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(54) **Title:** HEPATOPROTECTIVE ACTIVITY OF 10-O-P-HYDROXYBENZOYIAUCUBIN

(57) **Abstract:** ABSTRACT: A method for treating and/or preventing hepatic disease conditions in mammals including human beings, said method comprising the steps of administering to the mammal an effective amount of 10-O-p-hydroxybenzoylaucubin compound of Formula 1, also called Agnuside, optionally individual or in combination with one or more pharmaceutically acceptable additives.

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HEPATOPROTECTIVE ACTIVITY OF 10-O-P-HYDROXYBENZOYIAUCUBIN

Field of the invention

This invention relates to hepatoprotective activity of an iridoid glycoside, 10-O-p-hydroxybenzoylaucubin (agnuside) of the formula 1 isolated from *Vitex negundo* by extracting the aerial parts/whole plant with polar solvent like 95% ethanol, methanol, aqueous ethanol or water, removing fatty non polar constituents by triturating the extract with solvents such as ethylene chloride, methylene chloride, chloroform or ethyl acetate to get a fraction from which agnuside is separated by column chromatography. The hepatoprotective activity of agnuside has been confirmed by evaluation of its protective action against *CCl* and galactosamine induced liver damage models.

Background and Prior art references

Vitex negundo Linn (family Verbenaceae) is widely used in the indigenous system of medicine in India. Various medicinal properties are ascribed to leaves and roots of this plant. The leaves are aromatic, tonic and vermifuge and the roots are used as expectorant, febrifuge and tonic.(Chopra, R.N., Nayar, S.L. and Chopra, I.C., Glossary of Indian Medicinal Plants, CSIR, New Delhi, 1956, p. 256; Wealth of India : Raw Material, CSIR, New Delhi, 1976, vol. X. p. 522) A number of compounds have been isolated from various parts of this plant From the leaves Ghosh and Krishna isolated a number of compounds viz. glucononitol, p-hydroxybenzoic acid, 5-hydroxyisophthalic acid and' 3, 4 - dihydroxybenzoic acid along with two glucosides and an amorphous alkaloid [Ghosh, T.P. and Krishna, S.,7. *Indian Chem. Soc.* 1936, **13,** 634]. Masilungan reported the presence of the terpenes, oc-pinene, camphene, citral and β- caryophyllene in the essential oil of the leaves Masilungan, V.A. *Philip. J. Sci.* 1955, 84, 275]. A large number of flavonoids have been isolated from the leaves and twigs. These are casticin, orientin, isoorientin, luteolin, luteolin-7-0-glucoside, corymbosin, gardenins A and B, 3-0-desmethylartemetin,5-0-desmethylnobiletin,3',4',5,5',6,7,8-heptamethoxy-flavone, 3',5-dihydroxy-4',7,8-trimethoxyflavanone and 3',5-dihydroxy-4',6,7-trimethoxy flavanone [Sirait.L.M., Rimpler, H. and Haensal, R., *Experientia* 1962, 18, 72; Haensal, R.et al. *Phytochemistry* 1965,4 ,19; Banerji A. *el al. Phytochemistry* 1969, 8,511; Ferdous, A. J. *et al. Bangladesh Acad.Sci.* 1984, 8,23; Dayrit, *F.M..et al. Philipp. J.Sci.* 1987, 116, 403; Banerji, J. *et al. Indian J.Chem* .1988; 27B, 597; Achari, B. *et al. Phytochemistry.* 1984, 23, 703]. Stem-bark afforded five new flavone glycosides along with luteolin and acerosin. The new flavone glycosides are 6β - glucopyranosyl – 7 - hydroxy-3',4',5',8-

tetramethoxyflavone-5-O-a-L-rhamnopyranoside, 3', 7-dihydroxy-4',6, 8-trimethoxy flavone-5-O-(6"-O-acetyl-p-D-glucopyranoside),3,3',4',6,7-pentamethoxy flavone 5'-O-(4"-O-|3-D-glucopyranosyl-a-rhamnopyranoside, 4',5, 7-tri-hydroxyflavone-8-(2"-caffeoyl-a-glucopyranoside) and 3', 5,5',7-tetrahydroxy-4-methoxyflavone -3'-O-(4"-O-a-D- galactopyranosyl) galactopyranoside [Rao, *V.K.et al. Indian J. Pharm.* 1977,39, 41; Subramamian, P.M. and Misra, G.S. Indian J. Chem. 1978,16B, 615; Subramaniam, P.M. and Misra, G.S.J. Nat. Prod. 1979,42, 540]. A diterpenoid, 5 |3-hydro-8,ll,13-abieta-trien-6a-ol and three triterpenoids, 2a, 3a-dihydroxyoleana-5 12- dien-28-oic acid, 2a, 3a-diacetoxyoleana-5, 12-dien-28-oic acid, 2,3 cc-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid have been isolated from the seeds. These compounds exhibited anti inflammatory activity [Chawla, A.S., Sharma, A.K., Handa, S.S. and Dhar, K.L. Indian J. Chem. 1991, 30B, 773 and J.Nat. Prod. 1992,55,163]. Leaves, however, did not yield any triterpenoids but from the roots acetyloleanolic acid was isolated [Vishnol, S.P.,Shoeb, A., Kapil, R.S. and Popli, S.P. *Phytochemistry* 1983, 22, 597].

Five Iridoid glycosides have been reported from the leaves of *V. negundo.* These are aucubin, agnuside (Hansal *et al. Phytochemistry 4,* 1965, 9 negundoside 6'-p-hydroxybenzoyl mussaenosidic acid (Sehgal *et al. Phytochemistry*, 21, 1982, 363) and nishindaside (Datta *et al. Tetrahedron* **39,**1983, 3067).

During our search for hepatoprotectives agents of plant origin, the aqueous alcoholic extract of *V. negundo* and a fraction isolated from it exhibited strong immuno-stimulating hepatoprotective activities. A process has been developed for the isolation of an immuno-stimulating agent from the leaves of *Vitex negundo* for which a patent has been granted to Regional Research Laboratory, Jammu [Suri, J.L. *et. al* Indian Patent No. 78388 dt. 19-03.97). Another patent application has been submitted by Regional Research Laboratory, Jammu for a process for isolation of a bioactive composition possessing hepatoprotective and immuno-stimulating activity (Application no. 16/DEL98 dt. 16.1.98) In view of the strong hepatoprotective activity exhibited by the iridoid glycosides of *Picrorhiza kurroa* (Ansari, R.A. *et al Indian J. Med. Research,* 1988, 87, 401) it was thought desirable to evaluate the iridoid glycosides of *V. negundo* for hepatoprotective activity. Agnuside, an iridoid glycoside, was isolated from *V. negundo* and evaluated for hepatoprotective activity alongwith the aqueous alcoholic extract of the plant. Both aqueous alcoholic extract (coded as 033) and agnuside (coded as 033 (1) showed marked hepatoprotective activity in experimentally induced hepatic damage with CCl_4 and galactosamine (GalN) in rats. A

comparison with the known hepatoprotective agent silymarin revealed that 033 and 033 (1) exhibited higher hepatoprotective potential in most of the parameters with respect to their effect on elevated levels of serum and liver homogenate parameters (Table 1 and 2).

Description of the invention

Thus the main objective of the present invention is to provide hepatopro tec live activity of a defined bioactive molecule isolated from leaves of *V. negundo* viz., agnuside of formula 1, as shown in the diagram accompanying this specifications.

Accordingly, the present invention provides hepatoprotective activity of a compound of formula 1 accompanying the specifications which comprises :

(a) powdering the plant material by known methods

(b) preparing the aqueous alcoholic extract by percolation

(c) concentrating the alcoholic extract by conventional method,

(d) removing fatty non polar constituenls by triturating the extract with solvents such as ethylene chloride, methylene chloride, chloroform or ethyl acetate

(e) adsorbing the residue extract over silica gel,

(f) isolation of agnuside from the adsorbed extract by column chromatography and

(g) evaluating for hepatoprotective activity

The solvent used for extraction in step (b) is ethanol, aqueous ethanol, methanol, aqueous methanol or water.

The hepatoprotective activity of the compound is expressed in 50 mg/kg^{-1} oral dose in rals which on extrapolation comes to be 300-4QO mg daily human dose (70 kg) in single or divided doses.

Characterisation of agnuside 1

1 obtained as crystalline compound, mp 148-50°C IvT : -466, UV-232 nm, IR (KBr) spectrum showed absorptions at 3400, 1700, 1642 and 1618 cm^{-1}. ^{1}H NMR (200 MHz, CD_3OD) 5 6.35 {dd, *J 2,6,* H-3) 5.12 (dd, *J,* 4,6, H-4) 2.70 (m-H-5) 4.48 (m, H-6) 5.82 (s, H-7) 2.94 (m, H-9) 5.05 (s, H-10) 4. 69 (d, *J* 8, H-l') 3.65 (m, H-2') 7.92 fdd, / 2,7,H-2 "6") 6.84 (dd, 7,2,7, H-3 ") ^{13}CNMR δ 97.92(C-1), 141.50 (C-3), 105.35 (C-4), 46.04 (C-5) 82.58 (C-6) 132.09 (C-7), 142.55 (C-8), 46.04 (C-9) 63.46 (C-10), 100.07 (C-1') 74.56 (C-2'), 77.61 (C-3') 71.01 (C-4'), 77.82 (C-5') 62.49 (C-6') 121.81 (C-1") 132.73 (C-2",C-6"), 16.08 (C-3", 5"), 163.50 (C-4 "), 167.70 (CO)

Detailed description of the invention

Accordingly, the present invention provides a method for treating and/or preventing hepatic disease conditions in mammals including human beings, said method comprising the steps of administering to the mammal an effective amount of 10-O-p-hydroxybenzoylaucubin compound of Formula 1, also called Agnuside, optionally individual or in combination with one or more pharmaceutically acceptable additives.

Another embodiment of the present invention wherein the said composition reduces the elevated levels of serum glutamin-pyruvic transaminase (GPT) about 70%.

In yet another embodiment of the present invention wherein the said composition reduces the elevated levels of serum glutamin-oxalo acetic transaminase (GOT) about 60%.

In still another embodiment of the present invention wherein the said composition reduces the elevated levels of serum alkaline phosphatase (ALP) about 62%.

In yet another embodiment of the present invention wherein the said composition reduces the elevated levels of serum tryglycerides about 70%.

In still another embodiment of the present invention wherein said composition against the elevated level of bilirubin about 72%.

In yet another embodiment of the present invention wherein compound agnuside is obtained from the whole plant.

In yet another embodiment of the present invention wherein 10-O-p-hydroxybenzoylaucubin is of concentration ranging between about 20 to 200 mg /kg-body weight.

In still another embodiment of the present invention wherein 10-O-p-hydroxybenzoylaucubin is of concentration is about 50 mg/kg-body weight.

In yet another embodiment of the present invention wherein the pathological condition is selected from liver disorder

In still another embodiment of the present invention wherein the subject is selected from mammals and animals preferably humans.

In yet another embodiment of the present invention wherein said composition is used singly or in combination with pharmaceutically acceptable carriers.

In still another embodiment of the present invention wherein said composition is administered to subject in combination with pharmaceutically acceptable additives, carriers, diluents, solvents, filters. Lubricants, excipients, binder or stabilizers.

In yet another embodiment of the present invention wherein the desired dosage is administered for both preventive and curative properties.

In still another embodiment of the present invention wherein said composition is administered, orally or by any clinically/medically accepted methods.

In yet another embodiment of the present invention wherein the preferred dosage for human beings is about 5 mg/Kg of body weight.

In still another embodiment of the present invention wherein the various physical forms in which the composition is available, e.g. powder, tablet, capsule, syrup, granules, emulsion, aerosoal, or beads.

In yet another embodiment of the present invention is useful for Liver cirrhosis, Galactosemia, Hemoanigoma, Hemochromatosis, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Hepatitis G, Alcholic Liver disease, Autoimmune hepatitis, Cancer of Liver, Biliary Atresia, Glycogen Storage Disease 1, Alpha-1-antitrypsin deficiency, Alagille syndrome, Byler Disease, Caroli disease, Fatty liver, Itching in Liver, Primar Biliary Cirrhosis, Sclerosing Cholangitis or Protoporphyria Erythroepatic.

One more embodiment of the present invention a process for the isolation of compound 10-O-p-hydroxybenzoylaucubin compound of Formula 1, also called Agnuside, said compound isolated from aerial part/whole body comprising of steps:

(a) powdering the plant material by known methods
(b) preparing the aqueous alcoholic extract by percolation
(c) concentrating the alcoholic extract by conventional method
(d) removing fatty non-polar constituents by triturating the extract with solvents such as ethylene chloride, methylene chloride, chloroform or ethyl acetate
(e) adsorbing the residue extract over silica gel
(f) isolating of 2′-p-Hydroxy benzoyl mussaenosidic acid from the adsorbed extract by column chromatography

Brief Description of the accompanying drawings

Figure 1 represents the compound 10-O-p-hydroxybenzoylaucubin (Agnuside) of formula 1.

Figure 2 represents flow-sheet for isolation of 10-O-p-hydroxybenzoylaucubin) (Agnuside).

The invention is described in detail by the examples given below which should not be construed to the limit of scope of the present invention

Example 1

The shade dried and powdered leaves of (1 kg) *V. negundo* were extracted with 80% ethanol (4 x 3L) by percolation. The pooled extract was concentrated under reduced

pressure at below 50°C to 1 litre of aqueous concentrate. The aqueous concentrate was washed with ethyl acetate (3 x 500 ml) and further concentrated to 400 ml. This syrupy residue was adsorbed over silica gel (400 g) and allowed to dry at room temperature. The slurry was put on silica gel column and eluted with mixture of chloroform-methanol (19:1), furnished 1 (2.1g) (coded as 033 (1)

Example 2

The finely ground leaves (200 g) of *Vitex negundo* were extracted thrice with petroleum ether (60-80°) (1 litre for the first extraction and 600 ml each for two subsequent x extractions) The drug was freed of solvent at room temperature. It was then extracted with ethanol (1 litre for the first extraction and then thrice with the same solvent 600 ml each time) Evaporation of ethanol in a rotary film evaporator yielded 30 g of extract (coded as

Example 3

Treatment of experimental animals with the 033 and 033(1) against CCU induced liver damage reduced the elevated levels of serum GPT, GOT, ALP, bilirubin, TG and hepatic lipid peroxidation and increased the GSH level. A comparison with the known hepatoprotective agent silymarin revealed that 033 and 033(1) exhibited higher protective potential in most of the parameters with respect to their effect on elevated levels of above parameters by CCU The hepatoprotective activity observed with 033 and 033(1} was: serum GPT- 67.52 & 59.52, GOT- 58.91 & 57.42, ALP- 58.26 & 60.17, Bilirubin- 60.00 & 71.79, TG- 46.47 & 38.53 and in liver homogenate LP- 55.71 & 41.48 and GSH- 61.42 & 38.12% respectively. The same with silymarin was: 58.44, 51.63, 52.26, 57.50, 41.30, 62.05 & 60.15 respectively (Table-1). **Example 4**

Treatment of animals against the galactosamine (GaIN) induced hepatic damage also reduced the elevated levels of serum GPT, GOT, ALP, bilirubin, TG and hepatic lipid peroxidation and increased the GSH level . The hepatoprotective activity observed with 033 and 033(1) was 59.58 & 44.97, 56.62 & 44.05, 54.28 & 47.66, 57.71 & 66.66, 57.89 & 49.33 percent in serum GPT, GOT, Bilirubin, ALP, and TG respectively and 67.51 & 48.34, 66.56 & 55.13 percent in hepatic lipid peroxidation (LP) and GSH respectively. The same with silymarin was 57.38, 55.40, 60.00, 53.54,46.17, 69.59, 67.18 percent respectively (Table-2).

Advantages of the present invention over currently used plant based hepatoprotectives:

1. Agnuside is more potent than the commercially available herbal hepatoprotective agent silymarin.

2. Silymarin is a mixture of three constituents whose relative proportion varies from batch to batch while agnuside is a pure compound.

Table 1: Hepatoprotective activity (in vivo) of 033, 033 (1) **and** Silymarin fed at 48h, 24h, 2h before and 6h after CCU (1 ml/kg, p.o.) induced hepatic injury in rats[3].

Treatment	Dose	Serum parameters					Hepatic parameters	
	mg/kg p.o.	OPT (Units)	GOT (Units)	A1.F	Bihrubm (mg %)	Triglycerides (m_s %)	Lipid Peroxidation[c]	Glutathione[d]
033 + CCl_4	400	67.52	58.91	58.26	60.00	46.47	55.71	61.42
033(1)+ CCl_4	50	59.52	57.43	6017	71.79	38.53	41.48	38.12
Silymarin + CCl_4	50	58.44	51.63	52.26	57.50	41.30	62.05	60.15

a: Values represent the mean percent hepatoprotective activity of six animals in each group.

H: Hepatoprotective activity was calculated as {1-(T-V/C • V)} x 100

where'T' is mean value of drug and CCU,

"C" is mean value of CCU alone and "V" is the mean value of vehicle treated animals.

Unit: each unit is (mole pyruvate/min/L.

b: is 11 mole of p-nitrophenol *formed/mini* U

c: is n moles MDA/g liver.,

d: is n mole GSH/g liver

Table 2: Hepatoproteclive activity (m vivo) of **033,033 (1) and Silymarin** fed at 48 h, 24h, **2h** before and 6h after GalN (300 mg/kg, s.c.) induced hepatic injury in rats[a].

Treatment	Dose	Serum parameters					Hepatic parameters	
	mg/kg, p.o	GPT (Units)	GOT (Units)	Bilirubin (mg%)	ALP[b]	Triglycerides (mg %)	Lipid peroxidation[c]	Glulathi One[d]
033 + GalN	400	59.58	56.62	54.28	57.71	57.89	67.51	66.56
033(1) + GalN	50	44.97	44.05	47.66	66.66	49.33	48.34	55.13
Silymarirri-GalN	50	57.38	55.40	60.00	53.54	46.17	69.59	67.18

a: Values represent the mean percent hepatoproteclive activity of six animals in each group.

H: Hepatoprotective activity was calculated as {I- (T - V / C - V)} x 100 where "T" is mean value of drug and GalN, 'C' is mean value of GalN alone and "V" is the mean value of vehicle treated animals.

Unit: each unit is pinole pyruvate/min/L.

b: is M, mole of /j-nitrophenol formed/min/ L,

c: is n moles MDA/g liver.,

d: is p. mole GSH/g liver.,

CLAIMS

1. A method for treating and/or preventing hepatic disease conditions in mammals including human beings, said method comprising the steps of administering to the mammal an effective amount of 10-O-p-hydroxybenzoylaucubin compound of Formula 1, also called Agnuside, optionally individual or in combination with one or more pharmaceutically acceptable additives.
2. A method as claimed in claim 1, wherein the said composition reduces the elevated levels of serum glutamin-pyruvic transaminase (GPT) about 70%.
3. A method as claimed in claim 1, wherein the said composition reduces the elevated levels of serum glutamin-oxalo acetic transaminase (GOT) about 60%
4. A method as claimed in claim 1, wherein the said composition reduces the elevated levels of serum alkaline phosphatase (ALP) about 62%.
5. A method as claimed in claim 1, wherein the said composition reduces the elevated levels of serum tryglycerides about 70%.
6. A method as claimed in claim 1, wherein said composition against the elevated level of bilirubin about 72%.
7. A method as claimed in claim 1, wherein compound agnuside is obtained from the whole plant.
8. A method as claimed in claim 1, wherein 10-O-p-hydroxybenzoylaucubin is of concentration ranging between about 20 to 200 mg /kg-body weight.
9. A method as claimed in claim 1, wherein 10-O-p-hydroxybenzoylaucubin is of concentration is about 50 mg/kg-body weight.
10. A method as claimed in claim 1, wherein the pathological condition is selected from liver disorder
11. A method as claimed in claim 1, wherein the subject is selected from mammals and animals preferably humans.
12. A method as claimed in claim 1, wherein said composition is used singly or in combination with pharmaceutically acceptable carriers.
13. A method according to claim1 wherein said composition is administered to subject in combination with pharmaceutically acceptable additives, carriers, diluents, solvents, filters. Lubricants, excipients, binder or stabilizers.
14. A method as claimed in claim 1, wherein the desired dosage is administered for both preventive and curative properties.

15. A method as claimed in claim 1, wherein said composition is administered, orally or by any clinically/medically accepted methods.

16. A method as claimed in claim 1, wherein the preferred dosage for human beings is about 5 mg/Kg of body weight.

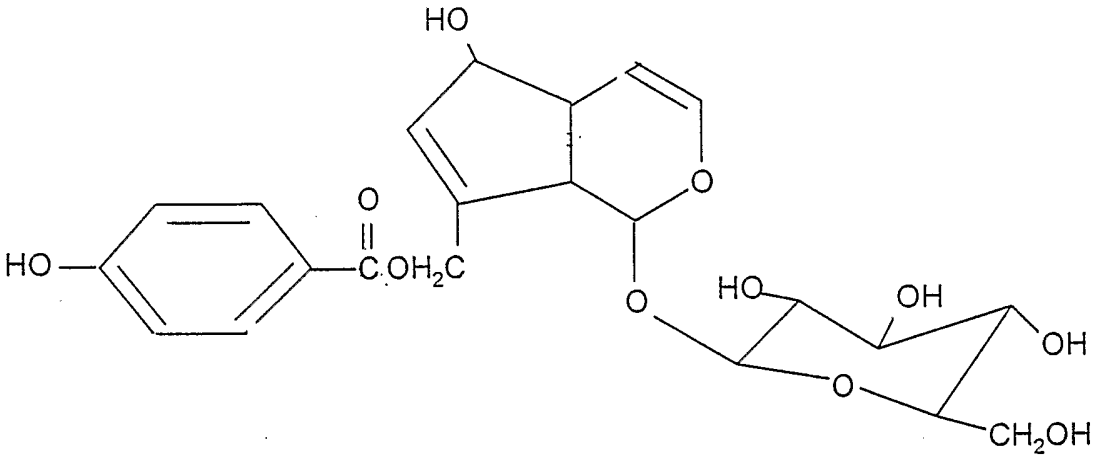
17. State the various physical forms in which the composition is available, e.g. powder, tablet, capsule, syrup, granules, emulsion, aerosoal, or beads.

18. A method as claimed in claim 1, is useful for Liver cirrhosis, Galactosemia, Hemoanigoma, Hemochromatosis, Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E, Hepatitis G, Alcholic Liver disease, Autoimmune hepatitis, Cancer of Liver, Biliary Atresia, Glycogen Storage Disease 1, Alpha-1-antitrypsin deficiency, Alagille syndrome, Byler Disease, Caroli disease, Fatty liver, Itching in Liver, Primar Biliary Cirrhosis, Sclerosing Cholangitis or Protoporphyria Erythroepatic.

19. A process for the isolation of compound 10-O-p-hydroxybenzoylaucubin compound of Formula 1, also called Agnuside, said compound isolated from aerial part/whole body comprising of steps:

1. powdering the plant material by known methods
2. preparing the aqueous alcoholic extract by percolation
3. concentrating the alcoholic extract by conventional method
4. removing fatty non-polar constituents by triturating the extract with solvents such as ethylene chloride, methylene chloride, chloroform or ethyl acetate
5. adsorbing the residue extract over silica gel
6. isolating of 2′-p-Hydroxy benzoyl mussaenosidic acid from the adsorbed extract by column chromatography

Figure 1

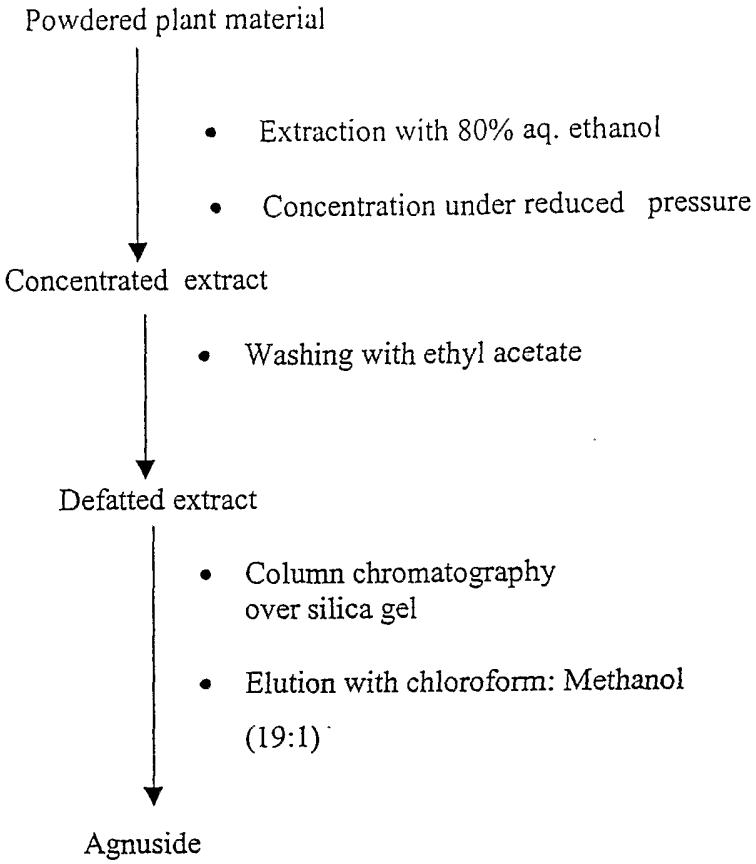


1

Agnuside

Figure 2

Flowsheet for isolation of 10-O-p-hydroxybenzoylaucubin) (Agnuside)



INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED

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IPC 7 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)

CHEM ABS Data, EMBASE, EPO-Internal, INSPEC, FSTA, SCISEARCH, BIOSIS, MEDLINE, 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.
X	DUTTA P ET AL: "STUDIES ON INDIAN MEDICINAL PLANTS-PART LXXV. NISHINDASIDE, A NOVEL IRIDOID GLYCOSIDE FROM VITEX NEGUNDO" TETRAHEDRON (1983) 39 (19) P. 3067-3072., XP001154066 INDIAN INST OF CHEM BIOLOGY, CALCUTTA 700 032 INDIA page 3071, left-hand column, line 10 -page 3071, right-hand column, line 13; table 1 --- -/--	19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 August 2003	11/09/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Venturini, F

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HOBERG, E. ET AL.: "An analytical high performance liquid chromatographic method for the determination of agnuside and p-hydroxybenzoic acid contents in Agni-casti fructus" PHYTOCHEMICAL ANALYSIS, vol. 11, pages 327-329, XP002251551 page 327, right-hand column, line 25 -page 328, left-hand column, line 17 ---	19
X	GÖRLER, K. ET AL.: "Iridoidführung von Vitex agnus-castus" PLANTA MEDICA, vol. 6, 1985, pages 530-531, XP002251552 the whole document ---	19
A	DAYRIT F M ET AL: "IDENTIFICATION OF FOUR IRIDOIDS IN THE PHARMACOLOGICALLY-ACTIVE FRACTION OF VITEX NEGUNDO, L" PHILIPPINE J SCI (1994) 123 (4) P. 293-304., XP002251549 PHILIPPINE INST PURE APPL CHEM, MANILA UNIV, QUEZON CITY MANILA the whole document ---	1-19
A	HOUGHTON P J ET AL: "ANTI-HEPATOTOXIC ACTIVITY OF EXTRACTS AND CONSTITUENTS OF BUDDLEJA SPECIES" PLANTA MEDICA, THIEME, STUTTGART, DE, vol. 55, no. 2, 1989, pages 123-126, XP009012740 ISSN: 0032-0943 the whole document ---	1-19
A	CN 1 258 545 A (WEN TIANCHENG) 5 July 2000 (2000-07-05) abstract ---	1-19
A	CN 1 200 290 A (WANG HUAIYUAN) 2 December 1998 (1998-12-02) abstract ---	1-19
A	CN 1 264 595 A (WANG HUAIYUAN) 30 August 2000 (2000-08-30) abstract ---	

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INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/IB 03/01810

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	OKUYAMA, E. ET AL.: "Pharmacologically active components of Viticis Fructus (Vitex rotundifolia). II. The components having analgesic effects" CHEM. PHARM. BULL, vol. 46, no. 4, pages 655-662, XP001154068 Chart 1. page 655, right-hand column, line 10-21; figures 4,5	1-18
X	page 660, right-hand column, line 8 -page 661, left-hand column, line 26	19
A	KURUÜZÜM-UZ, A. ET AL.: "Glucosides from Vitex agnus-castus" PHYTOCHEMISTRY, XP002251550 page 962, right-hand column, line 17-35	19
A	HÄNSEL, R. ET AL.: "Chemotaxonomische Untersuchungen in der Gattung Vitex L." PHYTOCHEMISTRY, vol. 4, 1965, pages 19-27, XP009015821 the whole document	19

INTERNATIONAL SEARCH REPORT

Int. tional application No.
PCT/IB 03/01810

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/IB 03/01810

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 1258545 A	05-07-2000	NONE	
CN 1200290 A	02-12-1998	NONE	
CN 1264595 A	30-08-2000	NONE	