



(51) International Patent Classification:

A61K 9/20 (2006.01) *A61P 3/04* (2006.01)
A61K 36/53 (2006.01) *A61P 3/00* (2006.01)

(21) International Application Number:

PCT/KR2013/006393

(22) International Filing Date:

17 July 2013 (17.07.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

10-2012-0078349 18 July 2012 (18.07.2012) KR

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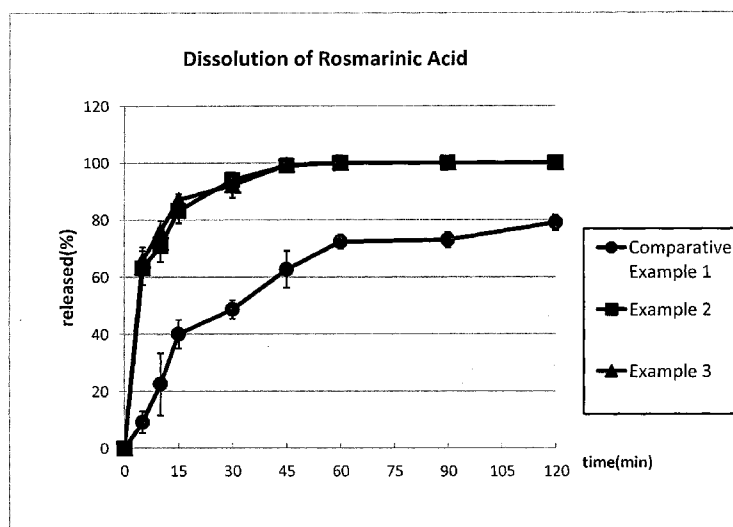
(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KZ,
LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: TABLET COMPRISING MELISSA OFFICINALIS FOLIUM EXTRACT FOR PREVENTING OR TREATING ANGIOGENESIS OR MMP ACTIVITY-MEDIATED DISEASE



(57) Abstract: The present invention relates to a tablet for preventing or treating angiogenesis or matrix metalloproteinase (MMP) activity-mediated disease, the tablet comprising Melissa (*Melissa officinalis*) folium extract having rosmarinic acid, caffeic acid, and rutin as major active ingredients, and a pharmaceutically acceptable disintegrator. The tablet in which the dissolution rates of the rosmarinic acid or the caffeic acid are 80% or more within 30 minutes has an excellent release rate and is stable under accelerated conditions, and thus the tablet may be used effectively for the prevention or the treatment of angiogenesis or MMP activity-mediated disease, in particular, obesity.

DESCRIPTION

5 **TABLET COMPRISING MELISSA OFFICINALIS FOLIUM EXTRACT FOR PREVENTING OR TREATING ANGIOGENESIS OR MMP ACTIVITY-MEDIATED DISEASE**

FIELD OF THE INVENTION

10 The present invention relates to a tablet for preventing or treating angiogenesis or matrix metalloproteinase (MMP) activity-mediated disease, the tablet comprising *Melissa officinalis* folium extract in which rosmarinic acid, caffeic acid, and rutin are major active ingredients, and a pharmaceutically acceptable disintegrator.

15

BACKGROUND OF THE INVENTION

 Melissa (*Melissa officinalis*), a perennial herb in a Labiatae family, is also called lemon balm.

20

 Melissa folium extract contains flavonoids, terpene acids, volatile oils, glycosides of alcoholic and phenolic compounds, and caffeic acid derivatives. In particular, flavonoids which are contained abundantly in *Melissa officinalis* folium include cynaroside, cosmosin, rhamnocitrin, and isoquercitrin; and ursolic acid is contained as terpene acid. Among nonvolatile components, rosmarinic acid is contained, and caffeic acid derivatives are contained in an amount of about 4.7%. Among volatile oils, geraniol, neral, citronellal, and eugenol are contained. Above this, coumaric acid, a kind of polyphenol, and cinnamic acid having weak aromaticity, and so on, are contained in Melissa folium extract.

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 Roles of major pharmacological components in Melissa folium extract are described as follows:

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 Rosmarinic acid has strong anti-inflammatory and antipyretic effect, and essential oils contained in folium have been used in the treatment of diseases, in particular, depression, neurogenic headache, memory loss, neuralgia, fever, and the like. Above this, rosmarinic acids are also known to

have sedative, antibacterial, antiviral, and antihormonal effects. Currently, dried *Melissa folium* extract which is known to contain a large amount of rosmarinic acid has been used as a blood circulation enhancer component.

5 Caffeic acid is contained in coffee and plants, such as fruits, vegetables, herbs, and so on. It is known to act as a carcinogenic inhibitor, and as an excellent antioxidant both *in vivo* and *in vitro*. Absorption of caffeic acid is limited when its chlorogenic acid form is esterified to quinic acid, and oral administration of high doses of caffeic acid in rats has caused stomach papillomas, and caffeic acid was recognized as a carcinogen in those rats.
10 Administration of high doses of caffeic acid combined with antioxidants showed a decrease in growth of colon tumors.

Rutin is a flavonol glycoside substance, and is widely distributed in nature. Its major physiological activity is a capillary contraction effect, and has been used as a hemostatic; however, the action is not so strong, and
15 temporary. Also, it has a capillary-strengthening effect, and can be used for preventing or treating cerebral hemorrhage, radiation sickness, hemorrhagic diseases, etc.

Meanwhile, angiogenesis refers to the process through which new capillaries form from pre-existing vessels. Under normal circumstances,
20 angiogenesis occurs during embryo development, tissue regeneration and wound healing, and development of corpus luteum, the periodic changes in the female reproductive system, and is strictly regulated during these processes.

For adults, vascular endothelial cells grow very slowly, and do not divide very well in a relative sense as compared to other kinds of cells.
25 However, adult angiogenesis is induced as necessary by hormone signal response, release of pro-angiogenic cytokines from inflammatory cells, activation of hydrolytic enzymes which release isolated angiogenic mediators to extracellular matrix, or stimulation of angiogenic factors. The process of angiogenesis is generally composed of degradation of basement membrane on
30 blood vessels induced by proteases, migration of vascular endothelial cells, lumen formation via proliferation and differentiation of vascular endothelial cells to reconstruct blood vessels, and formation of new capillaries. One of the most important events in the process of angiogenesis is an enzymatic breakdown of basement membrane on blood vessels which surrounds vascular
35 endothelial cells. Enzymes playing an important role therein are the enzymes

which belong to matrix metalloproteinase (MMP).

It is known that when angiogenesis is not autonomically regulated, or MMP which plays an important role in angiogenesis is over-activated, blood vessels grow abnormally, and thereby causing various diseases, *e.g.*, cancer growth and metastasis; cardiovascular diseases such as angioma, angiofibroma, 5 vascular deformity, atherosclerosis, angiostenosis, edemic sclerosis and restenosis; ophthalmological diseases, such as diabetic retinopathy, macular degeneration (including senescence-related macular degeneration such as age-related macular degeneration), pterygium, retinal degeneration, angiogenesis 10 associated with corneal transplantation, angiogenic glaucoma, angiogenic corneal diseases, retrolental fibroplasias, granular conjunctivitis, corneal ulcer, proliferative vitreous body retinopathy, immature retinopathy, eye inflammation, conical cornea, Sjogren's syndrome, myopia, eye tumors, and rejection in corneal transplantation; obesity, and the like (*see* Polverini PJ, 15 *Critical Reviews in Oral Biology*, 6(3), 1995, 230-247; Arup Das, *et al.*, *Progress in Retinal and Eye Research*, 22, 2003, 721-748; Nick Di Girolamo, *et al.*, *IOVS*, Vol. 42, No.9, August 2001, 1963-1968; Patricia Lee, *et al.*, *Survey of ophthalmology*, vol 43, No. 3, Nov-Dec 1998, 245-269; D.B. Holland, *et al.*, *British Journal of Dermatology*, 150, 2004, 72-81; Anthony H Vagnucci Jr, *et al.*, *The Lancet*, vol 361, Feb. 15, 2003, 605-608; Berislav V. Zlokovic, *Trends in Neuroscience*, Vol. 28, No.4, April 2005, 202-208; Jaap G. Neels, *et al.*, *The FASEB Journal express article* 10. 1096/fj.03-1101fje. Published online April 14, 2004; D.L. Crandall, *et al.*, *Microcirculation*, 4, 1997, 211-232; G. Voros, *et al.*, *Endocrinology*, 146, 2005, 4545-4554; M.A. Rupnick, *et al.*, *PNAS*, 99, 20 2002, 10730-10735; E. Brakenhielm, *et al.*, *Circ. Res.*, 94, 2004, 1579-1588; H.R. Lijnen, *et al.*, *Arterioscler Thromb Vasc Biol.*, 22, 2002, 374-379; and D. Demeulemeester, *et al.*, *Biochem. Biophys. Res. Commun.*, 329, 2005, 105-110).

Therefore, development of angiogenesis inhibitors or MMP activity inhibitors can lead to treatment of these diseases.

30 In this regard, Korean Patent No. 10-550298 discloses that *Melissa folium* extract has an anti-angiogenic effect through an experiment of suppressing formation of tubes such as capillaries using human umbilical vein endothelial cells (HUVEC), and *in vivo* assay using a mouse Matrigel model and chorioallantoic membrane (CAM) of chick embryo experiment.

35 Korean Patent No. 10-473688 further discloses that *Melissa folium*

extract has an inhibitory effect on the activity of MMP (matrix metalloproteinase), which plays an important role in degradation of the basement membrane, which surrounds vascular endothelial cells, the important process of angiogenesis.

5 Korean Patent No. 10-903030 discloses a method for increasing efficacy of Melissa folium extract, which includes the steps of: (i) Melissa folium extract is extracted with 50 to 100% ethanol or methanol, followed by drying; (ii) the alcohol extract is suspended in water, fractionated with ethyl acetate, followed by drying; and (iii) the ethyl acetate fraction is resuspended in
10 water, followed by drying to obtain the ethyl acetate fraction of Melissa folium. In addition, examples of hard capsules including Melissa folium extract are disclosed therein; however, when Melissa folium extract is prepared in a capsule formulation, release of active ingredients is not easy due to capsule base compounds, and drug release is nonfavorable due to agglomeration with
15 capsule base compounds. When the Melissa folium extract-containing capsule formulation is left under the condition of high temperature and humidity, the release rates of active ingredients become slower, and therefore, it is not suitable for Melissa folium extract-containing capsule to exhibit desired medicinal effects.

20 However, the above prior art does not consider sufficiently active ingredients which exhibit medicinal effects derived from Melissa folium extract and suitable formulations to administer to the body.

Therefore, the present inventors have studied the effects of drug metabolism depending on the release rates of major active ingredients derived
25 from Melissa folium extract, and observed that efficacies vary depending on dissolution profiles and formulations of pharmaceutical compositions comprising Melissa folium extract, thereby completing the present invention.

SUMMARY OF THE INVENTION

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Therefore, it is an object of the present invention to provide a pharmaceutical formulation including Melissa folium extracts, in which rosmarinic acid, caffeic acid, and rutin are major active ingredients and which has angiogenic inhibitory and abdominal obesity-treating effects, and showing
35 excellent drug metabolism.

To achieve the above object, the present invention provides a tablet for preventing or treating angiogenesis or MMP activity-mediated disease, wherein the tablet comprises Melissa folium extract in which rosmarinic acid, caffeic acid, and rutin are major active ingredients, and a pharmaceutically acceptable
5 disintegrator, and the dissolution rates of rosmarinic acid or caffeic acid are 80% or more within 30 minutes.

Since tablets in accordance with the present invention are stable even under accelerated conditions, have excellent release rates of active ingredients, and exhibit dissolution profiles which can maximize the effectiveness in
10 treating or preventing angiogenesis or MMP activity-mediated disease, the tablets can be used effectively for the prevention or the treatment of the above diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

15 The above and other objects and features of the present invention will become apparent from the following description of the invention, when taken in conjunction with the accompanying drawings, which respectively show:

FIG. 1 and FIG. 2 show the test results of rosmarinic acid and caffeic
20 acid dissolution from formulations in Examples 2 and 3, and Comparative Example 1, respectively;

FIGs. 3 to 5 show the results of content stability test for rosmarinic acid, caffeic acid, and rutin from formulations in Examples 2 and 3, and Comparative Example 1, respectively, according to storage period under
25 accelerated conditions;

FIG. 6 and FIG. 7 show the results of dissolution stability test for rosmarinic acid and caffeic acid from formulations in Examples 2 and 3, and Comparative Example 1, respectively, according to storage period under
accelerated conditions; and

30 FIG. 8 shows the changes in rat body weight by administration of formulations in Comparative Examples 2 and 3, and Example 4.

DETAILED DESCRIPTION OF THE INVENTION

35 The present invention provides a tablet for preventing or treating

angiogenesis or MMP activity-mediated disease, wherein the tablet comprises Melissa folium extract having rosmarinic acid, caffeic acid, and rutin as major active ingredients, and a pharmaceutically acceptable disintegrator, and the dissolution rates of rosmarinic acid or caffeic acid are 80% or more within 30 minutes.

In the present invention, "dissolution rate" refers to the value measured as follows: 900 mL of purified water was used as a dissolution test liquid and the dissolution test was performed at 37 ± 0.5 °C, 50 rpm of rotation speed in accordance with Paddle method, Method 2 in the general test methods of the Korean Pharmacopoeia. After a specific time, the dissolution liquid was filtered with a 0.45 µm filter. Using liquid chromatography (HPLC) analysis method in the general test methods of the Korean Pharmacopoeia, mobile phases were operated at a rate of 1 mL per minute according to concentration gradient condition in mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol) described in the following. 10 µL of a sample was injected into an analytical column, *e.g.*, Zorbax Eclipse Plus C18 (150 x 4.6 mm) column, and effective ingredients were analyzed to measure at 285 nm wavelength.

<Concentration gradient in the mobile phase>

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
5	100	0
25	0	100
35	0	100
35.1	100	0
45	100	0

In accordance with one aspect of the present invention, the dissolution rate of rosmarinic acid or caffeic acid in the tablet according to the present invention is 80% or more within 15 minutes (see FIGs. 6 and 7).

In addition, the tablet in the present invention comprises Melissa folium extract in an amount of 10 to 90% by weight, based on the total weight of the tablet. In one embodiment of the present invention, the tablet of the present invention comprises Melissa folium extract in an amount of 50% by weight to

90% by weight, based on the total weight of the tablet.

The tablet containing Melissa folium extract of the present invention can make the release of active ingredients easy, and thus can release 80% or more of active ingredients such as rosmarinic acid or caffeic acid within 30 minutes, and besides, even if it is left under the accelerated conditions of high temperature and humidity, release rates of its ingredients hardly change and are stable, thus it can exhibit excellent medicinal effects. Medicines prepared with natural product extracts are characterized in that active ingredients are admixed therein, and thus, formulations having rapid disintegration ability are favorable so that effective ingredients may exert their medicinal effects simultaneously. Therefore, a pharmaceutical composition comprising Melissa folium extract may be in a form of tablet, similar to the present invention.

In the present invention, Melissa folium extract may be prepared in accordance with the following procedure using conventional extraction methods which use ethanol. First, Melissa folium is reflux-extracted with 10 to 40 times its amount by weight of 50 to 100% ethanol, preferably 70 to 80% ethanol, and more preferably 75% ethanol, and then filtered to complete a first concentration procedure. Subsequently, a procedure for fractionation of the above extract with a mixture of ethyl acetate and water, *e.g.*, ethyl acetate:water = 1:1 (v/v), is repeated two times. The resulting ethyl acetate layer from the above procedures is collected, concentrated secondarily, dried and pulverized to prepare Melissa folium extract according to the present invention. The extract prepared above is used as a dried ethyl acetate-extract of Melissa folium in the present invention.

Dried Melissa folium, non-dried Melissa folium, or a mixture thereof may be used. For effective extraction, Melissa folium may be cut into small pieces to use.

According to the present invention, the Melissa folium extract comprises 5% by weight or more of rosmarinic acid, 0.5% by weight or more of caffeic acid, and 1% by weight or more of rutin, based on the total weight of the extract, and may comprise preferably 5 to 10% by weight of rosmarinic acid, 0.5 to 1% by weight of caffeic acid, and 1 to 2% by weight of rutin. In accordance with one aspect of an exemplary embodiment of the present invention, the dried ethyl acetate-extract of Melissa folium according to the present invention comprises about 5.5% by weight of rosmarinic acid, about

0.65% by weight of caffeic acid, and about 1.42% by weight of rutin (*see* Table 2).

Also, the *Melissa folium* extract may comprise rosmarinic acid and caffeic acid at a weight ratio of 1:0.1 to 1:0.2, caffeic acid and rutin at a weight ratio of 1:1 to 1:4, more specifically at a weight ratio of 1:1 to 1:3, and rosmarinic acid and rutin at a weight ratio of 1:0.1 to 1:0.3.

In addition, the tablet according to the present invention comprises a pharmaceutically acceptable disintegrator so as to achieve the aforementioned dissolution rate, *i.e.*, the dissolution rates of 80% or more of rosmarinic acid or caffeic acid within 15 minutes. The disintegrator may be selected from the group consisting of sodium starch glycolate (SSG), crospovidone, croscarmellose sodium, low-substituted hydroxypropyl cellulose, and a mixture thereof, and may be comprised in an amount of 1 to 10% by weight, based on the total weight of the tablet.

The tablet according to the present invention may further comprise a pharmaceutically acceptable additive selected from the group consisting of excipients, lubricants, binders, and a mixture thereof, and may be prepared according to conventional methods, by mixing the dried ethyl acetate-extract of *Melissa folium* and the pharmaceutically acceptable additive, and then performing direct compression, or tablet pressing in a dry or wet way.

The excipient may be selected from the group consisting of microcrystalline cellulose (MCC), light anhydrous silicic acid, mannitol, lactose, calcium dihydrogen phosphate, starch, low-substituted hydroxypropyl cellulose, and a mixture thereof, and may be comprised in an amount of 7 to 87% by weight, based on the total weight of the tablet.

The lubricant may be selected from the group consisting of stearic acid, metal salts of stearic acid such as magnesium stearate and calcium stearate, talc, sucrose fatty acid esters, hydrogenated vegetable oil, high melting point waxes, glyceryl fatty acid esters, glycerol dibehenate, and a mixture thereof, and may be comprised in an amount of 0.2 to 5% by weight, based on the total weight of the tablet.

The binder may be selected from the group consisting of hydroxypropyl cellulose (HPC-L), hydroxypropyl methylcellulose, polyvinylpyrrolidone, copovidone, and a mixture thereof, and may be comprised in an amount of 1 to 10% by weight, based on the total weight of the tablet.

The tablet according to the present invention can be used for the prevention or the treatment of angiogenesis-related diseases or MMP-mediated diseases, *e.g.*, a disease selected from the group consisting of cancer growth and metastasis; cardiovascular diseases, such as angioma, angiofibroma, vascular deformity, atherosclerosis, angiostenosis, edemic sclerosis and restenosis; ophthalmological diseases such as diabetic retinopathy, macular degeneration (including senescence-related macular degeneration such as age-related macular degeneration), pterygium, retinal degeneration, angiogenesis associated with corneal transplantation, angiogenic glaucoma, angiogenic corneal diseases, retrolental fibroplasias, granular conjunctivitis, corneal ulcer, proliferative vitreous body retinopathy, immature retinopathy, eye inflammation, conical cornea, Sjogren's syndrome, myopia, eye tumors, and rejection in corneal transplantation; and obesity, and preferably obesity.

Therefore, the present invention provides a method for preventing or treating angiogenesis or matrix metalloproteinase (MMP) activity-mediated disease in a subject, comprising administering the aforementioned tablet to the subject in need thereof, *e.g.*, a mammal, including human.

EXAMPLES

Hereinafter, the present invention will be described in more detail with reference to the following examples. However, the following examples are provided for illustrative purposes only, and the scope of the present invention should not be limited thereto in any manner.

Example 1: Analyses of Components and Ratio in Dried Ethyl Acetate-Extract of *Melissa Folium*

To extract effective ingredients from raw *Melissa folium*, extracts were prepared as follows and used for component analysis.

Specifically, 200 kg of dried leaves of *Melissa officinalis* was reflux-extracted with 75% ethanol amounting to 20 times the weight thereof for 4 hours, and filtered through a cartridge filter, and concentrated firstly under reduced pressure. The thus obtained concentrate (amount: 50 kg) was fractionated with a mixture of ethyl acetate and water (1:1(v/v)) (amount: 50 kg), which was repeated two times. Subsequently, the ethyl acetate layer was

collected, concentrated secondarily under reduced pressure, dried and pulverized to obtain an ethyl acetate extract of *Melissa folium* (amount: 8.2 kg). The above procedures were performed in triplicate to determine reproducibility for the extract, and the components comprised in the *Melissa folium* extract and the ratio of components were analyzed as follows.

The *Melissa folium* extract was analyzed by a liquid chromatography (HPLC). 10 μ L of the ethyl acetate extract of *Melissa folium* prepared above was injected into a Zorbax Eclipse Plus C18 (150 $\times$ 4.6 mm) column, and then, mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol) were eluted according to the ratio described in the following Table 1 at a flow rate of 1 mL/min, and the eluted effective ingredients were analyzed at 285 nm.

[Table 1]

Time (min)	Mobile phase A	Mobile phase B
0	100	0
5	100	0
25	0	100
35	0	100
35.1	100	0
45	100	0

As a result of analyzing effective ingredients in the *Melissa folium* extract, the *Melissa folium* extract contained on average about 5.5% by weight of rosmarinic acid, about 0.65% by weight of caffeic acid, and about 1.42% by weight of rutin, as shown in the following Table 2. Based on the analysis results, it is confirmed that rosmarinic acid and caffeic acid may be present at a weight ratio of 1:0.1 to 1:0.2, caffeic acid and rutin may be present at a weight ratio of 1:1 to 1:3, and rosmarinic acid and rutin may be present at a weight ratio of 1:0.1 to 1:0.3 in the *Melissa folium* extract.

It is considered that when the weight ratio of rosmarinic acid, caffeic acid, and rutin is appropriately balanced, and is maintained to be constant, the ethyl acetate extract of *Melissa folium* can exhibit desirable medicinal effects.

It is also considered to be preferable for the reproducibility of medicinal effects that 5.0% by weight or more of rosmarinic acid, 0.5% by weight or

more of caffeic acid, and 1.0% by weight or more of rutin are contained, based on the total weight of the Melissa folium extract, and this suggests that it is important to manage the contents continually and reproducibly to maintain the reproducibility of medicinal effects.

5

[Table 2]

	Content (% by weight)		
	Rosmarinic acid	Caffeic acid	Rutin
1	5.8	0.63	1.38
2	5.7	0.69	1.41
3	5.0	0.63	1.47
Mean	5.5	0.65	1.42

Example 2: Preparation of Tablets Comprising Melissa Folium Extract – (1)

10

According to the amounts (unit: mg) described in the following Table 3, tablets were prepared by admixing microcrystalline cellulose (MCC, Avicel PH101, FMC bio polymer) and light anhydrous silicic acid (Aerosil 200, Degussa) as excipients, sodium starch glycolate (SSG, YUNG ZIP Chem) as a disintegrator, and magnesium stearate (S-Mg, NOF Corporation) as a lubricant to the dried ethyl acetate-extract of Melissa folium prepared in Example 1, and then performing direct compression.

15

Example 3: Preparation of Tablets Comprising Melissa Folium Extract – (2)

20

According to the amounts (unit: mg) described in the following Table 3, granules were prepared by mixing the dried ethyl acetate-extract of Melissa folium prepared in Example 1 with MCC and SSG, mixing the mixture homogeneously with a binder liquid, hydroxypropyl cellulose (HPC-L, Shin-Etsu) dissolved in water, and then granulating the resulting mixture. The prepared granules were dried at 50 °C for 2 hours, passed through a 20-mesh sieve, mixed with light anhydrous silicic acid as an excipient and S-Mg as a lubricant, and then compressed to prepare a tablet.

25

Comparative Example 1: Preparation of Capsules Comprising Melissa Folium Extract

According to the amounts (unit: mg) described in the following Table 3, capsules were prepared by mixing the dried ethyl acetate-extract of Melissa folium prepared in Example 1 with MCC and light anhydrous silicic acid as excipients and S-Mg as a lubricant, and then, filling the mixture into Size No. 0 capsules.

[Table 3]

Components	Comparative Example 1	Example 2	Example 3
Dried ethyl acetate-extract of Melissa folium	300.0	300.0	300.0
Microcrystalline Cellulose	165.0	225.0	175.0
SSG	-	54.0	54.0
HPC-L	-	-	6.0
(Purified water)	-	-	(60.0)
Light anhydrous silicic acid	5.0	15.0	15.0
Magnesium stearate (S-Mg)	5.0	6.0	6.0
Capsule	77.0	-	-
Total weight	552.0	600.0	556.0

Comparative Example 2: Preparation of Tablets for Rat Administration Not Including Melissa Folium Extract

Tablets prepared in Examples 2 and 3 have doses and sizes which are applicable to real humans (about 60 kg). For a body weight measurement experiment through rat administration, tablets for rat administration not including Melissa folium extract were prepared in a ratio which is nearly proportional to the ratio of a rat body weight (about 250 g) to a human body

weight.

According to the composition described in the following Table 4, tablets for rat administration were prepared by the same methods as in Example 3, except that the dried ethyl acetate-extract of *Melissa folium* is not added.

5

[Table 4]

Components	Comparative Example 2
Microcrystalline Cellulose	6.0
SSG	1.8
HPC-L	0.2
(Purified water)	(3.0)
SiO ₂	0.5
S-Mg	0.2
Total weight	8.7

Comparative Example 3: Preparation of Capsules for Rat Administration Including *Melissa folium* Extract

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According to the amounts (unit: mg) described in the following Table 5, capsules for rat administration were prepared by mixing the dried ethyl acetate-extract of *Melissa folium* prepared in Example 1 with MCC and light anhydrous silicic acid as excipients and S-Mg as a lubricant, and then, filling the mixture into Size No. 5 capsules.

15

Example 4: Preparation of Tablets for Rat Administration Including *Melissa folium* Extract

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According to the amounts (unit: mg) described in the following Table 5, tablets for rat administration were prepared by the same method as in Example 3.

[Table 5]

Components	Comparative Example 3	Example 4
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Dried ethyl acetate-extract of Melissa folium	10.0	10.0
Microcrystalline Cellulose	5.5	6.0
SSG	-	1.8
HPC-L	-	0.2
(Purified water)	-	(3.0)
SiO ₂	0.2	0.5
S-Mg	0.2	0.2
Capsules	10.0	-
Total weight	25.9	18.7

Test Example 1: Rosmarinic Acid and Caffeic Acid Dissolution Test

Dissolution tests for rosmarinic acid and caffeic acid, major active ingredients of Melissa folium extract, were performed on capsules of Comparative Example 1 and tablets of Examples 2 and 3.

For the dissolution test, 900 mL of water was used as a dissolution test medium and the dissolution test was performed at 37 ± 0.5 °C, 50 rpm of rotation speed in accordance with Paddle method, Method 2 in the general test methods of the Korean Pharmacopoeia. After 5, 10, 15, 30, 45, 60, 90, and 120 minutes from the start of the dissolution test, the dissolution medium were taken and filtered through a 0.45 µm filter, and subsequently, the dissolution rates of rosmarinic acid and caffeic acid were measured by the same method as HPLC analysis method described in Example 1. The results are shown in FIGs. 1 and 2.

As shown in Figs. 1 and 2, the release rate and the final dissolution rate of each active ingredient were higher in tablets prepared in Examples 2 and 3 as compared to capsules prepared in Comparative Example 1. In addition, it was found that both tablets prepared by a direct dry-tableting process in Example 2 and tablets prepared by a wet granulation process in Example 3 released 80% or more of rosmarinic acid and caffeic acid within 30 minutes.

Test Example 2: Test for Amounts of Effective Components under Accelerated Conditions

Capsules prepared in Comparative Example 1, and tablets prepared in Examples 2 and 3 were packed in HDPE bottles, and kept in the accelerated conditions (40 °C and 75% relative humidity) for 6 months, and then, the amounts of major active ingredients of the Melissa folium extract, *i.e.*, rosmarinic acid, caffeic acid and rutin, were determined to confirm stability of formulations. After 1, 3 and 6 months of storage under the accelerated conditions, the amounts of rosmarinic acid, caffeic acid, and rutin were measured, and the results are shown in FIGs. 3 to 5.

Consequently, there was no significant difference in stability between capsule formulation and tablet formulations prepared by the direct dry-tabletting and the wet granulation, and the amount of each component did not change significantly. In particular, the tablet according to the present invention met the standard of 5.0% by weight or more of rosmarinic acid, 0.5% by weight or more of caffeic acid, and 1.0% by weight or more of rutin, and accordingly, it was confirmed that they are stable under the accelerated storage condition as well.

Test Example 3: Determination of Changes in Dissolution under Accelerated Conditions

Capsules prepared in Comparative Example 1, and tablets prepared in Examples 2 and 3 were packed in HDPE bottles, and kept in the accelerated conditions (40 °C and 75% relative humidity) for 6 months, and then, the dissolution rates of rosmarinic acid and caffeic acid were measured by the same way as in Test Example 1. After 15, 30, and 60 minutes from the start of the dissolution test, medium samples were taken to evaluate the dissolution rates, which were then compared with the initial value to determine the degree of change, and the results are shown in FIGs. 6 and 7.

As shown in FIGs. 6 and 7, capsules prepared in Comparative Example 1 showed a slow initial release rate, and when they were kept under the accelerated conditions, the release rate slowed down consistently over time. It is considered that the release rate of the Melissa folium extract was affected by capsules. Since there is a possibility that the medicinal effects could be changed as dissolution slows down, a capsule is considered to be a

pharmaceutically undesirable formulation. All tablets of the present invention showed no significant changes in release rate and were stable.

Test Example 4: Administration to Animals and Measurement of Body Weight Change

To examine *in vivo* efficacy of the Melissa folium extract on high-fat diet-induced obesity, the following experiment was carried out. After a one-week acclimation period, 7-week-old rats were divided into groups of 3 animals per group. The rats fed with 45 kcal% high-fat feed for 8 weeks, while being administered with the tablets of Comparative Examples 2 and 3, and Example 4 one time per day (a daily dose of the Melissa folium extract: 10 mg) using zoned. Then, body weights of the rats were measured, and the results are shown in FIG. 8. Comparison and analysis of the mean body weight of individuals showed that each result was significant in every case ($p < 0.05$).

As shown in FIG. 8, body weight of the group administered with the tablet not including the Melissa folium extract prepared in Comparative Example 2 increased rapidly over time. Meanwhile, the group administered with the capsule comprising the Melissa folium extract prepared in Comparative Example 3 showed the relatively high amount of body weight gain as compared to the group administered with the tablet prepared in Example 4. This result is considered to be interrelated with the dissolution test results in Experimental Example 1, and suggests that the release rates of rosmarinic acid and caffeic acid, which are effective components of the Melissa folium extract, play an important role in exhibiting the medicinal effects of the Melissa folium extract. In other words, if the Melissa folium extract is prepared in a capsule formulation as prepared in Comparative Example 3, and pharmaceutically active ingredients are not released well, the amounts of the components absorbed into a body would be small, and thus the medicinal effects would not be exhibited.

Therefore, the most appropriate formulation for the Melissa folium extract was found to be the tablet formulation, from which rosmarinic acid and caffeic acid, the major ingredients of the Melissa folium extract, are released 80% or more within 30 minutes, thereby rapidly exhibiting the medicinal effects, and which show little changes in dissolution during storage.

What is claimed is:

1. A tablet for preventing or treating angiogenesis or matrix metalloproteinase (MMP) activity-mediated disease, wherein the tablet comprises Melissa (*Melissa officinalis*) folium extract having rosmarinic acid, caffeic acid, and rutin as major active ingredients, and a pharmaceutically acceptable disintegrator, and the dissolution rates of rosmarinic acid or caffeic acid thereof are 80% or more within 30 minutes.

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2. The tablet of claim 1, wherein the dissolution rate of the rosmarinic acid or the caffeic acid is 80% or more within 15 minutes.

3. The tablet of claim 1, wherein the Melissa folium extract is prepared by reflux-extracting Melissa folium with 50 to 100% ethanol, followed by filtering and concentrating; collecting an ethyl acetate layer produced by repeating a procedure of fractionation of the obtained concentrate with ethyl acetate and water two times; and subsequently, concentrating, drying, and pulverizing the ethyl acetate layer.

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4. The tablet of claim 1, wherein the Melissa folium extract comprises 5 to 10% by weight of the rosmarinic acid, 0.5 to 1% by weight of the caffeic acid, and 1 to 2% by weight of the rutin, based on a total weight of the extract.

5. The tablet of claim 1, wherein the Melissa folium extract comprises the rosmarinic acid and the caffeic acid at a weight ratio of 1:0.1 to 1:0.2.

6. The tablet of claim 1, wherein the Melissa folium extract comprises the caffeic acid and the rutin at a weight ratio of 1:1 to 1:4.

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7. The tablet of claim 1, wherein the Melissa folium extract comprises the rosmarinic acid and the rutin at a weight ratio of 1:0.1 to 1:0.3.

8. The tablet of claim 1, wherein the pharmaceutically acceptable disintegrator is selected from the group consisting of sodium starch glycolate,

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crospovidone, croscarmellose sodium, low-substituted hydroxypropyl cellulose, and a mixture thereof.

9. The tablet of claim 1, wherein the pharmaceutically acceptable
5 disintegrator is comprised in an amount of 1 to 10% by weight, based on a total weight of the tablet.

10. The tablet of claim 1, which further comprises a pharmaceutically
acceptable additive selected from the group consisting of excipients, lubricants,
10 binders, and a mixture thereof.

11. The tablet of claim 10, which comprises 10 to 90% by weight of the
Melissa folium extract, 1 to 10% by weight of the disintegrator, 7 to 87% by
weight of the excipient, 0.2 to 5% by weight of the lubricant, and 1 to 10% by
15 weight of the binder, based on a total weight of the tablet.

12. The tablet of claim 1, wherein the angiogenesis or MMP activity-
mediated disease is a disease selected from the group consisting of cancer growth
and metastasis; angioma, angiofibroma, vascular deformity, atherosclerosis,
20 angiostenosis, edemic sclerosis and restenosis; diabetic retinopathy, macular
degeneration, pterygium, retinal degeneration, angiogenesis associated with
corneal transplantation, angiogenic glaucoma, angiogenic corneal diseases,
retrolental fibroplasias, granular conjunctivitis, corneal ulcer, proliferative
vitreous body retinopathy, immature retinopathy, eye inflammation, conical
25 cornea, Sjogren's syndrome, myopia, eye tumors, and rejection in corneal
transplantation; and obesity.

13. The tablet of claim 12, wherein the angiogenesis or MMP activity-
mediated disease is obesity.
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14. A method for preventing or treating angiogenesis or matrix
metalloproteinase (MMP) activity-mediated disease in a subject, comprising
administering the tablet of claim 1 to a subject in need thereof.

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FIG. 1

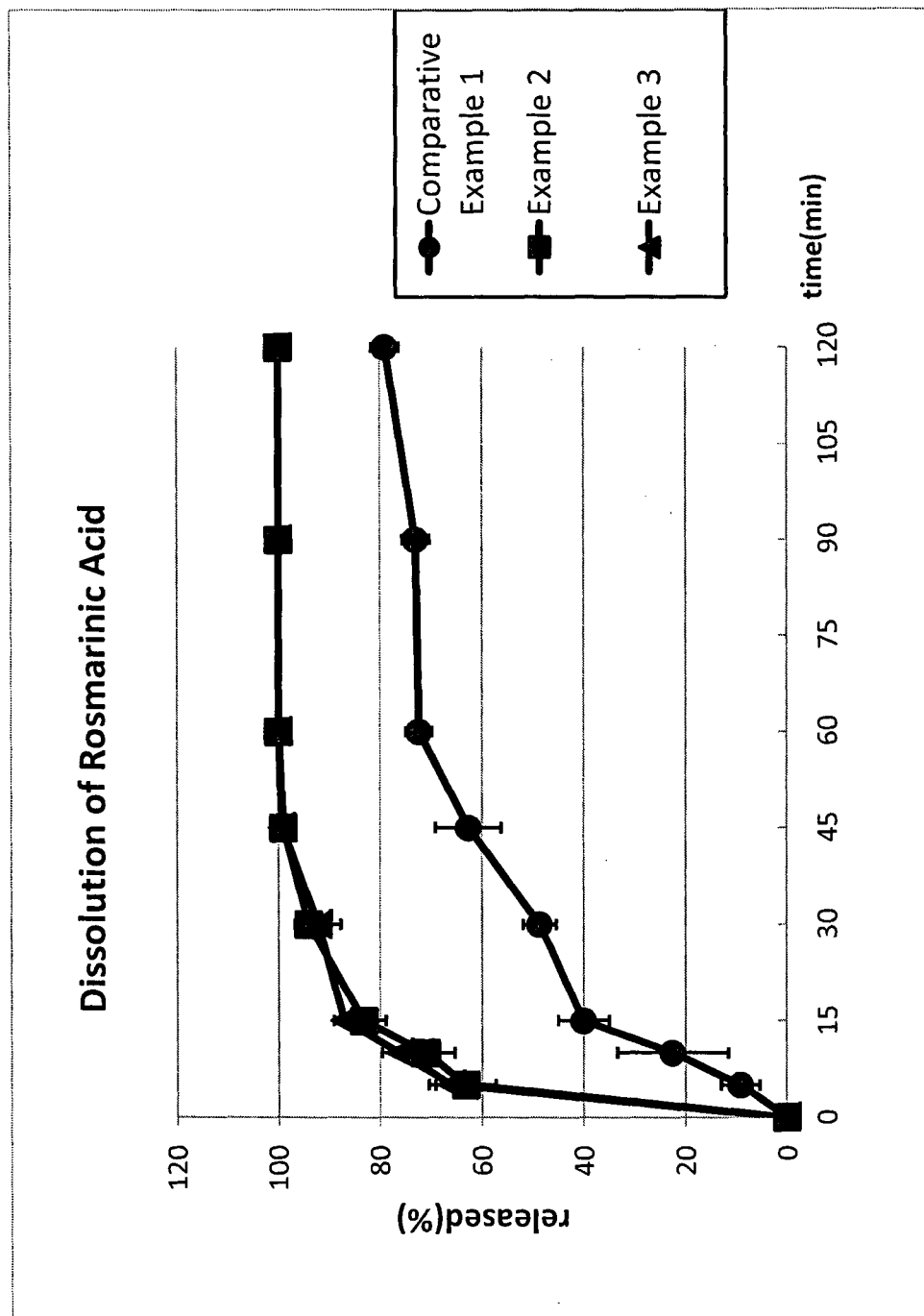


FIG. 2

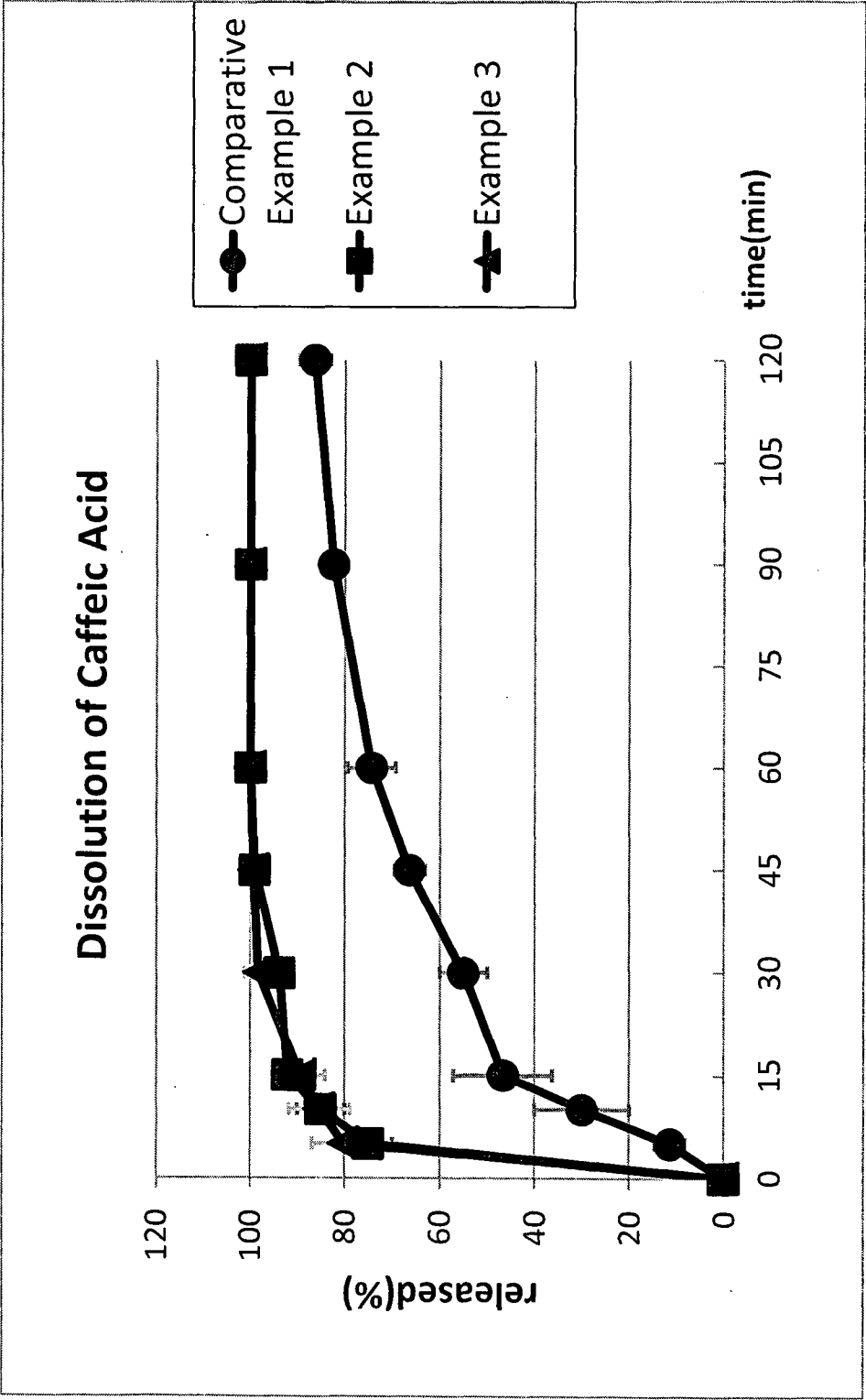


FIG. 3

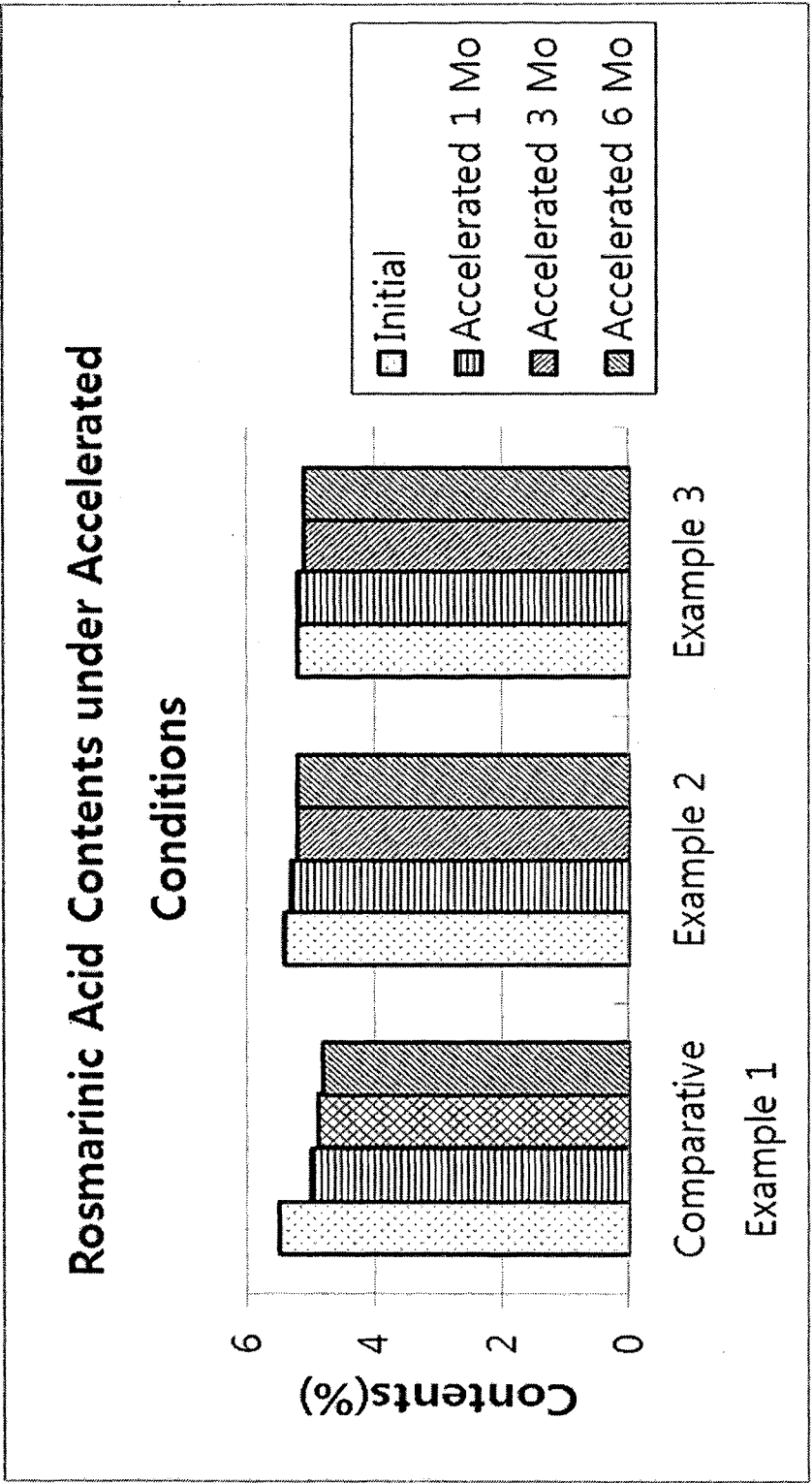
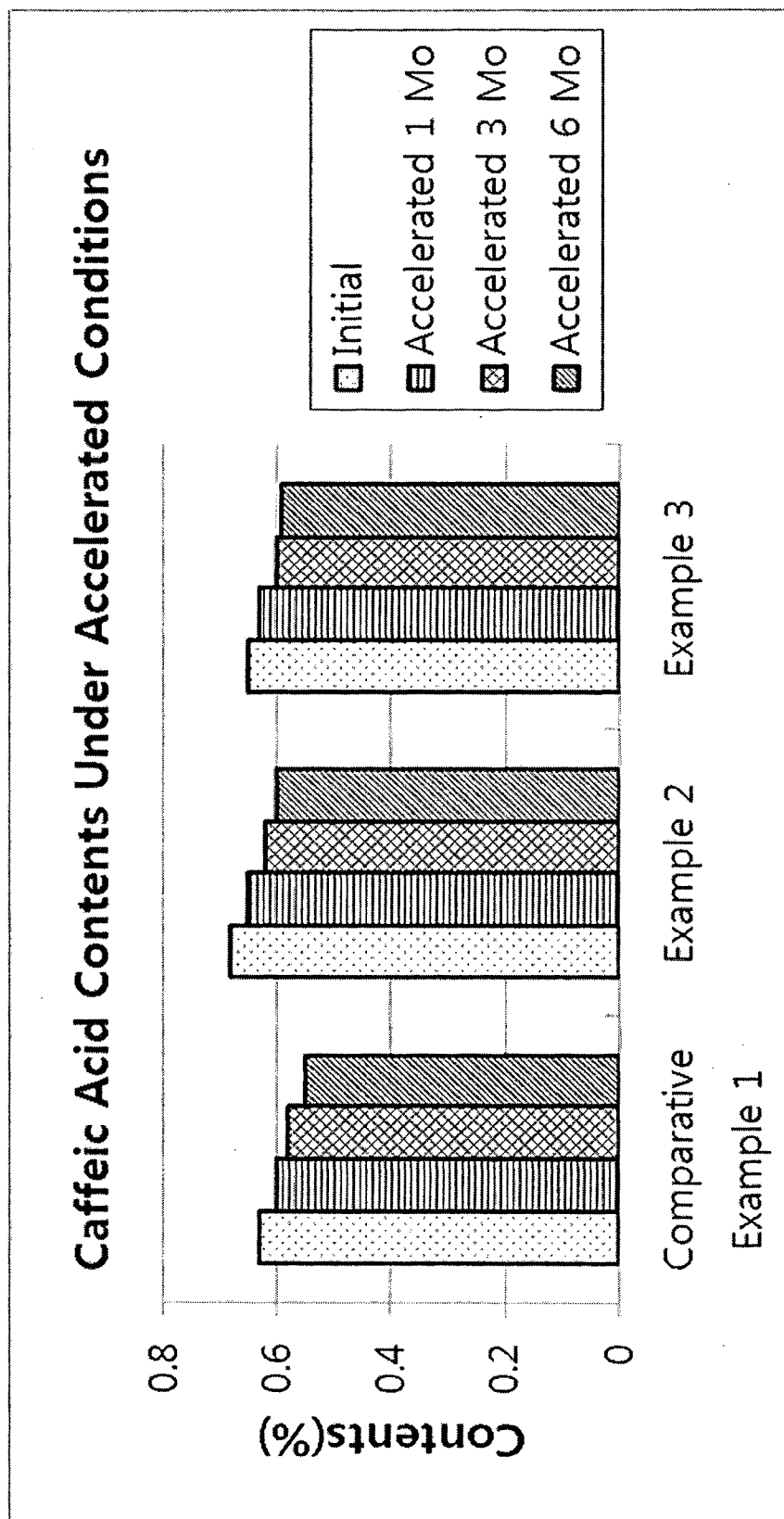
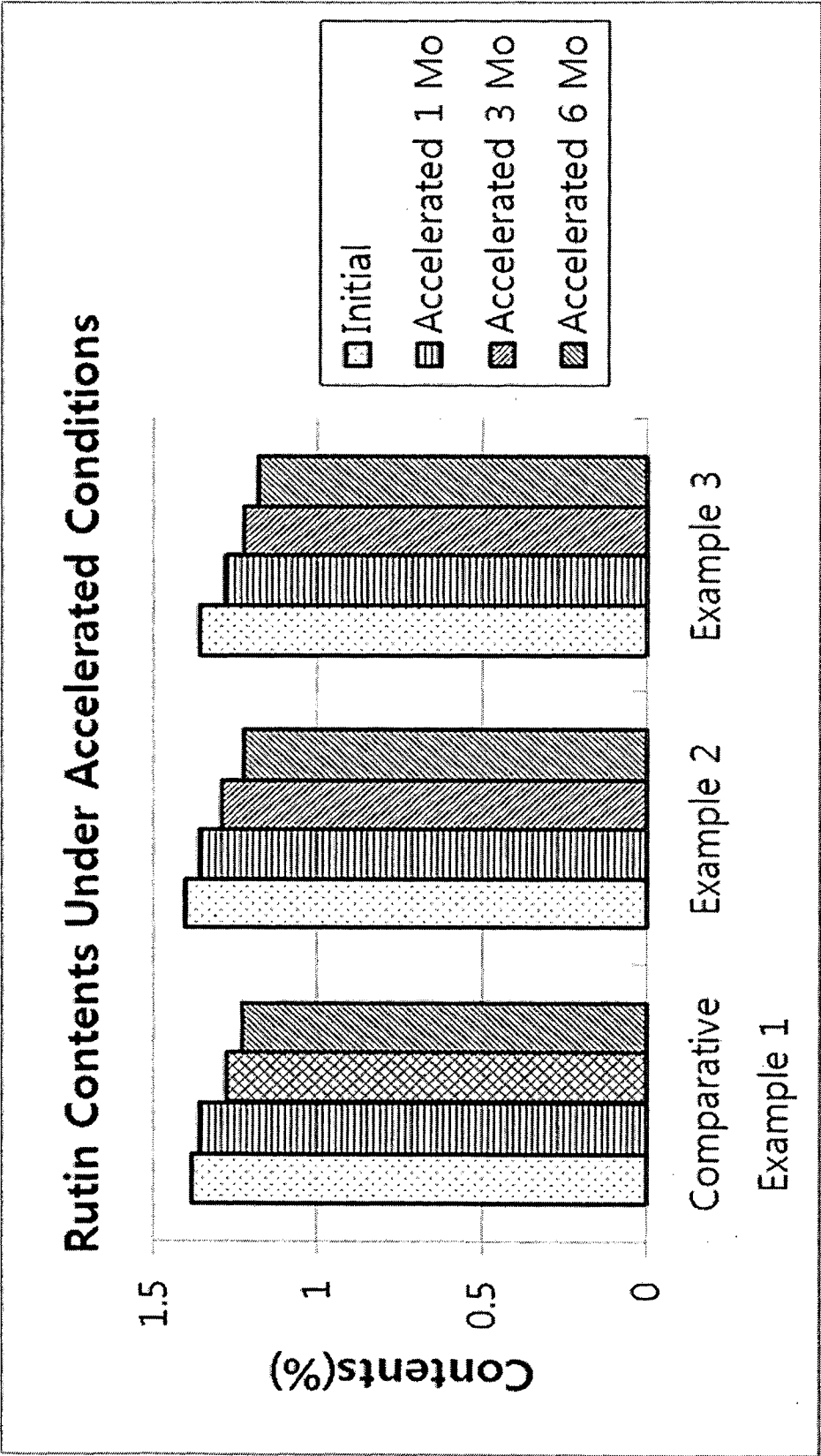


FIG. 4



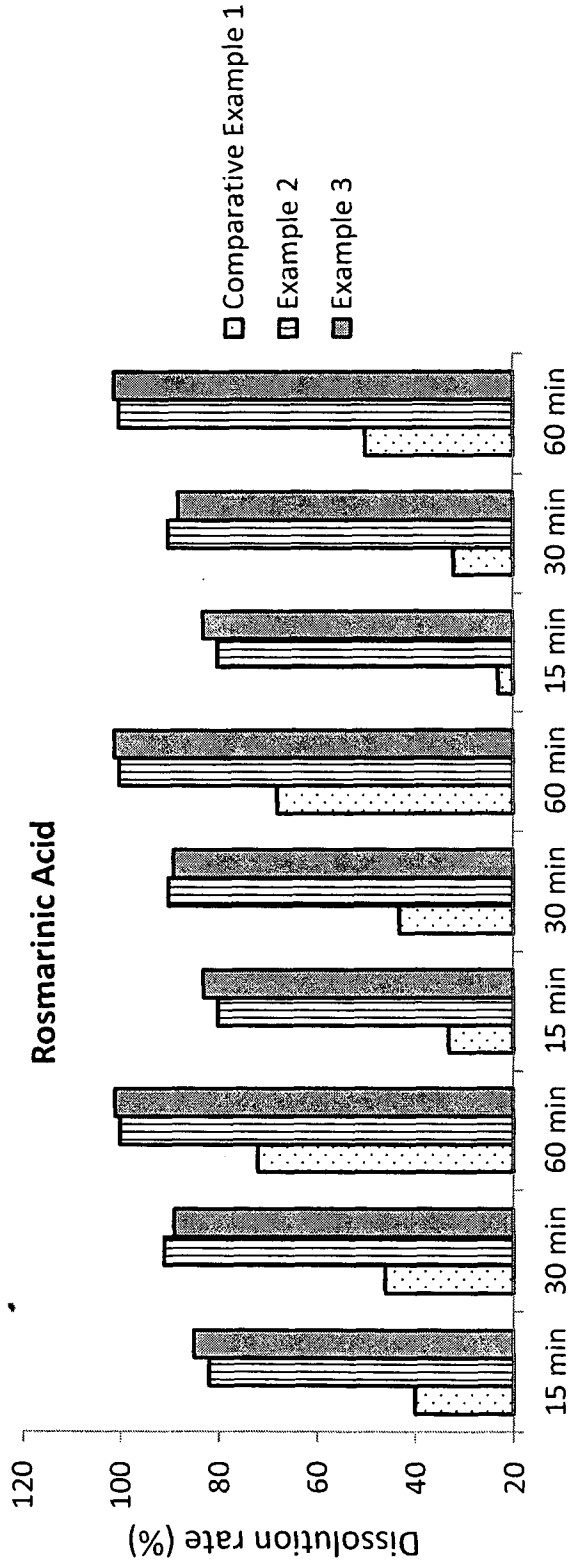
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FIG. 5



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FIG. 6



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FIG. 7

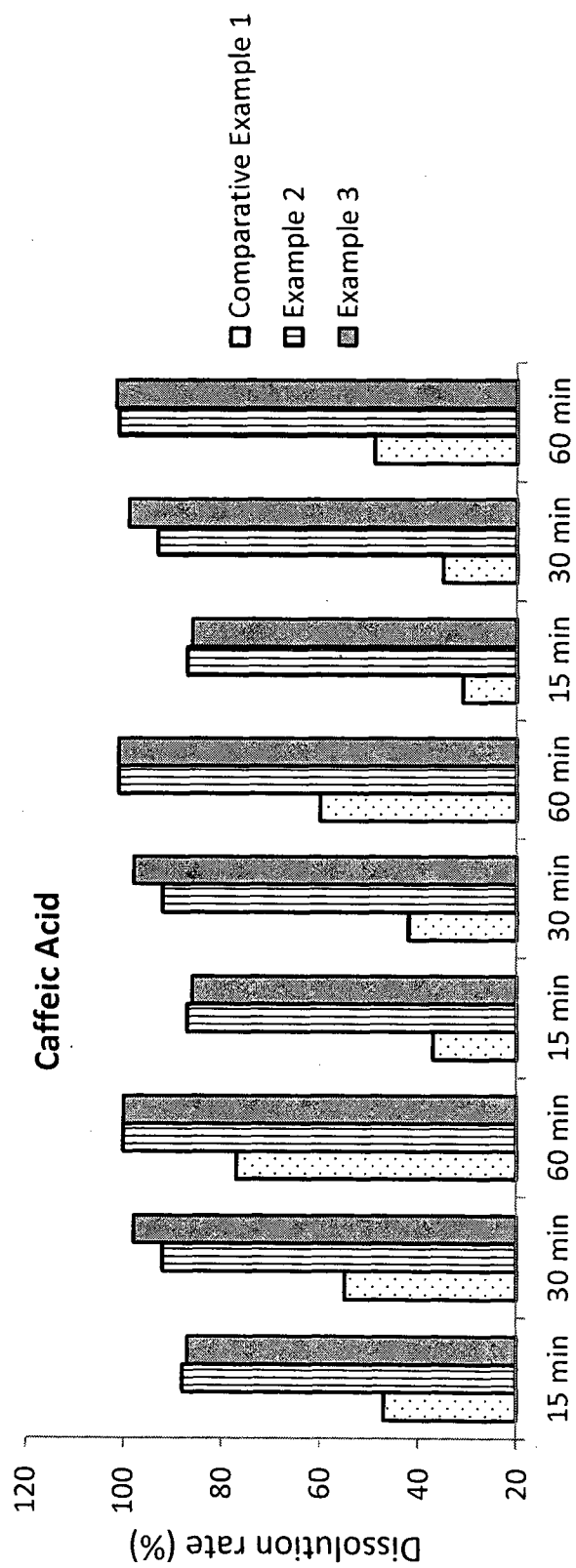
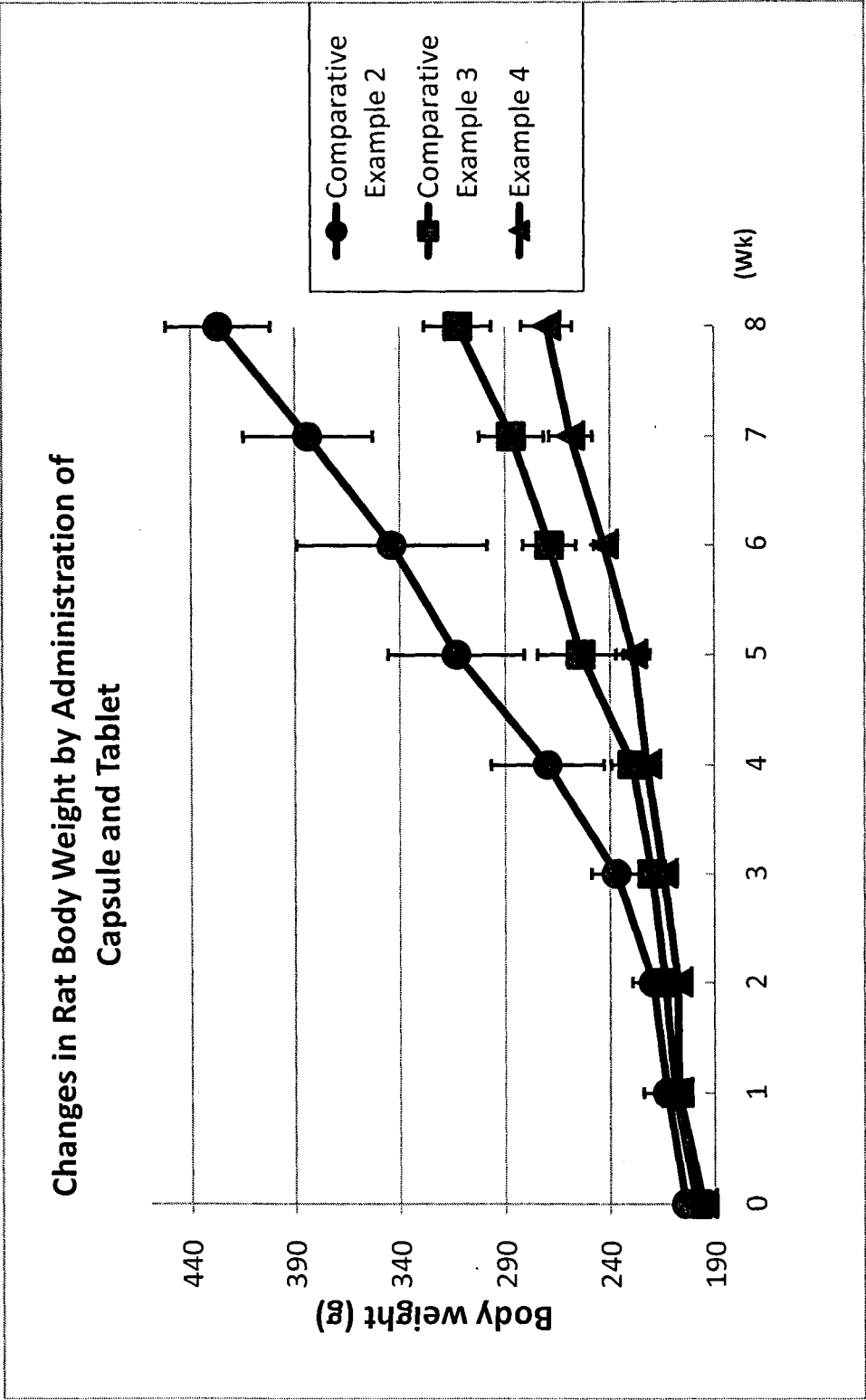


FIG. 8



A. CLASSIFICATION OF SUBJECT MATTER**A61K 9/20(2006.01)i, A61K 36/53(2006.01)i, A61P 3/04(2006.01)i, A61P 3/00(2006.01)i**

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)

A61K 9/20; A61P 25/00; A61K 36/752; A61K 31/22; A23L 1/30; A61K 9/28; A61K 36/53; A61P 3/04; A61P 3/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: Melissa officinalis, rosmarinic acid, caffeic acid, rutin, tablet, angiogenesis, matrix metalloproteinase (MMP), obesity

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010-0278914 A1 (KIM, M.-Y. et al.) 04 November 2010 See abstract; paragraphs [0005]–[0007], [0038] and [0070]–[0073]; and claims 1–8 and 14–17.	1–13
Y	SOFIC, E. et al., `Screening of medicinal plant extracts for quercetin-3-rutinoside (rutin) in Bosnia and Herzegovina`, Medicinal Plants, 2010, Vol. 2, No. 2, pages 97–102. See abstract and tables 1 and 2.	1–13
A	YOON, M. et al., `The anti-angiogenic herbal composition Ob-X from Morus alba, Melissa officinalis, and Artemisia capillaris regulates obesity in genetically obese ob/ob mice`, Pharmaceutical Biology, 2011, Vol. 49, No. 6, pages 614–619. See abstract.	1–13
A	KR 10-2004-0052213 A (ANGIOLAB, INC. et al.) 22 June 2004 See abstract; page 2, lines 13–21; and claims 1–4.	1–13
A	KR 10-2011-0120760 A (HALLYM UNIVERSITY INDUSTRY ACADEMIC COOPERATION FOUNDATION) 04 November 2011 See abstract and claims 1–6.	1–13



Further documents are listed in the continuation of Box C.



See patent family 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 application or patent 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 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

28 October 2013 (28.10.2013)

Date of mailing of the international search report

28 October 2013 (28.10.2013)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2013/006393

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. ☒ Claims Nos.: 14
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 14 pertains to a method for treatment of the human body by therapy and thus relates to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
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 No. III Observations where unity of invention is lacking (Continuation of item 3 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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2013/006393

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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