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(54) Title: HERBAL NUTRITIOUS COMPOSITION AND ITS PROCESSING

(57) Abstract: The present invention provides a novel herbal nutritious composition composed of *Tinospora cordifolia*, *Glycyrrhiza glabra*, *Adhatoda vasica*, *Madhuca indica* and *Cassia occidentalis*. The solid or semisolid nutritious composition is rich in protein with standardized herbs with proven pharmacological activities such as anti-oxidant, anti stress and adaptogenic property.



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HERBAL NUTRITIOUS COMPOSITION AND ITS PROCESSING

FIELD OF INVENTION

The present invention relates to development of chocolate with novel herbal composition(s) with proven pharmacological activities such as anti-oxidant, anti stress, adaptogenic and immune enhancing property.

BACKGROUND AND PRIOR ART OF THE INVENTION

The origins of chocolate can be traced back to the ancient Maya and Aztec civilizations in Central America, who first enjoyed 'chocolate'; a much-prized spicy drink made from roasted cocoa beans. Chocolate was exclusively for drinking until the early Victorian era, when a technique for making solid 'eating' chocolate was devised. Throughout its history, whether as a cocoa, a drinking chocolate beverage or confectionery treat, chocolate has been a much sought after food.

As conventional chocolate is assumed to be a causative factor of migraines, Acne and pimples, diabetes and obesity and also considered as a luxury food where as Cocoa beans contain a type of antioxidant that may have a role in the prevention of certain diseases. The *catechins* found in cocoa beans aid resistance against degenerative diseases such as cancer and heart disease. In this context the thought has aroused of providing a chocolate type of edible substance which can provide positive effects with lesser or no side effect, for it the proper blending of modern knowledge with traditional Indian system of medicine, preparing medicine in the form of food which is therapeutically effective & economical too.

OBJECTS OF THE INVENTION

The main object of the present invention to provide a novel herbal nutritious chocolate and it's processing.

SUMMARY OF THE INVENTION

Accordingly the present invention provides a novel herbal nutritious composition useful in the treatment of anti fatigue, anti stress agent and provides vitality and energy.

The novel herbal nutritious composition comprises of decoction of *Tinospora cordifolia*, *Glycyrrhiza glabra*, *Adhatoda vasica*, and paste of *Madhuca indica* with roasted seeds of *Cassia occidentalis*. In a battery of tests like hypoxia and swimming endurance the synergistic effect of

the composition showed 90% potency confirms the adaptogenic activity. The detail description of the plants used in the invention are having the following properties as reported.

Tinospora cordifolia

Family: Menispermaceae

Botanical Descriptions: A large glabrous climbing shrub. Stems rather succulent with long filiform, aerial roots arising from branches. Bark; warty, papery thin, creamy white or grey brown. Peels off easily. Wood, soft, perforated. Leaves; membranous, cordate with broad sinus. Pointed at the tip. Flowers; unisexual and greenish, in long clusters. Seeds; curved. Drupes; ovoid, succulent, lustrous, red, pea sized. Fruits; fleshy, one seeded. Flowers during the summer and fruits during the winter. It is found throughout tropical India, ascending to an altitude of 300m.

Medicinal Uses: Useful in bilious fever, rheumatism, general debility, seminal weakness, splenic diseases and urinary affections. Fresh plant is considered more efficacious. It is mostly used for preparing a kind of starch known as Guduchi satva or Sat giloe.

Phytochemistry: Sesquiterpene tinocordifolin, sesquiterpene glucoside tinocordifolioside, tinosponone, tinocordioside, cordioside, furanoid diterpenes, a new clerodane furano-diterpene viz. columbin, tinosporaside, an immunologically active arabinogalactan, two phytoecdyyones viz., ecdysterone and makisterone and several glycosides isolated as polyacetates. Other alkaloids viz., jatrorrhizine, palmatine, berberine, tembeterine, phenylpropene disaccharides cordifolioside A, B and C, choline, tinosporic acid, tinosporal, tinosporon, 20- β -hydroxyecdysone, palmatoside C and F, cordifolisides D and E, diterpenoid furanolactones.

Pharmacology: The water and ethanolic extract inhibited the cyclophosphamide induced immunosuppression. Aqueous extract of the stem showed anti-inflammatory, analgesic and antipyretic properties in rats. In clinical studies, it also showed immunosuppressive effect in obstructive jaundice patients, antioxidant activity and amelioration of cyclophosphamide-induced toxicity.

Glycyrrhiza glabra

Family: Papilionaceae, Fabaceae.

Botanical description: A perennial herbs and under shrubs distributed in the sub – tropical and warm temperate regions of the world, chiefly in the Mediterranean countries and china. The drug is imported into India on a considerable scale from Asia Minor, Iraq, Persia and other Central Asian countries.

Phytochemistry:- Glycyrrhizin, Glycyrrhizic acid, and glycyrrhetic acid, the characteristic sweet taste is due to Glycyrrhizin (2-14%).

Pharmacology: Rhizomes and roots tonic, expectorant, demulcent, and laxative, used for allaying coughs and catarrhal affections, and irritable conditions of the membranes of the urinary organs.

Madhuca indica (Koenig) Gmelin

Family: Sapotaceae

Botanical description: A medium sized to large deciduous tree, leaves clustered near the ends of stout branches 5-25 cm long and 3.5- 12 cm wide, apex shortly acuminate, base rounded to cuneate; petioles 2-4 cm long; stipules 0.6-1 cm long, subulate, densely pubescent. Flowers numerous, scented, clustered on leafless shoots; fruits ovoid or subglobose, 2.5-5 cm long, greenish turning reddish- yellow or orange when ripe, fleshy; seeds 1-4, brown or ochre, oblong – ellipsoid, shiny, 2.5 – 3.8 cm long and 1.5 cm wide. Flowers appear with new leaves from February to April and fruits ripen from May to August in northern and central India.

Pharmacology: The seed oil has emollient properties and is useful for external application in treating skin diseases, rheumatism and headache. It is also laxative and used to treat chronic constipation, piles and haemorrhoids and is sometimes used as an emetic and galactagogue. The ground flowers are applied as a paste to relieve aching muscles. The bark is used for treating rheumatism, ulcers, itches, bleeding and spongy gums, tonsillitis and diabetes mellitus. The roots are applied externally to promote healing of ulcers.

Cassia occidentalis (L)

Family: Caesalpiniaceae

Botanical description: An erect fetid, woody herb or undershrub, 60-150 cm tall, with a smooth, purplish or green stem. Leaves alternate, pinnate, 15-20 cm long, stipulate with a sessile dark brown gland near the base of the petiole; leaflets 3-5 pairs, opposite, short-stalked, membranous, ovate or lanceolate, 3-9 cm long and 1.5-4 cm wide, base rounded, apex acute or attenuate, glabrous above glaucous beneath. Flowers yellow, in short, few flowered axillary or terminal racemes. Flowers and fruits throughout the year depending on locality, but mainly between August and January in central India.

Phytochemistry: The seeds contain tannic acid, mucilage (36%), fatty acid (2.56%), emodin and a toxalbumin. Chrysarobin (methyl – di hydroxy anthranol. M. P. 203-4⁰C) has been isolated from the benzene extract of the seeds. The fatty oil contains: saturated fatty acids 19.7; linoleic acid, 31.4; oleic acid 30.7, linolenic acid, 6.3; volatile constituents, 0.7; unsapon matter 7.4 %.

Pharmacology: All parts of the plant have similar medicinal properties and are considered purgative, tonic, febrifugal, expectorant and diuretic. The plant is used as a vermifuge and to treat sore eyes, haematuria, rheumatism, dropsy, typhoid, asthma, blood disorders and is reportedly effective against leprosy. A decoction of the plant is used for the treatment of hysteria, dysentery and other stomach troubles. The roots are considered bitter, tonic, purgative, anthelmintic and diuretic. In ayurveda a paste made from the roots is considered specific ringworm and other skin ailments. The leaves are also used externally to relieve inflammatory swellings, rheumatism, wounds and sprains. The root bark is used as a quinine substitute to cure fever, and its decoction is used as a stimulant and as a specific medicine for gonorrhoea and hepatic troubles.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly the present invention provides a herbal nutritious chocolate as a nutrient source, comprising the extracts obtained from *Tinospora cordifolia*, *Glycyrrhyza glabra*, *Adhatoda vasica*, *Madhuca indica* and *Cassia occidentalis* with acceptable additives.

In another embodiment, an herbal nutritious chocolate used in the fields of foods and pharmaceuticals as a nutrient source, comprising the extracts obtained from *Tinospora cordifolia*, *Glycyrrhyza glabra*, *Adhatoda vasica*, *Madhuca indica* and *Cassia occidentalis* with acceptable additives.

In still another embodiment, herbal nutritious composition as, wherein the extracts/juice of the plants are mixed in the ratio viz. *Tinospora cordifolia* (4-7 %), *Glycyrrhyza glabra* (4-7 %), *Adhatoda vasica* (4-7 %), *Madhuca indica* (8-15 %), and *Cassia occidentalis* (2-4 %).

In another embodiment, herbal nutritious composition wherein said composition is in conventional form of a semisolid/solid/granules or any other form.

In another embodiment, herbal nutritious composition wherein the extracts of plants may be alcoholic or aqueous extract or mixture thereof.

In yet another embodiment, herbal nutritious composition wherein the alcohol used is ethanol.

In another embodiment, herbal nutritious composition wherein the said composition comprises plant extract about 20-40% wt of the total formulation.

In still another embodiment, herbal nutritious composition wherein the additives selected are starch, lactose, sugar, gum acacia and known lubricant or mixture thereof

In another embodiment, herbal nutritious composition wherein the plant extracts are obtained from plant parts selected from leaf, seeds, floral parts rhizome and aerial parts.

In yet another embodiment, herbal nutritious composition wherein the lubricants may be selected from milk or its derivatives.

In another embodiment, a method of preparing a herbal nutritious composition as claimed in claim 1 wherein the said method comprising:

- a) obtaining the part of medicinal plants from a group comprising leaves, rhizome and aerial parts including flowers,
- b) drying the plant material in shade,
- c) powdering the dried plant material to a coarse powder,
- d) preparation of decoction: the coarsely powdered plant material was sieved and added water and boiled to concentrate,
- e) to the concentrate *Madhuca indica* (fl.parts) and roasted seeds of *Cassia occidentalis* is added, followed by spray dried milk Sugar & Gum acacia,
- f) the above is heated gently, after attaining proper consistency the paste is kept in molds and cool at a temperature ranging from -4 to -20 °C to obtain solid or semisolid.

In yet another embodiment, herbal nutritious composition wherein the formulation can be stored at room temperature

In yet another embodiment, herbal nutritious composition wherein formulation has a shelf life of 2 years.

In another embodiment, herbal nutritious composition wherein the formulation showed as a potent antioxidant activity.

In still another embodiment, herbal nutritious composition wherein the formulation showed as a potent adaptogenic activity.

In another embodiment, herbal nutritious composition wherein the formulation showed as a potent anti stress activity.

In yet another embodiment, herbal nutritious composition wherein the formulation showed as a potent enzymatic super oxide dismutase enhancing activity.

In another embodiment, herbal nutritious composition wherein the formulation showed as a potent lipid peroxidation inhibition.

In still another embodiment, herbal nutritious composition wherein the herbal nutritious composition showed as a potent enzymatic enhancing catalase activity.

In another embodiment, herbal nutritious composition wherein the herbal nutritious composition showed an enhanced protein content.

In yet another embodiment, herbal nutritious composition wherein the herbal nutritious composition helpful in defending the body against the effects of stressful environment and provide vitality and energy.

Following example are given by illustration

Formulation1 (F1):

1. <i>Tinospora cordifolia</i> (stem) –	7 %
2. <i>Adhatoda vasica</i> (Wl. Pl.) –	0 %
3. <i>Glycyrrhiza glabra</i> (Rhizome) –	0 %
4. <i>Madhuca indica</i> (fl.parts) –	14 %
5. <i>Cassia occidentalis</i> -	3 %
6. Spray dried milk powder –	10 %
7. Sugar –	10 %
8. Gum acacia powder-	1 %
9. Lactose	to make up 100 %

Formulation2 (F2):

1. <i>Tinospora cordifolia</i> (stem) –	0 %
2. <i>Adhatoda vasica</i> (Wl. Pl.) –	6 %
3. <i>Glycyrrhiza glabra</i> (Rhizome) –	0 %
4. <i>Madhuca indica</i> (fl.parts) –	12 %
5. <i>Cassia occidentalis</i> -	3 %
6. Spray dried milk powder –	10 %
7. Sugar –	10 %
8. Gum acacia powder-	1 %
9. Lactose	to make up100 %

Formulation3 (F3):

1. <i>Tinospora cordifolia</i> (stem) –	0 %
2. <i>Adhatoda vasica</i> (Wl. Pl.) –	0 %
3. <i>Glycyrrhiza glabra</i> (Rhizome) –	7 %
4. <i>Madhuca indica</i> (fl.parts) –	14 %
5. <i>Cassia occidentalis</i> -	3 %
6. Spray dried milk powder –	10 %
7. Sugar –	10 %
8. Gum acacia powder-	1 %
9. Lactose	to make up 100 %

Formulation4 (F4):

1. <i>Tinospora cordifolia</i> (stem) –	6 %
2. <i>Adhatoda vasica</i> (Wl. Pl.) –	6 %
3. <i>Glycyrrhiza glabra</i> (Rhizome) –	6 %
4. <i>Madhuca indica</i> (fl.parts) –	12 %
5. <i>Cassia occidentalis</i> -	3 %
6. Spray dried milk powder –	9 %
7. Sugar –	9 %
8. Gum acacia powder-	1 %
9. Lactose	to make up 100 %

The above (1,2,3) dried material coarsely powdered with 16 x screen and water is added which is heated on to concentrate. Out of which 300 ml of decoction is taken in it of *Madhuca indica* (fl.parts) added along with powder of roasted seeds of *Cassia occidentalis* followed by spray dried milk and gum acacia. Mixed properly through mixer, and sieved through fine mesh no. 60, and consistency to paste is kept in molds & in oven to remove excess of moisture. Altering the first three ingredients, and taking them altogether has provided four sets of combination which has been termed as

Table 1- Effect of formulation on chronic stress-induced changes in lipid peroxidation, superoxide dismutase and catalase in rats.

S.No	Treatment groups (mg/kg, p.o.)	Lipid peroxidation (nmoles of TBARS)	Superoxide dismutase	Catalase
1	Vehicle	0.45 ± 0.02	105.3 ± 11.2	33.9 ± 2.4
2	Vehicle + stress	0.71 ± 0.04	256.8 ± 7.5	19.5 ± 1.9
3	F1	0.51 ± 0.02	235.6 ± 6.9	22.7 ± 3.1
4	F2	0.48 ± 0.02	201.3 ± 5.1	25.3 ± 2.7
5	F3	0.39 ± 0.02 ^a	185.6 ± 6.9 ^b	26.7 ± 3.1
6	F4	0.28 ± 0.02 ^c	162.3 ± 5.1 ^c	31.3 ± 2.7 ^a

Values are mean ± S.E.M.

P: ^a<0.05, ^b<0.01 and ^c<0.001 compared to respective control group.

NOTE:

No mortality was found in any of the treated group.

No gross abnormality in behavior was observed in the animal exposed with herbal preparation.

Formulation (F1)- consisting of only extract of *Tinospora cordifolia* (stem) Formulation (F2) consisting of only extract *Adhatoda vasica* (Wl. Pl.) Formulation (F3) consisting of only *Glycyrrhiza glabra* (Rhizome) where as Formulation (F4) consisting of all the above.

The above table 1 states that the herbal formulation F4 has potent antioxidant activity as evident by its ability to prevent lipid peroxidation from 0.71 to 0.28 and by the augmentation of the antioxidant enzymes viz. superoxide dismutase (256.8 in vehicle under stress to 162.3 at F4) and catalase (19.5 in vehicle under stress to 31.3 at F4) when compared to the vehicle which is plain water. Thus the herbal formulation prepared exhibits potent antioxidant activity under stress conditions and hence is a good rejuvenator.

Lipid peroxidation: The fundic part liver was homogenized (5%) in ice-cold 0.9% NaCl in glass homogenizer for 30 s. The homogenate was centrifuged for 10 min A volume of the homogenate (0.20 ml) was transferred to a vial and was mixed with 0.2 ml of a 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 ml of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.50 ml of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to

4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged. The absorbance of the supernatant fraction was measured at 532 nm. Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water. 1,1,3,3-Tetraethoxypropan was used as standard for calibration of the curve and is expressed as nanomoles per milligram protein.

SOD and Catalase:

Decomposition of H_2O_2 in presence of catalase (CAT) was followed at 240 nm. One unit (U) of catalase was defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 per minute, at 25 $^{\circ}\text{C}$ and pH 7.0. Results are expressed as units of CAT activity per milligram of protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system. One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25 $^{\circ}\text{C}$) and the results have been expressed as units of SOD activity per milligram of protein.

Table 2: Effect of formulation on hypoxia and swimming performance time in mice.

S.No	Treatment	Dose (mg/kg,p.o)	Hypoxia time	Swimming endurance
1.	Control+ stress	-	22.34 $\pm$ 1.15	236.21 $\pm$ 25.11
2.	F1	200	28.11 $\pm$ 1.55	295.12 $\pm$ 27.41
3.	F2	200	31.54 $\pm$ 2.11	325.45 $\pm$ 27.05
4.	F3	200	40.01 $\pm$ 2.68	366.52 $\pm$ 26.74
5.	F4	200	45.89 $\pm$ 3.05 ^a	423.21 $\pm$ 28.85 ^a
6.	Commercially available sample	200	24.35 $\pm$ 2.1	246.25 $\pm$ 23.18

Values are mean $\pm$ S.E.M.

P: ^c<0.001 compared to respective control group.

NOTE:

No mortality was found in any of the treated group.

No gross abnormality in behavior was observed in the animal exposed with herbal preparation.

Formulation (F1)- consisting of only extract of *Tinospora cordifolia* (stem) Formulation (F2) consisting of only extract *Adhatoda vasica* (Wl. Pl.) Formulation (F3) consisting of only *Glycyrrhiza glabra* (Rhizome) where as Formulation (F4) consisting of all the above.

As evident from table 2 the herbal formulation F4 exhibits potent adaptogenic activity as evident from increase in the hypoxia time from 28.11 under stress to 45.89 upon administration of F4 and increase in swimming endurance from 295.12 to 423.21 upon administration of F4.

Commercially available sample did not significant activity in hypoxia time and swimming endurance tests of the adaptogenic activity.

Procedures for adaptogenic activity**Hypoxia time:**

The animals were placed in an empty glass jar of 300 mL capacity attached with an electronic watch; the jars were made air tight with greased glass stoppers and the time until the onset of convulsion was recorded (Singh B et al, 2001, Phytotherapy Research, 15; 311-318).

Swimming performance time:

The animals were allowed to swim inside a perspex glass beaker (30 cm high with 20 cm diameter containing water up to 25cm high) maintained at $26^{\circ} \pm 1^{\circ}$ C with a continuous air current from the bottom. The end point of swimming endurance was taken as when the mice remained at the bottom for more than 10s (Singh B et al, 2001, Phytotherapy Research, 15; 311-318).

What is claimed is:

1. A herbal nutritious chocolate as a nutrient source, comprising the extracts obtained from *Tinospora cordifolia*, *Glycyrrhiza glabra*, *Adhatoda vasica*, *Madhuca indica* and *Cassia occidentalis* with acceptable additives.
2. Herbal nutritious composition as claimed in claims 1 & 2, wherein the extracts/juice of the plants are mixed in the ratio viz *Tinospora cordifolia* (4-7 %), *Glycyrrhiza glabra* (4-7 %), *Adhatoda vasica* (4-7 %), *Madhuca indica* (8-15 %), and *Cassia occidentalis* (2-4 %).
3. Herbal nutritious chocolate composition as claimed in claim 1 wherein said composition is in conventional form of a semisolid/solid/granules or any other form.
4. Herbal nutritious composition as claimed in claim 1 wherein the extracts of plants is alcoholic extract, aqueous extract or mixture thereof.
5. Herbal nutritious composition as claimed in claim 1 wherein the alcohol used is ethanol.
6. Herbal nutritious composition as claimed in claim 1 wherein the said composition comprises plant extract about 20-40% wt of the total formulation.
7. Herbal nutritious composition as claimed in claim 1 wherein the additives selected from starch, lactose, sugar, gum acacia and known lubricants or mixture thereof.
8. Herbal nutritious composition as claimed in claim 1 wherein the plant extracts are obtained: from plant parts selected from leaf, seeds, floral parts rhizome and aerial parts.
9. Herbal nutritious composition as claimed in claim 1 wherein the lubricants used is selected from milk or its derivatives.
10. A method of preparing a herbal nutritious composition as claimed in claim 1 wherein the said method comprising:
 - g) obtaining the part of medicinal plants from a group comprising leaves, rhizome and aerial parts including flowers,
 - h) drying the plant material in shade,
 - i) powdering the dried plant material to a coarse powder,
 - j) preparation of decoction: the coarsely powdered plant material was sieved and added water and boiled to concentrate,
 - k) to the concentrate flower parts *Madhuca indica* and roasted seeds of *Cassia occidentalis* is added, followed by spray dried milk Sugar & Gum acacia,

- 1) the above is heated gently, after attaining proper consistency the paste is kept in molds at cool temperature ranging from -4 to -20 °C to obtain solid or semisolid.
11. Herbal nutritious composition as claimed in claim 1 wherein the formulation can be stored at room temperature
12. Herbal nutritious composition as claimed in claim 1 wherein the formulation has a shelf life of 2 years.
13. Herbal nutritious composition as claimed in claim 1 wherein the formulation showed as a potent antioxidant activity.
14. Herbal nutritious composition as claimed in claim 1 wherein the formulation showed as a potent adaptogenic activity.
15. Herbal nutritious composition as claimed in claim 1 wherein the formulation showed as a potent anti stress activity.
16. Herbal nutritious composition as claimed in claim 1 wherein the formulation showed as a potent enzymatic super oxide dismutase enhancing activity.
17. Herbal nutritious composition as claimed in claim 1 wherein the formulation showed as a potent lipid peroxidation inhibition.
18. Herbal nutritious composition as claimed in claim 1 wherein the herbal nutritious composition showed as a potent enzymatic enhancing catalase activity.
19. Herbal nutritious composition as claimed in claim 1 wherein the herbal nutritious composition showed an enhanced protein content.
20. Herbal nutritious composition as claimed in claim 1 wherein the herbal nutritious composition helpful in defending the body against the effects of stressful environment and provide vitality and energy.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23G A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, COMPENDEX, FSTA, EMBASE, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE MEDLINE 'Online! US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; March 1987 (1987-03), SADIQUE J ET AL: "Biochemical modes of action of Cassia occidentalis and Cardiospermum halicacabum in inflammation." XP002342444 Database accession no. NLM3613609 abstract ----- -/--	1, 17



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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