected to comprise in a preferred form: between 5% and 95% isoflavones, between 0% and 70% lignans, and between 2% and 70% saponins and saponogenins. In a more preferred form, the composition will be extracted from soy. In another preferred form, the composition will contain a ratio of (saponins plus saponogenins) to isoflavones from 1:100 to 100:1, with the isoflavones consisting predominantly of naturally occurring derivatives of genistein and/or its precursor biochanin A and daidzein and/or its precursor formononetin, with a ratio of the genistein derivatives to daidzein derivatives from 100:1 to 1:100. Preferably, the isoflavones are predominantly glycosylated derivatives.

The composition's ratios may be readily varied by changing the plant source or by combining several plant sources for extraction. Thus, as further study shows which phytochemical combinations are more efficacious for certain health effects, the particular composition will also vary.

It is known that isoflavones, lignans, and saponins can be used advantageously to treat or prevent various cancers, including breast cancer, prostate cancer, skin cancer, and colon cancer.

It is believed that the improved composition will provide increased benefits in the form of chemoprevention. Recent experiments appear to confirm this belief.

EXAMPLE 1

An initial series of animal studies was made to investigate the effects of dietary soy products on the growth of s.c. (SUBCUTANEOUS) implanted LNCaP in male SCID mice. A high isoflavone-containing soy protein isolate (SPI) (2.0 mg isoflavones/g SPI) is provided by Protein Technology International (St. Louis, Mo.) A soy phytochemicals extract, soy phytochemicals concentrate (SPC) which contains 28.5% total soy isoflavones and a diverse amount of other soy phytochemicals, is provided by Archer Daniels Midland Company (Decatur, Ill.) These materials were used to prepare six experimental diets. Table 1 shows ingredients of the diets.

Eight-week-old male SCID mice were s.c. injected on the right flank with 2×10^4 LNCaP cells from hosts, randomized into six groups (n=10) and assigned to one of the experi-

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mental diets. Food intake, body weight, and tumor volume were measured. At the termination of the experiment, blood samples were collected and serum separated for PSA analysis. An aliquot of tumor tissues was formalin-fixed, paraffin-embedded, and cut into 4 μm sections for in situ histochemical detection of apoptotic cells, and immunohistochemical analyses of angiogenesis and proliferation. Another aliquot was prepared for cell lysates for western blot to determine the expression of apoptosis-related gene products.

Table 2 summarizes the effects of treatment on food intake, body weight, isoflavone intake and tumor volume. Soy products did not significantly alter food intake or body weight. Compared to casein-fed controls, tumor volumes from mice treated with SPI (20%), SPC (1.0%), and SPI and SPC (1.0%) were reduced by 12%, 28% (P<0.04), or 40% (P<0.005), respectively. Factorial analysis indicated that there was no significant effect of protein source on tumor growth. Linear regression analysis indicated that tumor volumes were inversely correlated to total dietary isoflavones (Tumor volume (cm³) = -0.008 + 2.121 × Isoflavones (mg), R² = 0.76, p < 0.03).

Table 3 shows the effects of SPC at 1.0% of the diet on apoptosis, proliferation, and angiogenesis of tumors from a pilot study. It indicates that dietary supplementation of soy phytochemicals inhibits the growth of LNCaP tumor in vivo by enhancing apoptosis and inhibiting proliferation of tumor cells. Its inhibitory effect on tumor angiogenesis is not significant which may be due to small sample size (n=2).

Results from in vitro study showed that genistein and soy phytochemical concentrate inhibited secretion of PSA by LNCaP cells into media. PSA concentrations were reduced 68% and 74% by 25 and 50 μM of genistein treatment respectively, and 31% and 42% by 25 and 50 μM of soy phytochemical concentrate treatment respectively.

TABLE 1

	Ingredients of experimental diets (grams)					
	Diet 1 casein	Diet 2 SPI	Diet 3 Casein/LSPC	Diet 4 SPU/LSPC	Diet 5 Casein/HSPC	Diet 6 SPU/HSPC
SPI	0	200	0	200	0	200
Casein	200	0	200	0	200	0
DL-methionine	3	3	3	3	3	3
Corn starch	150	150	150	150	150	150
Sucrose	500	500	500	500	500	500
Cellulose, BW200	50	50	50	50	50	50
Corn oil	50	50	50	50	50	50
Mineral Mix, SD0001 ¹	35	35	35	35	35	35
Vitamin Mix, V10001 ¹	10	10	10	10	10	10
Choline Bitartrate	2	2	2	2	2	2
Soy phytochemicals	0	0	2	2	10	10
Total (g)	1000	1000	1002	1002	1010	1010
Isoflavones, mg/kg diet	0	245	341	585	705	950

¹AIN formulation with minor modification by Dr E A Ulman, Research Diets, Inc

TABLE 2

	Final body weight, total food intake, total isoflavone intake, and tumor volume			
Treatment	Body weight	Food intake	Total isoflavone	Tumor volume
	grams/m	grams/m	micrograms	(cm ³)
Casein	22.4 ± 0.5 ¹	46.6 ± 3.1	0.00 ± 0.00	2.32 ± 0.31 ²
SPI	23.2 ± 0.7	46.2 ± 2.8	17.00 ± 6.37	2.06 ± 0.32

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TABLE 2-continued

	Final body weight, total food intake, total isoflavone intake, and tumor volume			
Treatment	Body weight	Food intake	Total isoflavone	Tumor volume
	grams/m	grams/m	micrograms	(cm ³)
Casein/LSPC	21.4 ± 0.7	41.2 ± 3.4	14.03 ± 3.4	1.88 ± 0.35
SPI/LSPC	22.6 ± 0.6	50.1 ± 4.7	29.36 ± 2.76	1.66 ± 0.28*
Casein/HSPC	22.2 ± 0.7	44.8 ± 6.3	76.38 ± 10.40	1.64 ± 0.22*
HSPC				
SPI/HSPC	23.0 ± 0.6	47.5 ± 1.7	92.53 ± 3.22	1.39 ± 0.30**

¹Values are means ± SE. There are no significant differences of food intake or body weight among treatment groups.
²Compared with control group, SPI/LSPC, casein/HSPC, and SPI/HSPC had significantly smaller tumor volumes (*p < 0.05, **p < 0.005).

TABLE 3

	Effects of treatment on apoptotic index (AI, % TUNEL), proliferation index (PI, % PCNA Staining) and angiogenesis (microvessel density)		
Treatment	AI (% TUNEL)	PI (% PCNA)	Microvessel Density
Control (n = 2)	6.07 ± 0.88	60.1 ± 3.1	12.5 ± 3.6
Casein/HSPC (n = 2)	10.75 ± 0.54	51.7 ± 3.3	9.7 ± 0.7
P value	<0.02	<0.01	>0.05

Values are means ± SE.

In summary, preliminary results indicate that soy products inhibit the s.c. growth of LNCaP tumor in SCID mice,

possibly via induction of apoptosis, and inhibition of angiogenesis and proliferation.

Isoflavones or lignans can alleviate menopausal-related symptoms such as hot flashes and osteoporosis as well as alleviate symptoms associated with menstruation. This is further believed to be due to their estrogenic activity. It is believed that the improved composition described here will alleviate these symptoms even more effectively.

Also, isoflavones positively affect various cardiovascular-related conditions, including heart disease, cholesterol (seponins also positively affect cholesterol), angiogenesis

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and other vascular effects. It is believed that the improved composition will produce results for these cardiovascular conditions at least as beneficial as those hitherto known and at a reduced cost.

As explained earlier, isoflavones, lignans, and saponins are known to individually positively affect various neurological and immunological symptoms. It is believed that the improved composition will result in alleviating neurological and immunological symptoms at least as well as those compounds hitherto known and at a reduced cost. Moreover, it would be expected that some synergism would arise out of the combination described herein.

The improved composition may be administered orally, parenterally, for instance, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation or by application of an aerosol spray to mucous membranes, or to the skin by an ointment or a cream.

Administering the improved composition may be done with any suitable carrier, in solid or liquid dosage form such as tablets, capsules, powders, soft gels, solutions, suspensions, emulsions, ointments, or creams. The improved composition may also be administered as a food supplement or as a food ingredient.

The amount of the improved composition administered will vary depending on the person, the mode of administration, and the desired result. An effective amount is expected to be 10 mg to 2000 mg/per dose.

EXAMPLE 2

Tablet Manufacture

The composition provided for in this patent may be used to prepare tablets or other dosage forms. An example of a capsule preparation is provided in Example 1. The higher the concentration of the active component, the easier it is to form a tablet or emulsion. This leads to an added ability to incorporate other dietary nutrients. An example would be to prepare a phytochemical tablet which incorporates calcium and vitamin E as a supplement to maintain bone health and/or reduce post menopausal symptoms such as hot flashes. In an example of this embodiment, a 600 mg dry compression tablet was prepared containing a total of 125 mg of isoflavones concentrate (50 mg isoflavone compound). Included in the tablet formulation was a source of calcium and magnesium.

Dry compression tablets were produced by first blending the following ingredients: 4 kg of the improved composition (39.83% isoflavones), 1.91 kg sorbitol, 0.095 kg magnesium

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stearate, and 13.11 kg dicalcium phosphate in a 120 quart capacity Hobart mixer. This blend of ingredients was then dry compressed at 1 ton pressure with a Stokes BE2 simple press into tablets having a total weight of 600 mg containing 125.53 mg of the improved composition and therefore 50 mg of total isoflavones.

Alternatively, a phytochemical concentrate may be provided in a single dosage form, a skin cream or as a food ingredient added to conventional food in amounts from 10 mg to 2000 mg/per dose, the purpose of which is to exert a positive effect on health and well being. These benefits include: cancer prevention, estrogen and sex hormone related maladies, inhibition of the pituitary-thyroid-gonadotrophic axis, alcohol dependency reduction, modulation of the cardiovascular, immune and nervous systems, antiviral effects and analgesic effects.

EXAMPLE 3

Two-piece gelatin capsules were produced by filling the receiving end of the empty size "0" capsules with 0.106 g of the improved composition (44.35% isoflavones) and closed with the capping end, providing a capsule containing 47.2 mg of total isoflavones.

EXAMPLE 4

A comparison between various sources of phytochemical preparations is given in Table 4. It is readily seen that the phytochemical components of the composition of the "Isoflavone Concentrate" of this invention is substantially higher than the corresponding amounts in the natural vegetable materials. Notably, the amount of glycone isoflavones and saponins are over 100 times more concentrated compared to the food source and over twenty times more concentrated compared to the germ of the plant which naturally concentrates these phytochemicals. Comparison of the "Isoflavone Concentrate" of this invention to other concentrates shows that the isoflavone fraction predominates in these latter products, reducing the amount of other healthful phytochemicals. Additionally, the extraction methods of these other products employ techniques which modify the components, particularly the isoflavones, so that they are not identical to the substances found in the natural vegetable material (U.S. Pat. No. 5,637,562).

One version of the improved composition was compared to other previously described compositions. The results are shown in Table 4.

TABLE 4

Product Example	Comparative Product to the Invention					
	Isoflavone Glycosides in Product (mg/g)	Isoflavone Aglycones in Product (mg/g)	Genistein/Daidzein Ratio	Lignans (mg/g)	Saponins (mg/g)	Phenolic Acids (mg/g)
Improved composition	401.0	3.37	1.06 to 1	0.2	460.7	25.47
Soybean	1,748-2,776 ^a	0.044 ^a -0.075	1.59-2.7	NA	0.9-3.2 ^a	2.870 ^a
Soy Flour (defatted)	1.969 ^a	0.045 ^a	3.58	0.0013		
Soy germ Product ^a	24.32 ^d	0.65 ^d		NA	16.7-1.98 ^b	
Product ^a (P1)	NA	2.5-6.5 ^a	0.5-3.5	NA	NA	NA
Product ^a (P2)	NA	5.1-14.7 ^e	0.43-3.48	NA	NA	NA
Product ^a (P3)	NA	1.7-3.5 ^a	0.66-2.86	NA	NA	NA
Product ^a (P4)	NA	970	12.8	NA	NA	NA

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TABLE 4-continued

Comparative Polyphenol to the Invention						
Product Example	Isoflavone Glycosides in Product (mg/g)	Isoflavone Aglycones in Product (mg/g)	Genistein/Daidzein Ratio	Lignans (mg/g)	Saponins (mg/g)	Phenolic Acids (mg/g)
product ^a						
FTI	NA	640	2.0	NA	NA	NA
product ^b						
Soy Molasses (dried)	27.6	0.1	1.87	NA	NA	8.788
Novogen ^c	0.0	550	3-1.7 to 1	NA	NA	NA

^aWong H and Murphy P A, *J Agric Food Chem* 1994, 42, 1666-1673^bAnderson R L and Wolf W J, *J Nutr* 125: S81S-S88S, 1995^cSee A and Mori C V, *J Agric Food Chem* 1984, 32, 530-533^dSoy Life™ promotional literature

*WO 95/10530, PCT/US94/10597

*WO 95/10512, PCT/US94/10699

*WO 95/10529, PCT/US94/10606

^eNCI paper^fNovogen promotional literature

EXAMPLE 5

The improved composition, containing the glycoside forms of isoflavones, has as one aspect an improved solubility at body temperature over the previously described compositions containing the aglycoside forms.

Separate solutions (0.02% in distilled water) were made for genistein, genistin, daidzein, daidzin, and isoflavone concentrate in volumetric flasks. Samples were then placed in a 37° C. water bath for 17 hours, followed by rapid filtration through a 0.2 micron syringe-type filter to remove particulates. Filtered samples were then analyzed for isoflavone concentration by HPLC. Results are tabulated as shown in Table 5.

TABLE 5

Differential Solubility of Isoflavone Glycosides vs. Aglycones				
Isoflavone sample	Genistein (ppm)	Genistin (ppm)	Daidzein (ppm)	Daidzin (ppm)
Genistein	7.82			
Genistin		33.69		
Daidzein			3.64	
Daidzin				48.51
Isoflavone Concentrate	0.492	30.075	0.672	37.69

The glycoside forms, genistin and daidzin, are at least 4.57 and 13.32 fold higher in concentration at 37° C. than their corresponding aglycone forms, respectively.

The modifications made to the isoflavones are to remove the carbohydrate attached to the isoflavone moiety. This modification renders the isoflavone less soluble in water. Maintenance of the natural modification, glycosylation, enhances solubility. This fact is shown in the comparative solubility chart of Table 5. This chart shows that the genistin isoflavone is 4.6 times higher and the daidzin isoflavone is 13.3 times higher than the corresponding non-glycosylated form. Higher solubility can lead to better bioavailability to intestinal organisms. The glycosylation does not inhibit absorption in the gut because the intestinal microflora convert the glycone form to the aglycone form before absorption occurs.

EXAMPLE 6

Extraction of Lignans from Flax

Lignans can be readily extracted from flax using this following method.

978 g of defatted flax meal (F1) was extracted with 2000 g of 85% ethanol at 40° C. for 10 minutes, forming a slurry. The resulting slurry was filtered and extraction was repeated twice with a total of 6000 g of ethanol.

The ethanolic fraction was then evaporated under vacuum at 70° C., resulting in an aqueous fraction of 1186 g. The aqueous fraction was combined with 1000 g of water and mixed.

The mixed sample was then ultra-filtered through a 5000 molecular weight cutoff membrane, resulting in a 767 g permeate fraction and a retentate fraction of 1283 g.

The retentate fraction was freeze-dried, resulting in a 27.84 g sample (F2).

The 767 g permeate fraction at 50° C. was fed to a 35 ml bed volume, XAD-4 resin column at a rate of 10 ml/min. The column effluent was collected and dried, resulting in a 14.8 g sample (F3). XAD-4 is a trademark for an absorbent resin, available from Rohm & Haas.

The column was then eluted with four bed volumes (140 ml) of 70% ethanol at 50° C. The eluent sample was evaporated under vacuum at 70° C. and dried, resulting in a 1.79 g sample (F4). The four fractions were then analyzed for their lignan content, measured as the concentration by weight of secoisolariciresinol. As Table 6 shows, this extraction method enriches lignan concentration.

TABLE 6

LIGNAN CONCENTRATIONS AS SECOISOLARICRESINOL				
FRACTION	F1	F2	F3	F4
SECO. CONC. (mg/g)	2.3	1.9	4.8	13.4
PHENOLIC ACID				

While the present invention has been disclosed in terms of the preferred embodiment in order to facilitate a better understanding of the invention, it should be appreciated that the invention can be embodied in various ways without departing from the principles of the invention. Therefore the invention should be understood to include all possible embodiments, modifications, and equivalents to the described embodiment which do not depart from the principles of the inventions as set out in the appended claims.

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What is claimed is:

1. A method of treating female symptoms selected from the group consisting of hot flashes, osteoporosis, sleep disorders, menopausal symptoms, vaginal dryness, premenstrual syndromes and related problems relating to a circulation of sex hormones, comprising administering to a person an amount therapeutically effective in treating said female symptom of a composition comprising at least two phytochemical fractions selected from the group consisting of isoflavones, lignans, saponins and saponogenins, catechins, and phenolic acids, said at least two phytochemical fractions being different from each other.
2. The method of claim 1 wherein said phytochemical fractions are extracted from plant matter selected from the group consisting of soy, wheat, psyllium, rice, oats, red clover, kudzu, flax, alfalfa, tea, and cocoa.
3. The method of claim 2 in which said plant matter is soy.
4. The method of claim 3, in which said soy is selected from the group consisting of soybean, soy foods, soy molasses, soy whey, soy protein, and soy flour.
5. The method of claim 1 wherein said treatment is for hot flashes.
6. The method of claim 1 wherein said treatment is for osteoporosis.
7. The method of claim 6 wherein said phytochemical fractions are in a form for topical application.
8. The method of claim 1 wherein said phytochemical fractions are mixed with a carrier selected from a group consisting of skin cream or skin lotion.
9. The method of claim 1 wherein said treatment is for vaginal dryness.
10. The method of claim 1 wherein said treatment is for sleep disorders.
11. The method of claim 1 wherein said treatment is for menopausal symptoms.
12. The method of claim 1 wherein said treatment is for pre-menstrual syndromes.
13. The method of claim 1 wherein said therapeutically effective amount is selected to inhibit the pituitary-thyroid-gonadotrophic axis.
14. The method of claim 1 wherein said composition is formed into a product for oral delivery comprising between about 10 milligrams and about 2000 milligrams of said composition.
15. The method of claim 1 wherein said composition is formed into a product for oral delivery selected from a group consisting of:
 - a. a predetermined dosage of said composition;
 - b. a gelatin capsule;
 - c. a liquid; and
 - d. a food supplement composition in a concentrated, easy to consume dosage.
16. The method of claim 1 including the further step of forming said composition into a product selected from a group consisting of a concentrate, dried powder, liquid, capsule, pellet, pill, a food supplement, health bar, intranasal, and spray.
17. The method of claim 1 in which said composition is comprised of at least 70% by weight of said phytochemical fractions.
18. The method of claim 1 in which at least one of the selected phytochemical fractions comprises at least 10% by weight of said composition.
19. The method of claim 1 in which said composition is comprised of at least 80% by weight of said phytochemical fractions.
20. The method of claim 1 in which said composition is comprised of at least 90% by weight of said phytochemical fractions.

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21. The method of claim 1 wherein said phytochemical fractions are in the ranges of about 5%–95% isoflavones; 0%–70% lignans; 2%–70% saponins and saponogenins.
22. The method of claim 21 wherein the ratio of saponins plus saponogenins to isoflavones in the composition is in the range of about 1:100 to about 100:1.
23. The method of claim 1 in which the ratio of isoflavones to lignans in the composition is in the range of about 1000:1 to about 1:50.
24. The method of claim 1 wherein said first phytochemical fraction is isoflavones and said second phytochemical fraction is saponins and saponogenins.
25. The method of claim 24 wherein said isoflavone fraction is selected from a group consisting of malonyl, acetyl, glucoside, and aglycone forms.
26. The method of claim 1 wherein said therapeutically effective amount is selected to promote circulation of sex hormones.
27. A method for the treatment of female conditions comprising the steps of: providing a composition made from plant matter in which said composition is formed by selecting at least two phytochemical fractions selected on a basis of effectiveness in treating said female condition from the group consisting of isoflavones, lignans, saponins and saponogenins, catechins, and phenolic acids, said at least two phytochemical fractions being different members of said group; and combining said at least two phytochemical fractions; and administering a therapeutically effective amount of said composition to a person.
28. The method of claim 27 in which said female condition is selected from the group consisting of hot flashes, osteoporosis, sleep disorders, menopausal symptoms, vaginal dryness, pre-menstrual syndrome, and menstrual problems.
29. The method of claim 27 in which said plant matter is selected from one or more of the group consisting essentially of soy, wheat, psyllium, rice, oats, red clover, kudzu, alfalfa, tea, and cocoa.
30. The method of claim 29 in which said plant matter is soy.
31. The method of claim 30 in which said soy is selected from the group consisting of soybean, soy foods, soy molasses, soy whey, soy protein, and soy flour.
32. The method of claim 27 further comprising a dietary supplemental nutrient selected from the group consisting of vitamins and minerals.
33. The method of claim 32 wherein said dietary supplemental nutrient is selected from the group consisting of dicalcium phosphate, magnesium stearate, calcium citrate, calcium malate, and other calcium sources.
34. The method of claim 27 wherein said composition is formed into a product for oral delivery selected from the group consisting of tablets, capsules, pills, concentrates, powders, liquids, and added food ingredients.
35. The method of claim 34 wherein said product is a tablet comprising
 - a. said composition; and
 - b. a filler selected from the group consisting of sorbitol, lactose, cellulose and dicalcium phosphate.
36. The method of claim 35 wherein said tablet comprises between about 15% and about 25% by weight of said composition and between about 65% and about 85% by weight of said filler.
37. The method of claim 35 wherein said tablet comprises:
 - a. between about 15% and about 25% by weight of said composition;

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- b. between about 60% and about 84% by weight of said filler; and
 - c. between about 1% and about 25% by weight of a dietary supplemental nutrient selected from a group consisting of vitamins and minerals.
38. The method of claim 37 wherein said dietary supplemental nutrient is selected from the group consisting of dicalcium phosphate, magnesium stearate, calcium citrate, calcium malate, and other calcium sources.
39. The method of claim 34 wherein said product for oral delivery is a capsule comprising:

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- a. a predetermined dosage of said composition; and
 - b. a gelatin capsule.
40. The method of claim 27 wherein said composition comprises between about 10 milligrams and about 2000 milligrams of said phytochemical fractions.
41. The method of claim 27 wherein said plant matter is fractionated to substantially isolate individual ones of said phytochemical fractions.

* * * * *

EXHIBIT 5



(12) **United States Patent**
Kelly

(10) Patent No.: **US 6,562,380 B1**
(45) Date of Patent: **May 13, 2003**

(54) **METHODS FOR TREATING OR REDUCING PREDISPOSITION TO BREAST CANCER, PRE-MENSTRUAL SYNDROME OR SYMPTOMS ASSOCIATED WITH MENOPAUSE BY ADMINISTRATION OF PHYTO-ESTROGEN**

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(75) Inventor: **Graham Edmund Kelly, Northbridge (AU)**
(73) Assignee: **Novogen Research Pty Limited, New South Wales (AU)**
(*) Notice: **Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 493 days.**
(21) Appl. No.: **08/910,837**
(22) Filed: **Aug. 13, 1997**

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(57) **ABSTRACT**

Phyto-estrogen-containing health supplement compositions containing any two or more of Genistein, Daidzein, Formononetin and Biochanin A, or the natural glycosides thereof are administered for treating or reducing predisposition to breast cancer, pre-menstrual syndrome or symptoms of menopause.

23 Claims, No Drawings

Related U.S. Application Data

(63) Continuation of application No. 08/338,567, filed as application No. PCT/AU93/00230 on May 19, 1993, now Pat. No. 5,830,887.

(30) **Foreign Application Priority Data**

May 19, 1992 (AU) PL2511

(51) Int. Cl.⁷ **A61K 35/78**

(52) U.S. Cl. **424/757; 424/423; 424/449; 424/451; 424/464; 514/182**

(58) Field of Search **514/54, 182; 424/423, 424/449, 451, 464, 757**

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