

COMPOSITION FOR ENHANCING BIOAVAILABILITY OF SILYMARIN

BACKGROUND OF THE INVENTION

Field of the Invention

The present disclosure relates to a composition for enhancing bioavailability of silymarin and a food composition comprising the same.

Description of the Related Art

Silymarin is an ingredient extracted from Holy Thistle of *Carduus marianus* Linne (*Silybum mariannum*), which is a medicinal plant belonging to the family of Asteraceae, and is a kind of a flavonoid-based compound. This plant is native to Southern Europe and North Africa, and has settled also in the American Continent, and thus, this plant has been cultured as ornamental plants or medicinal plants.

Silymarin has been known to stabilize hepatocyte membranes, thereby inhibiting the influx of harmful substances into the cells, and activate the protein synthesis of hepatocytes, thereby promoting the regeneration of hepatocytes. Thus, it has been widely applied to the treatment of various liver diseases caused by smoking, drinking, overwork and environmental pollution due to various pollutions.

Preparations containing silymarin as an active ingredient have already been clinically widely applied for the treatment of liver diseases. However, its active ingredient, silymarin, is a water-insoluble substance that is rarely soluble in water, and has a low absorption when administered orally, and thus, its bioavailability is only about 20-40%. Therefore, enhancing bioavailability by increasing solubility and dissolution rate can be considered important in the development of

silymarin-containing preparations.

In the related art, in order to enhance low solubility and bioavailability of silymarin, a self-emulsifying drug delivery system consisting of drugs, oils, surfactants, co-surfactants, and solvents, etc. has been applied. However, in this case, there are disadvantages that the use of a large amount of synthetic surfactants (e.g., polysorbates, etc.) and a large amount of organic solvents (e.g., hexane, etc.), which are not used for food, can cause more irritation to users with gastrointestinal disorders such as gastritis and gastric ulcers, or cause side effects such as diarrhea, abdominal pain, etc. Moreover, the volume of the preparation becomes larger which is inconvenient to ingest.

In addition, in the case of taking silymarin as a tablet, there is a demand for soft capsules because of low consumer preference due to the particular smell, etc., and in aspect of convenience of taking, a technology of comprising a high content of functional ingredients in a small size is required.

Citation List

Patent Literature

Patent Literature 1: Korean Patent Laid-Open No. 10-2005-0075074

SUMMARY OF THE INVENTION

In order to solve the aforementioned problems, the object of the present disclosure is to provide a composition for enhancing bioavailability of silymarin that effectively enhances solubility and bioavailability of silymarin while exhibiting no side effects as it does not comprise synthetic surfactants, and a food composition exhibiting excellent bioavailability of

silymarin.

In order to achieve the object, in one aspect, the present disclosure provides a composition for enhancing bioavailability of silymarin, comprising, as effective ingredients, one or more of lecithin and phosphatidylcholine, wherein the composition is added to a food composition comprising silymarin and vegetable oils such that the weight ratio of said silymarin : said one or more of lecithin and phosphatidylcholine is 1:0.08 or more and 1:2.31 or less.

In another aspect, the present disclosure provides a composition for enhancing bioavailability of silymarin, comprising, as effective ingredients, one or more of lecithin and phosphatidylcholine, wherein the composition is added to a food composition comprising silymarin and vegetable oils, and wherein the food composition has a dissolution concentration of silymarin of 6 to 31 µg/mL within 240 minutes, when testing the dissolution in purified water according to the paddled method of the dissolution test method pursuant to the Korean Pharmacopoeia.

In one another aspect, the present disclosure provides a use of one or more of lecithin and phosphatidylcholine for the preparation of the composition for enhancing bioavailability of silymarin, wherein the composition is added to a food composition comprising silymarin and vegetable oils.

In one another aspect, the present disclosure provides a use of one or more of lecithin and phosphatidylcholine for use in enhancing bioavailability of silymarin, wherein said one or more of lecithin and phosphatidylcholine is added to the food composition comprising silymarin and vegetable oils.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the dissolution rate test results according to Test Example 1.

FIG. 2 shows the disintegration test results according to Test Example 2.

FIG. 3 shows the bioavailability test results according to Test Example 3.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, the present disclosure will be explained in detail.

In one aspect, the present disclosure may relate to a composition for enhancing bioavailability of silymarin, comprising, as effective ingredients, one or more of lecithin and phosphatidylcholine.

In another aspect, the present disclosure may relate to a use of one or more of lecithin and phosphatidylcholine for the preparation of a composition for enhancing bioavailability of silymarin.

In yet another aspect, the present disclosure may relate to a use of one or more of lecithin and phosphatidylcholine for use in enhancing bioavailability of silymarin.

The composition for enhancing bioavailability of silymarin may be added to a food composition comprising silymarin and vegetable oils. In addition, said one or more of lecithin and phosphatidylcholine may be added to a food composition comprising silymarin and vegetable oils.

Silymarin has been known to stabilize hepatocyte membranes, thereby inhibiting the influx of harmful substances into the cells and to activate the protein synthesis of hepatocytes, thereby promoting the

regeneration of hepatocytes. In particular, silymarin has been known to have strong antioxidant action, reduce phospholipid synthesis, prevent hyper accumulation of neutral lipids, reduce serum LDL, and promote recovery of patients with anti-inflammatory, anti-cancer, acute viral hepatitis, complications of chronic hepatitis, cirrhosis. Therefore, silymarin has been widely applied to the treatment of various liver diseases caused by smoking, drinking, overwork and environmental pollution due to various pollutions.

In addition, said silymarin is an aggregate of flavolignans in which silybin which is a main ingredient, silicristin which is an isomer of silybin, silidianin and isosilybin are mixed.

In one embodiment, said silymarin may be obtained from a milk thistle extract. Here, "milk thistle" refers to a plant *Silybum marianum*, and "milk thistle extract" refers to a substance obtained by isolating from milk thistle, and more specifically, the extract refers to a substance obtained by isolating from milk thistle using typical extraction solvents known in this technical field, for example, water, C1-C4 alcohol (e.g., methanol, ethanol, butanol, etc.), or a mixture solvent of said alcohol and water.

In addition, said milk thistle extract may be obtained by known various extraction methods, for example, hot water extraction, organic solvent extraction or supercritical extraction, etc., and may be prepared in powders by additional process such as distillation under reduced pressure and freeze-drying or spray-drying, etc.

In one embodiment, said lecithin may be one or more soybean lecithin and yolk lecithin.

The present disclosure may comprise natural surfactants such as lecithin and phosphatidylcholine and

not use synthetic surfactants such as polysorbate, thereby exhibiting no side effects caused by a large amount of the synthetic surfactants.

In one embodiment, said vegetable oils may be one or more selected from soybean oils, sunflower oils, rapeseed oils, avocado oils, corn oils, palm oils, coconut oils, rice oils, cottonseed oils, castor oils, sesame seed oils, flax seed oils, olive oils, canola oils, grape seed oils, almond oils, peanut oils, hazelnut oils and walnut oils.

In one embodiment, said composition may be added to a food composition such that the weight ratio of silymarin : one or more of lecithin and phosphatidylcholine is 1:0.08 or more and 1:2.31 or less.

In another embodiment, in said food composition, the weight ratio of silymarin : one or more of lecithin and phosphatidylcholine may be 1:0.08 or more, 1:0.15 or more, 1:0.23 or more, 1:0.35 or more, 1:0.40 or more, 1:0.46 or more, 1:0.53 or more, 1:1 or more, 1:1.23 or more or 1:1.53 or more, and in addition, the weight ratio of silymarin : one or more of lecithin and phosphatidylcholine may be 1:2.31 or less, 1:2.3 or less, 1:2 or less, 1:1.7 or less, 1:1.53 or less, 1:1.4 or less, 1:1.23 or less, 1:1 or less, 1:0.7 or less, 1:0.53 or less or 1:0.46 or less. Preferably, in the food composition, the weight ratio of silymarin : one or more of lecithin and phosphatidylcholine may be 1:0.46 to 1:1.53, and within said range, bioavailability of silymarin may be higher.

In one embodiment, the food composition according to the present disclosure may be a soft capsule formulation. In another embodiment, said food composition can be prepared by suitably selecting ingredients generally used in the pertinent field, in addition to the effective ingredients, by a skilled person in the art, when

preparing the soft capsule formulation.

In one embodiment, said food composition may have a viscosity of 1800 cps or less. To be specific, the viscosity of said food composition may be 1800 cps or less, 1600 cps or less, 1400 cps or less, 1200 cps or less, 1000 cps or less, 800 cps or less, 600 cps or less, 400 cps or less or 200 cps or less, and in addition, may be 10 cps or more, 50 cps or more, 100 cps or more, 200 cps or more, 400 cps or more, 600 cps or more, 800 cps or more, 1000 cps or more, 1200 cps or more, 1400 cps or more or 1600 cps or more.

In one embodiment, said food composition may have a dissolution concentration of silymarin of 6 to 31 $\mu\text{g/mL}$ within 240 minutes by measuring the dissolution rate by rotating at the rotational frequency of 150rpm at 37°C using 500ml of purified water as a dissolution solution, when testing the dissolution in purified water according to the paddled method of the dissolution test method pursuant to the Korean Pharmacopoeia. In another embodiment, the dissolution concentration of silymarin may be 6 $\mu\text{g/mL}$ or more, 6.5 $\mu\text{g/mL}$ or more, 7 $\mu\text{g/mL}$ or more, 8 $\mu\text{g/mL}$ or more, 8.5 $\mu\text{g/mL}$ or more, 9 $\mu\text{g/mL}$ or more, 9.5 $\mu\text{g/mL}$ or more, 15 $\mu\text{g/mL}$ or more, 19 $\mu\text{g/mL}$ or more, 19.6 $\mu\text{g/mL}$ or more, 23 $\mu\text{g/mL}$ or more, 24 $\mu\text{g/mL}$ or more, 26 $\mu\text{g/mL}$ or more, 28 $\mu\text{g/mL}$ or more or 30 $\mu\text{g/mL}$ or more. In addition, the dissolution concentration of silymarin may be 31 $\mu\text{g/mL}$ or less, 28.5 $\mu\text{g/mL}$ or less, 25.5 $\mu\text{g/mL}$ or less or 22 $\mu\text{g/mL}$ or less. Preferably, the dissolution concentration of silymarin may be 19.6 to 31 $\mu\text{g/mL}$.

According to one embodiment, the food composition according to the present disclosure may be a health functional food composition, and can be used for improving liver function.

Herein, "improving" includes treatment, alleviation

and prevention of symptoms, and in the present disclosure, this term means that liver function, liver condition, liver illness, and liver disease are treated, alleviated or prevented.

In the present disclosure, "prevention" refers to all acts of inhibiting or delaying the outbreak of liver diseases by an administration of the composition according to the present disclosure.

In the present disclosure, "liver diseases" include, without any limitation, all liver diseases that can be treated, alleviated or prevented by the composition of the present disclosure, and the examples thereof include non-alcoholic fatty liver, alcoholic fatty liver, hepatitis, hepatic cirrhosis, liver cancer, etc.

The health functional food composition of the present disclosure may comprise supplementary ingredients for nourishment and additives for enhancing sensual acceptability and forming a formulation within the range that does not impair the effects of the present disclosure.

Hereinafter, the contents of the present disclosure will be explained in more detail with reference to the examples and test examples. However, these examples and test examples are provided only for understanding of the present disclosure, and the scope of the present disclosure is not limited to the examples and test examples. Any modification, substitution and insertion, etc. generally known in the pertinent art may be carried out, and those are also covered by the scope of the present disclosure.

[Examples and Comparative Examples]

Examples 1 to 21 and Comparative Examples 1 to 4,

which are compositions with a total content of 600 mg, were prepared according to the composition of the following Table 1 (unit: mg).

Table 1

	Silymarin	Phosphatidylcholine	Soybean lecithin	Vegetable oils		Silymarin : one or more of phosphatidylcholine and soybean lecithin (weight ratio)
				Soybean oil	Sunflower oil	
Comparative Example 1	130	-	-	Balance	-	-
Comparative Example 2	130	300	-	Balance	-	1:2.31
Comparative Example 3	130	240	60	Balance	-	
Comparative Example 4	130	-	300	Balance	-	
Example 1	130	10	-	Balance	-	1:0.08
Example 2	130	8	2	Balance	-	
Example 3	130	-	10	Balance	-	
Example 4	130	30	-	Balance	-	1:0.23
Example 5	130	24	6	Balance	-	
Example 6	130	-	30	Balance	-	
Example 7	130	60	-	Balance	-	1:0.46
Example 8	130	48	12	Balance	-	
Example 9	130	-	60	Balance	-	
Example 10	130	70	-	Balance	-	1:0.53
Example 11	130	56	14	Balance	-	
Example 12	130	-	70	Balance	-	
Example 13	130	160	-	Balance	-	1:1.23
Example 14	130	128	32	Balance	-	

Example 15	130	-	160	Balance	-	
Example 16	130	200	-	Balance	-	1:1.53
Example 17	130	160	40	Balance	-	
Example 18	130	-	200	Balance	-	
Example 19	130	70	-	-	Balance	1:0.53
Example 20	130	56	14	-	Balance	
Example 21	130	-	70	-	Balance	

[Test Example 1] Silymarin Dissolution Evaluation Test

The dissolution rates of Examples 1 to 21, Comparative Examples 1 to 4 and the milk thistle extract were compared and evaluated. The milk thistle extract is a raw material prepared by isolating and extracting from milk thistle (*silybum marianum*), and then filtering, concentrating and purifying it, and a milk thistle extract (HONSON INGREDIENTS®, Canada) in which the content of silymarin is 50% was used.

The dissolution evaluation test was conducted to evaluate the *in vitro* dissolution by the dissolution test method pursuant to the Korean Pharmacopoeia, and the amount of silybin, which is the representative ingredient of silymarin, was quantified and evaluated by the HPLC analysis method. More specifically, according to the paddled method of the dissolution test item pursuant to the Korean Pharmacopoeia, the dissolution rates were measured by taking 3 ml of the Examples 1 to 21, Comparative Examples 1 to 4 and the milk thistle extract, respectively, at the rotational frequency of 150rpm at 37°C using 500ml of purified water as a dissolution solution. The results are shown in following Table 2 (unit: µg/mL) and FIG. 1.

Table 2

	Time (minute)					
	5	15	30	60	120	240
Milk thistle extract	5.93	13.76	16.28	17.97	18.51	19.55
Comparative example 1	0.04	0.34	0.64	1.16	1.89	3.50
Comparative example 2	0.06	3.04	9.70	17.27	25.59	33.24
Comparative example 3	0.22	4.95	9.59	14.19	23.42	31.42
Comparative example 4	0.10	2.50	8.12	12.57	22.82	27.84
Example 1	0.37	1.35	2.27	3.57	4.84	7.48
Example 2	0.17	1.21	2.37	3.53	5.49	7.85
Example 3	0.27	1.50	2.48	4.25	6.24	8.49
Example 4	0.14	0.89	1.75	2.43	3.91	6.87
Example 5	0.09	0.77	1.71	2.64	4.27	6.56
Example 6	0.09	0.77	2.50	3.01	6.53	9.38
Example 7	0.29	0.92	1.95	4.60	9.73	19.63
Example 8	0.92	4.38	6.94	11.32	16.68	21.29
Example 9	1.50	3.45	9.39	16.81	18.77	21.70
Example 10	0.23	2.69	5.30	9.03	21.64	25.13
Example 11	0.89	7.80	12.44	17.27	22.18	24.91
Example 12	0.72	6.40	13.33	18.84	22.77	24.02
Example 13	3.80	11.24	17.46	22.83	27.42	28.25
Example 14	2.98	10.58	14.29	19.92	23.74	26.25
Example 15	2.30	9.38	12.98	16.54	24.05	26.72
Example 16	0.99	9.46	18.51	22.97	27.92	30.90
Example 17	1.45	11.02	18.38	22.74	27.72	30.48
Example 18	1.20	8.50	12.59	18.71	25.02	28.52
Example 19	0.20	3.50	4.80	10.10	22.64	26.29
Example 20	0.18	2.50	15.23	18.94	22.65	25.44
Example 21	0.15	1.50	9.75	14.55	20.01	23.08

The results shown in Table 2 and FIG. 1 show that as compared to Comparative Example 1 comprising only silymarin and soybean oil, Examples 1 to 21 comprising silymarin, soybean oil and one or more of lecithin and phosphatidylcholine exhibit a higher dissolution concentration of silymarin. Thus, it was confirmed that the dissolution rate of silymarin is increased by comprising one or more of lecithin and phosphatidylcholine.

In addition, it can be verified that Examples 7 to 21 comprising one or more of lecithin and phosphatidylcholine at a weight ratio of 1:0.46 to 1:1.53 have a greatly higher dissolution concentration of silymarin after 240 minutes of the dissolution test as compared to the milk thistle extract.

As can be seen from the above, it was verified that the health functional food composition comprising the composition for enhancing bioavailability of silymarin according to the present disclosure and silymarin has greatly enhanced bioavailability due to the excellent dissolution rate of silymarin.

On the other hand, it was confirmed that Comparative Examples 2 to 4 have increased dissolution concentration of silymarin as the amount of one or more of lecithin and phosphatidylcholine is increased, but as will be shown in Test Example 2 below, the test results of disintegration show that the formulation as a soft capsule is impossible.

[Test Example 2] Disintegration Test of Soft Capsule Formulation

The disintegration test was performed in order to evaluate whether the compositions of Example 11 and

Comparative Example 2 are suitable for the production as a soft capsule formulation. The disintegration test was performed according to the disintegration test method among General Test Methods in the Korean Food Standards Codex. Specifically, the test was performed using water as a test solution, using a disintegration tester (J-DTC2, JISICO®). In the case of soft capsule, disintegration should be completed within 20 minutes, and an auxiliary plate was put and moved up and down for 20 minutes, and then it was determined that disintegration was completed if no residue of the respective compositions remain in a glass tube or if the residues are a film even if residues remain.

The results are shown in FIG. 2. From the results of FIG. 2, it can be verified that the composition of Example 11 according to the present disclosure is suitable for the production as a soft capsule since the disintegration was completed within 20 minutes. By contrast, in the case of Comparative Example 2, it was verified that the weight ratio of silymarin to one or more of lecithin and phosphatidylcholine is 1:2.31, so the amounts of lecithin and phosphatidylcholine are too high, such that the viscosity is high, and thereby the disintegration was not completed within 20 minutes. Thus, the composition is not suitable for the production as a soft capsule.

[Test Example 3] Bioavailability Evaluation Test

The pharmacokinetics of each of the milk thistle extract of Test Example 1 and the composition of Example 11 were examined to evaluate the bioavailability of silybin, which is the main ingredient of silymarin. Specifically, 200 mg/kg of the milk thistle extract and the composition of Example 11 were orally administered to

the five SD rats on the basis of the silybin concentration, and then blood samples were taken 11 times in total (0 minutes, 2 minutes, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, 120 minutes, 180 minutes) over time and analyzed using high-performance liquid chromatography (HPLC).

The HPLC analysis conditions are as below.

- HPLC Model: Shimadzu HPLC-UV system Prominence model
- Analysis column: Kientex C18 Column (250 × 4.6mm)
- Mobile phase: Isocratic mode, 10 mM Ammonium acetate buffer : Acetonitrile = 74 : 26
- Flow rate: 1.0 mL/min
- UV wavelength: 288 nm
- Sample injection volume: 20 μ L

In the HPLC analysis method, specifically, 120 μ L of SD rat plasma was taken, 400 μ L of acetonitrile containing an internal standard substance (Phenacetin 3 μ g/mL) was added, and mixed for 5 minutes to deproteinize. The deproteinized sample was then centrifuged at 15,000 × g at 4°C for 10 minutes to obtain 400 μ L of supernatant, and then dried using a nitrogen concentrator and reconstituted with 60 μ L of the mobile phase. 20 μ L of the sample was then injected into HPLC for analysis.

The results are shown in the following table and FIG. 3.

Table 3

	AUC _{last} (μ g·min/ml)	C _{max} (μ g/ml)
Milk thistle extract	76.5 ± 19.2	1.02 ± 0.24
Extract 11	207 ± 28	3.13 ± 0.49

(AUC_{last} is an index for comparing the total blood absorption of the test drug, and C_{max} is the highest blood concentration index of the test drug.)

From the results of Table 3 and FIG. 3, it was

verified that in the composition of Example 11 according to the present disclosure, both the total blood absorption and the maximum concentration value of the silybin, which is the main ingredient of silymarin, were greatly increased compared to the milk thistle extract. That is, it was confirmed that in Example 11 according to the present disclosure, the bioavailability of silymarin is significantly higher than the milk thistle extract.

The composition for enhancing bioavailability of silymarin according to the present disclosure can effectively enhance bioavailability of silymarin while not comprising synthetic surfactants.

In addition, the food composition comprising the composition for enhancing bioavailability of silymarin according to the present disclosure can exhibit excellent solubility and bioavailability of silymarin while not using synthetic surfactants and organic solvents, and can also prevent side effects caused by the use of synthetic surfactants or organic solvents.

While the present disclosure has been described with respect to the specific embodiments, it will be apparent to those skilled in the art that various changes and modifications may be made without departing from the spirit and scope of the present disclosure as defined in the following claims.

What is claimed is:

1. An additive composition for enhancing bioavailability of silymarin, comprising, as effective ingredients,
phosphatidylcholine; or
one or more of lecithin and phosphatidylcholine;
wherein the composition is added to a food composition comprising silymarin and vegetable oils, and
wherein the composition is added to the food composition such that the weight ratio of said silymarin : said effective ingredients is more than 1:0.46 and less than 1:2.31.
2. The additive composition according to claim 1, wherein said silymarin is obtained from a milk thistle extract.
3. The additive composition according to claim 1, wherein said lecithin is one or more of soybean lecithin and yolk lecithin.
4. The additive composition according to claim 1, wherein said vegetable oils are one or more selected from soybean oils, sunflower oils, rapeseed oils, avocado oils, corn oils, palm oils, coconut oils, rice oils, cottonseed oils, castor oils, sesame seed oils, flax seed oils, olive oils, canola oils, grape seed oils, almond oils, peanut oils, hazelnut oils and walnut oils.
5. The additive composition according to claim 1, wherein said weight ratio of said silymarin : said effective ingredients is more than 1:0.46 and 1:1.53 or less.
6. The additive composition according to claim 1, wherein

said food composition has a viscosity of less than 1800 cps.

7. An additive composition for enhancing bioavailability of silymarin, comprising, as effective ingredients,

phosphatidylcholine; or

one or more of lecithin and phosphatidylcholine;

wherein the composition is added to a food composition comprising silymarin and vegetable oils, and

wherein said food composition has a dissolution concentration of silymarin of 24 to 31 µg/mL within 240 minutes, when testing the dissolution in purified water according to the paddled method of the dissolution test method pursuant to the Korean Pharmacopoeia.

8. The additive composition according to claim 7, wherein the composition is added to the food composition such that the weight ratio of said silymarin : said effective ingredients is more than 1:0.46 and less than 1:2.31.

9. The additive composition according to any one of claims 1 to 8, wherein said food composition is a soft capsule formulation.

10. A food composition comprising the additive composition for enhancing bioavailability of silymarin according to any one of claims 1 to 8, silymarin and vegetable oils.

11. The food composition according to claim 10, wherein said food composition is a soft capsule formulation.