IP HIGH COURT OF KOREA

FOURTH-TWO DIVISION

DECISION

Case No.	2022Heo1827 Rejection (Patent)
Plaintiff	A Foundation Corp.
	Delivered to:
	Representative Head of the Foundation B
	Counsel for Plaintiff Tae Woong IP Law Firm
	Patent Attorney in Charge Gyungchan KANG
Defendant	Commissioner of the Korean Intellectual Property Office
	Counsel for Defendant Byeongsook KIM
Date of Closing Argument	January 26, 2023
Decision Date	February 15, 2023

ORDER

1. The plaintiff's claims are dismissed.

2. The cost arising from this litigation shall be borne by the plaintiff.

PLAINTIFF'S DEMAND

The IPTAB Decision 2021Won1847 dated January 25, 2022, shall be revoked.

OPINION

1. Background

A. Plaintiff's Claimed Invention (Plaintiff's Exhibits 8 and 10)

1) Title of invention: Composition for Inhibiting Angiogenesis Using Extracts of *Zizania latifolia*

2) Filing date of application (Divisional) / Filing No.: April 14, 2020 / No. 2020-45169

3) Claims (As Finally Amended on October 15, 2020)

【Claim 1】 A composition for inhibiting angiogenesis, comprising extracts of *Zizania latifolia* as an active ingredient, wherein the extract is obtained using a mixed solvent of water and ethanol (hereinafter referred to as "Claim 1").

【Claim 2】 to 【Claim 5】: It is the same as the corresponding section in Appendix 1.[1)]

4) Summary of invention

The main content of the invention is as follows, and the detailed description for implementing the invention corresponds to the description provided in the relevant section of Appendix 1.

> **1 Background Technology**
>
> [0004] The formation of angiogenesis is known to date to be promoted by more than 20 angiogenic factors, among which vascular endothelial growth factor (VEGF) is secreted by various types of tumor cells and mast cells. VEGF is also recognized as the most potent angiogenic factor. VEGF, also known as vascular permeability factor, is believed to bind to its receptors

1) Errors in the specification, such as typos, are recorded as is.

VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), inducing endothelial cell proliferation and increasing vascular permeability, contributing to the growth and metastasis of tumors and mast cells (Leung DW et al., Science 246:1306-1309, 1989; Ferrara N & Davis-Smyth T Endocr. Rev. 18:4-25,1997; Liping Liu & Meydani Mohsen Nutrition Review, 61(11):384-387, 2003; Jaap G et al., The FASEB Journal 10.1096/fj.03- 1101fje. 2004; Hausman GJ & Tichardson RL, J. anim. Sci., 82:925-934, 2004). Additionally, enzymes such as matrix metalloproteinases (MMPs), which degrade the vascular basement membrane, play an importan role in angiogenesis (Haas TL et al., Am J Physiol Heart Circ Physiol. 2000 Oct;279(4):H1540-7.).

[0005] In a normal physiological state, factors that promote and inhibit angiogenesis interact to strictly regulate and participate in processes such as growth, development, and regeneration. However, when these factors fail to properly regulate angiogenesis, diseases can occur; Excessive angiogenesis has been reported in various conditions, including cancer, arthritis, diabetic retinopathy, retinopathy of prematurity, neovascular glaucoma, corneal diseases caused by angiogenesis, retinal degeneration, corneal graft rejection, posterior capsular fibrosis, granular conjunctivitis, psoriasis, telangiectasia, pyogenic granulomas, seborrheic dermatitis, and acne (Nature, 407:249, 2000; Ophthalmol 102:1261-1262, 1995; J Am Acad Derm 34(3):486-497, 1996; Circultion 93(4):632-682, 1996; Cell 86:353-364, 1996).

[0006] Also, angiogenesis plays a critical role in the growth and metastasis of tumors. Tumors can grow up to a size of approximately 1-3 mm on their own, but further growth requires nutrients from external sources, and this is when the continuous growth of capillaries is stimulated. When new capillaries are formed, they integrate into the tumor, providing pathways for the supply of oxygen and nutrients, enabling the tumor to grow and metastasize to other organs through these blood vessels (McDougall SR et al., JTheor Biol. 2006 Aug 7;241(3):564-89, Grant MB et al.,Expert Opin Investig Drugs. 2004 Oct;13(10):1275-93.;Tarzami ST and Singh JP., Expert Opin Investig Drugs. 2004 Oct;13(10):1319-26.;Bandello F et al., Acta Diabetol. 2013 Feb;50(1):1-20).

2 Problem to be solved

[0011] The present invention aims to provide a composition for inhibiting angiogenesis using extracts of *Zizania latifolia*.

3 Means for solving the problem

[0013] The inventors have confirmed, as demonstrated in the following embodiments and experimental examples, that extracts of *Zizania latifolia* inhibit the proliferation, adhesion, and migration of human umbilical vein endothelial cells. Additionally, the extract increases the expression of p27, a known cell cycle inhibitor (Cell, 1996, 85(5): 733-44; Trends Cell Biol. 2003, 13(2):65-70), while suppressing the expression of cyclin E and cdk2, which are involved in the transition from the G1 phase to the S phase of the cycle (Cell. 1992, 70: 993-1006;Cell Cycle. 2010, 9: 4900-4907). The extract was also found to inhibit the activity of MMP-2/9, a critical factor for new capillary formation along with VEGF (Curr. Opin. Hematol. 2003 10(2):136-141).

[0015] In this specification, "extracts of *Zizania latifolia*" refer to (i) extracts obtained by extracting parts of Zizania latifoli, such as stems, leaves, fruits, flowers, roots, or the whole plant, using a solvent such as water, methanol, ethanol, butanol or other C1–C4 lower alcohols, methylene chloride, ethylene, acetone, hexane, ether, chloroform, ethyl acetate, butyl acetate, N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), 1,3-butylene glycol, propylene glycol, or their mixed solvents; (ii) extracts obtained using supercritical fluid extraction solvents such as carbon dioxide or pentane; or (iii) fractions obtained by fractionating the extract. Depending on the polarity of the active substances, the degree of extraction, and the extent of preservation, various extraction methods can be employed, including cold steeping, reflux, heating, ultrasonic radiation, or supercritical fluid extraction. Fractionated extracts include (i) fractions obtained by suspending the crude extract in a specific solvent before mixing and settling with a solvent of differing polarity and (ii) fractions obtained by adsorbing the crude extract onto a column packed with silica gel, etc. and using a hydrophobic solvent, a hydrophilic solvent, or their mixed solvents as the mobile phase. Additionally, the extracts above refer to concentrated liquid extracts or solid extracts obtained by

removing the extraction solvent using methods such as freeze-drying, vacuum drying, hot air drying, or spray drying. The extracts desirably refer to extracts obtained using water, ethanol, or a mixed solvent of these as the solvent, or more desirably extracts obtained using a mixed solvent of water and ethanol as the extraction solvent.

4 Effect of the invention

[0044] As described above, the present invention provides a composition for inhibiting angiogenesis using *Zizania latifolia* extracts.

[0045] The composition for inhibiting angiogenesis according to the present invention can be commercialized as pharmaceuticals, foods, etc. for purposes such as improving angiogenesis-related diseases.

5 Detailed description for implementing the invention

[0051] <Embodiment 2> Example of preparing *Zizania latifolia* extract 2

[0052] The *Zizania latifolia* extract (G56 80%) was prepared in the same way as in <Embodiment 1>, except that 80% ethanol was used as the extraction solvent.

[0053] <Experimental Example> Evaluation of angiogenesis inhibitory activity

[0069] <2> Experiment results

[0071] When the effect of *Zizania latifolia* extract on the proliferation of vascular endothelial cells was examined, both the 70% (Embodiment 1) and the 80% ethanol extracts (Embodiment 2) exhibited cell proliferation inhibitory activity, but the 80% one demonstrated better activity (See Figure 1). The 80% ethanol extract was treated at different concentrations (25, 50, and 100 mg/mL) to compare the expression levels of cell cycle-related proteins. The results showed that G56 80% increased the expression of p27, a cell cycle inhibitor, while decreasing the expression of cyclin E and cdk2, which are involved in the transition from the G1 phase to the S phase of the cell cycle (See Figure 2). When the effect of the extract on vascular endothelial cell adhesion was examined using a cell adhesion assay, both the 70% and the 80% ethanol extracts showed adhesion inhibitory activity dependent on concentration (See Figure 3). The effects of the extracts on vascular endothelial cell migration were assessed using in vitro wound healing assay and in vitro transwell migration assay, and the results showed that the 80% ethanol extract exhibited stronger migration inhibitory activity than the 70% one (see Figures 4 and 5). Zymography revealed that the activity of MMP2/9, which is involved in cell migration, was inhibited at 100 mg/mL of the 80% ethanol extract (See Figure 6). These results indicate that the 80% ethanol extract regulates migration of vascular endothelial cell by moderating the activity of MMP2/9.

B. Prior Art[2] (Plaintiff's Exhibit 9 and Defendant's Exhibit 1)[3]

The prior invention relates to "Physiological Activities and Anticancer Effects of Ethanol Extracts of Euonymi Ramuli Suberalatum and *Zizania latifolia*" published in the doctoral dissertation submitted and successfully defended by D to the Department of Complementary and Alternative Medicine at C University Graduate School in April 2014. The main content is detailed in Appendix 2.

C. Procedural History

1) On June 17, 2020, the patent examiner of Korean Intellectual Property Office (hereinafter, the "KIPO") sent a notice of grounds for rejection regarding the subject invention, stating that "The claims are unclear and not supported by the description of the invention, and the specification does not provide sufficient information to enable a person having ordinary skill in the art (hereinafter, a "skilled person") to easily invent it. Therefore, the invention fails to meet the requirements of Articles 42(3)1, 42(4)1, and 42(4)2 of the Patent Act. Claims 1 to 4 are denied of novelty by Cited Art 1[4], and lack an inventive step as a skilled person could easily derive it from Cited Art 1."

2) Strictly speaking, it is "prior literature," but for the convenience of comparison with the subject invention, it will be referred to as "prior art."
3) The prior art in this case is identical to the comparable invention referenced during the trial stage of this case.
4) It is the same as the prior art in this case.

2) In response, the plaintiff submitted amendments to the specification along with written opinions on October 15, 2020, and January 25, 2021, but the KIPO examiner issued a rejection on June 16, 2021, stating that the reasons for rejection that “the invention lacks novelty and an inventive step” had not been resolved.

3) The plaintiff filed a petition in the Intellectual Property Trial and Appeal Board (hereinafter, the “IPTAB”) on July 19, 2021, for an administrative trial against the rejection above under IPTAB 2021Won1847, and submitted amendments to the specification and written opinions on August 25, 2021, November 15, 2021, and January 18, 2022. The IPTAB dismissed the plaintiff’s petition on May 19, 2017, (hereinafter, the “IPTAB Decision”) on the grounds that “Claim 1 of the present invention can be easily invented based on the cited invention, thereby lacking an inventive step, and is not patentable under Article 29(2) of the Patent Act. If any claim in an invention contains grounds for rejection, the entire patent application shall be rejected.”

[Factual Basis] Undisputed facts, the descriptions on Plaintiff's Exhibits 1 through 10, the description and images of Defendant's Exhibit 1, and the purport of the overall arguments

2. Summary of Parties' Arguments

A. Plaintiff

As Claim 1 is acknowledged to possess an inventive step, the IPTAB Decision that reached a different conclusion is erroneous and shall be overturned.

1) The pharmaceutical use of Claim 1 in this case is the “inhibition of angiogenesis” itself, with its pharmacological mechanism being the suppression of

endothelial cell proliferation, adhesion, migration, and the expression of angiogenic inducers (hereinafter, “endothelial cell proliferation, etc."). Since its pharmaceutical use and pharmacological mechanism are different from the anticancer effect disclosed in the prior art (cytotoxicity against cancer cells), it has an inventive step.

2) The prior art merely experimented on the cytotoxicity of *Zizania latifolia* extract against cancer cells, and a skilled person would not be able to confirm from this invention that the suppression of endothelial cell proliferation, etc. inhibits angiogenesis. Therefore, it cannot be concluded that Claim 1 is easily derivable from the prior art.

B. Defendant

Claim 1 lacks an inventive step due to the following reasons based on the prior art. Therefore, the IPTAB Decision consistent with this conclusion is well-grounded.

1) Claim 1 and the prior art share the same active ingredients, comprising a mixed solvent of Zizania latifoli, water, and ethanol (hereinafter, “*Zizania latifolia* extract”), and as angiogenesis is closely related to cancer, the inhibition of angiogenesis inherently encompasses the concept of cancer treatment. Therefore, the pharmaceutical use disclosed in Claim 1 and the prior art is substantially identical.

2) A skilled person could identify the pharmacological mechanism of the prior art by confirming its anti-angiogenic effects through experiments. Thus, the anti-angiogenic use of the invention in Claim 1 can be easily derived.

3) The pharmacological data presented in the specification of the present invention merely confirm the inhibitory effects on "angiogenesis" through

experimentation, which is already known to be closely associated with cancer occurrence and metastasis. Simply verifying this effect does not demonstrate that Claim 1 exhibits remarkable or qualitatively distinct effects that a skilled person could not have predicted from the prior art.

3. Whether IPTAB Erred

A. Claim Construction Regarding the Pharmaceutical Use of Claim 1

1) Relevant law

In an invention of pharmaceutical use, the invention is constituted by a specific substance and its pharmaceutical use (See Supreme Court Decision 2006Hu3564, dated January 30, 2009). The pharmacological mechanism is merely an inherent property inseparably tied to the substance and serves only as a means to derive the connection between the substance and its pharmaceutical use. Therefore, the pharmacological mechanism described in the claims of an invention of pharmaceutical use is meaningful as a constituent element of the invention only to the extent that it specifies the pharmacological use of the particular substance. The pharmacological mechanism itself should not be regarded as an element that limits the scope of the claims (See Supreme Court Decision 2012Hu3664, dated May 16, 2014). Therefore, in case the pharmaceutical use of a specific substance is already known for a particular disease or therapeutic effect, identifying the pharmacological mechanism and including it in the claims does not make the mechanism an element of the invention. Thus, it cannot serve as a basis for acknowledging its inventive step.

When evaluating the inventive step of a patented invention by referencing multiple prior art documents, if the prior art provides suggestions or motivations,

or even if this is not the case, if it can be acknowledged that a skilled person could easily achieve such combination based on the level of technology, technical common knowledge, fundamental challenges in the field, development trends, industry demands, etc. at the time of filing the patent application, the inventive step of the invention is denied. In the case of inventions of pharmaceutical use, if a skilled person can easily predict the therapeutic effect of a specific substance for a specific disease based on prior inventions, the inventive step is denied (See Supreme Court Decision 2016Hu502, dated January 31, 2019).

2) Discussion

Considering the following facts and circumstances recognized based on the descriptions of Plaintiff's Exhibit 2 and Defendant's Exhibits 2, 3, and 6, as well as the purport of the overall arguments, it is reasonable to interpret "inhibiting angiogenesis" in Claim 1 as including "prevention, inhibition, or delay of the onset of a disease caused by angiogenesis, such as cancer, etc. (hereinafter, the "treatment")" "Inhibiting angiogenesis" can be an element of the invention only to the extent that it specifies such a pharmaceutical use, and it is difficult to consider it to be an element that by itself limits the scope of the claims.[5)]

A) The specification of the present invention defines "angiogenesis inhibition" as including the prevention, suppression, or delay of diseases[6)] such as cancer, arthritis, and diabetic retinopathy, as described below.

Specification of the Claimed Invention (Plaintiff's Exhibit 8)

5) After the conclusion of arguments in this case, the plaintiff submitted several Patent Publications as reference materials, with the titles of the inventions ending in "composition for inhibiting angiogenesis." However, it is reasonable to conclude that each of these inventions specifies its pharmaceutical use by their claims or specifications, such as the treatment or prevention of particular diseases.

6) The specification of the subject invention states that "excessive angiogenesis has been reported in diseases such as cancer, arthritis, diabetic retinopathy, retinopathy of prematurity, neovascular glaucoma, corneal diseases caused by angiogenesis, retinal degeneration, corneal graft rejection, posterior capsular fibrosis, granular conjunctivitis, psoriasis, telangiectasia, pyogenic granuloma, seborrheic dermatitis, and acne" ([0005]).

[0017] Also, in this specification, "angiogenesis inhibition" includes the improvement (alleviation of symptoms), treatment, prevention, suppression, or delay of diseases caused by angiogenesis, as defined below.

[0018] Furthermore, in this specification, "diseases caused by angiogenesis" include all diseases related to angiogenesis. To be specific, it encompasses the aforementioned cancer, arthritis, diabetic retinopathy, (omitted) inflammatory diseases, and neurodegenerative diseases.

B) The documents published before the filing of the present invention describes that cancer cells stimulates angiogenesis to obtain a blood supply, and thus cancer can be treated by inhibiting angiogenesis. Therefore, it is deemed that angiogenesis inhibition is recognized as one of the various pharmacological mechanisms for treating cancer, which was widely known at the time of filing the application.

▸ Defendant's Exhibit 2 (p. 846, Hard Tissue and Oral Biochemistry, Molecular Cell Biology, published January 1, 2013)

In 2000, Hanahan D and Weinberg RA suggested 6 **fundamental capabilities of malignant tumors**: (i) to produce their own growth signals bypassing the need for external growth factors; (ii) to evade external anti-growth signals; (iii) to evade apoptosis; (iv) to avoid aging and achieve essentially infinite growth; **(v) to stimulate sustained angiogenesis;** and (vi) to invade surrounding tissues and form distinct tumors.

▸ Defendant's Exhibit 3 (p. 3 to 4, Trends in Cancer Vaccine Development, published around October 2005)

C. Characteristics of Cancer Cells

○ The transformation of normal cells into cancer cells through mutations alters cellular characteristics and leads to the development of tumors. Additional mutations cause benign tumors to progress into malignant ones, which acquire new characteristics in the process. Cancer cells secrete substances to **promote the growth of surrounding blood vessels, stimulating angiogenesis and being provided with blood, which enables the cancer cells to metastasize to other tissues.**

▸ Defendant's Exhibit 6 (p. 542 to 543, Journal of the Korean Medical Association, published around 2003)

Most anticancer drugs currently used in clinical practice exhibit cytotoxic effects by targeting the chromosomes or microtubules of cancer cells. However, as these drugs also damage normal cells, leading to side effects, there is a growing demand for anticancer drugs that target substances or mechanisms specific to cancer cells without harming normal cells. As a result, the development of such targeted drugs has become inevitable, and some of these drugs have already been commercialized and are currently used in clinical practice. Most newly developed drugs with advances in molecular biology are designed to target molecules unique to cancer cells, thereby demonstrating efficacy. **Drugs or anticancer drugs used in molecular targeted therapies** are designed to act on various targets, including signal transduction pathways, **angiogenesis,** matrix, cell cycle regulators, and **apoptosis**.

C) Considering the specification of the subject invention and the publicly disclosed literature prior to the filing, it is clear that the pharmaceutical use of Claim 1 includes the treatment of cancer through an angiogenesis inhibition mechanism. Thus, it is reasonable to interpret the scope of Claim 1 as including the therapeutic use of *Zizania latifolia* extract for the treatment of cancer, etc.

3) Discussion on the plaintiff's argument

A) The plaintiff argues that "inhibition of angiogenesis" itself can be recognized as a pharmaceutical use, and that "inhibition of proliferation, adhesion, and migration of vascular endothelial cells" and "inhibition of expression of angiogenesis-inducing factors" are its pharmaceutical mechanism, based on Supreme Court Decision 2003Hu1550, decided December 23, 2004, which held that the claims are described clearly even though the claims refer to "inhibition of vasculogenesis," not the treatment of a specific disease, as a pharmaceutical use.

B) However, the Supreme Court decision above only determines whether

the “inhibition of vasculogenesis” is percieved by a skilled person as a specific pharmaceutical effect and is clearly an expression of a pharmaceutical use considering the requirement of definiteness of the patent claims [Article 42(4)2 of the old Patent Act (before amended by Act No. 8197 of January 3, 2007; the same shall apply hereinafter)] and the requirement of practicability of the patent specification [Article 42(3) of the old Patent Act], and does not conclude that “inhibition of vasculogenesis” itself is an element that limits the scope of the claims. Furthermore, as previously examined, angiogenesis is one of the key mechanisms driving cancer progression, and while cancer may not be the only condition treated through angiogenesis inhibition, it undeniably falls within the category of diseases that can be treated by inhibiting angiogenesis. Moreover, it cannot be argued that "inhibition of angiogenesis" does not inherently encompass the treatment of diseases such as cancer. Considering these, the plaintiff's argument, which is based on the assumption that the therapeutic use of Claim 1 does not include the treatment of cancer and similar conditions, cannot be accepted.

Meanwhile, the pharmacological mechanisms claimed by the plaintiff for Claim 1, that is, "inhibition of endothelial cell proliferation, adhesion, and migration" and "suppression of the expression of angiogenesis-inducing factors," can be regarded as individual and specific sub-mechanisms of the broader pharmacological mechanism of "angiogenesis inhibition.” So, even if it is a higher-level pharmacological mechanism encompassing these subordinate mechanisms, that alone does not suffice to constitute a medical use on its own [Even if viewed differently, considering the description in the specification of the subject invention, the "inhibition of angiogenesis" in Claim 1 should be understood as an "efficacy" aimed at treating specific diseases such as cancer. Therefore, it is reasonable to interpret the pharmaceutical use of Claim 1 as "the

treatment of cancer, etc. (through angiogenesis inhibition).”]

B. Comparison Between Claim 1 and Prior Art

1) Element-by-element comparison

Element	Claim 1 (Plaintiff’s Exhibit 8)	Prior Art (Defendant’s Exhibit 1)
1	Including *Zizania latifolia* extract as an active ingredient, wherein the extract is characterized as being obtained using a mixed solvent of water and ethanol.	After grinding *Zizania latifolia* and obtaining sample through extraction with 80% ethanol (See 2. Sample Extraction on page 7)
2	Composition for angiogenesis inhibition	The anticancer effect of *Zizania latifolia* extract was evaluated against four types of cancer cells. - When *Zizania latifolia* extract was treated for 24 hours, the survival rates of cervical cancer cells, liver cancer cells, and breast cancer cells decreased (second paragraph on page 17). - When *Zizania latifolia* extract was treated for 48 hours, the survival rate of liver cancer cells decreased, and a minimal anticancer effect was observed for breast cancer cells (second paragraph on page 19). - When *Zizania latifolia* extract was treated for 72 hours, a low

		anticancer effect was observed for gastric cancer cells and a weak anticancer effect for breast cancer cells (second paragraph on page 21).

2) Commonalities and differences

A) Element 1

Element 1 and the corresponding component of the prior art are substantially identical in that both are *Zizania latifolia* extracts, and there is no dispute between the parties regarding this.

B) Element 2

Element 2 and the corresponding element in the prior art are different in that the former is limited the use to "angiogenesis inhibition," whereas the latter is an anticancer effect (reduction effect in cancer cell survival rate) “through apoptosis” (hereinafter, referred to as the “Difference”).[7)]

C. Analysis of difference

1) Pharmaceutical use of Claim 1

The scope of Claim 1 includes therapeutic uses such as the treatment of cancer, and "angiogenesis inhibition" shall be considered an element of the invention only to the extent that it specifies this pharmaceutical use, as previously discussed.

2) Whether the element can be easily derived

The prior art discloses experimental results showing a reduction in the

7) However, as previously discussed, if Claim 1 is considered to include the therapeutic use for cancer, it can be deemed substantially the same.

survival rate of cancer cells treated with *Zizania latifolia* extract (apoptosis). The close correlation between angiogenesis and cancer (Defendant’s Exhibits 2 and 3) and the fact that various targets, such as angiogenesis and apoptosis (or programmed cell death), are utilized in cancer treatment (Defendant’s Exhibit 6) were well-known in the relevant field at the time of the application.

Therefore, a skilled person could easily derive a use for cancer treatment from the prior art disclosing the results of tests showing that extracts of *Zizania latifolia* reduce cancer cell survival rates, and reviewing "inhibition of angiogenesis" is only an optional step in the process of reviewing the anticancer effects. Thus, Claim 1 has no difficulty in its configuration, and the difference in this case can be easily overcome.

It is true that the prior art discloses the experimental results showing that compared to *Euonymus alatus* extract, the anticancer effect of *Zizania latifolia* extract is weak, and that it exhibits little to no effect on certain types of cancer cells. However, it is difficult to conclude that the prior art includes negative teachings regarding the anticancer effects of *Zizania latifolia* extract for the following reasons: (i) While the experimental results indicate that the anticancer effect of *Zizania latifolia* extract is relatively lower than *Euonymus alatus* extract or decreases over time, it is not recognized that *Zizania latifolia* extract completely lacks anticancer effects and (ii) As the prior art infers that the efficacy of *Euonymus alatus* is attributed to the content of polyphenol and flavonoid, and it is disclosed that *Zizania latifolia* extract, though in a smaller amount compared to *Euonymus alatus* extract, also includes them, a skilled person would have sufficient motivation to investigate whether *Zizania latifolia* extract has anticancer effects through angiogenesis inhibition.

3) Analysis of effect in Claim 1

The active ingredient described in both Claim 1 and the prior art is *Zizania latifolia* extract, and they share the common characteristic of having anticancer effects. The only difference lies in their pharmacological mechanisms.

Claim 1 merely confirms anticancer activity through experiments, and simply verifying such effects through experiments does not establish that Claim 1 possesses remarkable or unexpected effects that could not have been predicted by a skilled person.

D. Summary of discussion

Therefore, Claim 1 can be easily derived by a skilled person based on the prior art and thus is denied of an inventive step. Meanwhile, in a patent application consisting of two or more claims, if even one claim has grounds for rejection, the entire application must be rejected (See Supreme Court Decision 2007Hu3820, dated December 10, 2009). Since the inventive step of Claim 1 is denied and it cannot be granted a patent, the subject application cannot be granted a patent and there is no need to further examine the remaining claims. Thus, the IPTAB Decision in line with this conclusion is lawful.

4. Conclusion

Therefore, the plaintiff's petition seeking the revocation of the IPTAB Decision is without merit, and accordingly, the decision is rendered as ordered.

Presiding Judge Taeksoo JUNG
Judge Sook Yeon LEE
Judge Jiyoung YI

[Appendix 1]

Claims and Detailed Description of Subject Invention

【Claim 2】 According to Claim 1,

the *Zizania latifolia* whole plant extract above is a composition for angiogenesis inhibition, characterized by being a 70% or an 80% ethanol extract.

【Claim 3】 According to Claim 1 or Claim 2,

the composition above is a composition for angiogenesis inhibition, characterized by being a pharmaceutical composition.

【Claim 4】 While including *Zizania latifolia* extract as an active ingredient,

the *Zizania latifolia* extract above is a food composition for inhibiting angiogenesis, characterized by being a mixed solvent extract of water and ethanol.

【Claim 5】 According to Claim 4,

the *Zizania latifolia* whole plant extract above is a food composition for angiogenesis inhibition, characterized by being a 70% or an 80% ethanol extract.

Detailed description for implementing the invention

[0048] <Embodiment> Preparation of *Zizania latifolia* extract

[0049] <Embodiment 1> Example of preparing *Zizania latifolia* extract 2

[0050] 100 g of dried and ground *Zizania latifolia* (whoe plant) was mixed with 1 L of 70% ethanol and underwent one repeated extraction at room temperature for 24 hours before being filtered with filter paper. The obtained 70% ethanol filtrate was vacuum-concentrated and then freeze-dried to produce the *Zizania latifolia* extract (G56 70%).

[0051] <Embodiment 2> Example of preparing *Zizania latifolia* extract 2

[0052] The *Zizania latifolia* extract (G56 80%) was prepared in the same way as in <Embodiment 1>, except that 80% ethanol was used as the extraction solvent.

[0053] <Experimental Example> Evaluation of angiogenesis inhibitory activity

[0054] <1> Experiment method

[0055] Cell culture

[0056] Human Umbilical Vein Endothelial Cells (HUVECs) were purchased from Lonza (Walkersville, MD, USA) and cultured in EGM-2 MV BulletKit (Lonza) medium. Only endothelial cells between passages 4 and 6 were used for the experiments. The medium was replaced every two days during the culture period.

[0057] Cell proliferation assay (Cell proliferation)

[0058] Endothelial cells were plated in 6-well plates (100,000 cells/well) and synchronized to the G1/G0 phase using serum-free basic EBM-2 medium. Then, the cells were treated with the extract in EGM-2 MV BulletKit medium (growth media) under the defined conditions to observe the inhibitory effect of the extract on cell proliferation. The inhibitory effect on cell proliferation was assessed using the tryphan blue exclusion assay (Curr Protoc Immunol. 2001 May; Appendix 3: Appendix 3B).

[0059] Cell adhesion assay (Cell adhesion)

[0060] The cultured endothelial cells were detached using trypsin/EDTA treatment and reacted in EGM-2 MV BulletKit medium for 1 hour to normalize cell surface and activity (recovery). Then, the medium was replaced with basic EBM-2 medium. The extract was applied in EGM-2 MV BulletKit medium (growth media) under experimental conditions, and the cells were cultured in a 96-well plate (15,000 cells/well) for 2 hours. Non-adherent cells were removed by washing with phosphate-buffered saline (PBS, pH 7.4), and the remaining ones were stained with Giemsa stain solution. The extent of cell adhesion was measured by manually counting the stained cells using a microscope (Hemacytometer, counter, chemidoc, and 37°C shaking incubator).

[0061] In vitro wound-healing assay (Cell migration)

[0062] The monolayer wound healing assay was performed through the following process: endothelial cells were cultured as a confluent monolayer (40,000 cells/well)

in a 48-well plate, and the layer was scratched using a 200 μl pipette tip; the cells were then synchronized to the G1/G0 phase by incubating them in serum-free basic EBM-2 medium for 2 hours; and the extract was applied in EGM-2 MV BulletKit medium (growth media) under experimental conditions, and changes in cell migration were observed based on the extract concentration and time (12 to 15 hours). The cells were stained using Giemsa stain solution, and the cell migration distance was measured.

[0063] In vitro transwell migration assay (Cell migration)

[0064] Endothelial cells cultured in basic EBM-2 medium for 2 hours were plated at 100 μl (4×10^4 cells/mL) in a transwell insert (Costar, 6.5 mm diameter insert). The lower wells were filled with 600 μl of either basic EBM-2 medium or EGM-2 MV BulletKit medium. The extract was applied under experimental conditions, and after 18 hours, the insert was fixed with methanol. Unmigrated cells on the upper surface of the insert were removed using a cotton-tipped swab. The cells were stained with Giemsa stain solution, and six different parts were observed under a microscope (×200) to manually count migrated cells using microscopy.

[0065] Zymography (MMPs enzyme activity)

[0066] Endothelial cells were plated in 6-well plates (100,000 cells/well) and synchronized to the G1/G0 phase using serum-free basic EBM-2 medium. Then, the extract was applied under experimental conditions (alternatively, the medium from the lower wells could be used as the sample after the transwell migration experiment), and the culture medium was mixed with a non-denaturing loading buffer and incubated at 37°C for 30 minutes. A 10% SDS-PAGE gel containing 0.1% gelatin as the substrate was then performed.

After the electrophoresis, the gel was washed with 2.5% Triton X-100 solution at room temperature for an hour to remove SDS. The gel was then reacted in developing buffer (50 mM Tris, pH 7.5, 10 mM $CaCl_2$, and 150 mM NaCl) at 37°C for 15 to 18 hours. After the reaction, the gel was stained for 2 hours with a staining solution containing 0.5% Coomassie Brilliant Blue (30% methanol-10% acetic acid). The gel was then destained using a destaining solution (30% methanol-10% acetic acid).

[0067] Western blot

[0068] Endothelial cells were cultured in a culture dish (100×104 cells/well) and synchronized to the G1/G0 phase using serum-free basic EBM-2 medium. Then, the extract was applied under experimental conditions, and the cells were then lysed using lysis buffer (50 mM Tris-HCl, [pH 7.4], 150 mM NaCl, 10% glycerol, 1% nonidet P-40, 1 mM EDTA, 100μg/ml 4-(2aminoethyl)benzenesulfonyl fluoride, 10μg/ml aprotinin, 1μg/ml pepstatin A, 0.5μg/ml leupeptin, 80mM β-glycerophosphate, 25mM NaF, and 1mM sodium orthovanadate) to be centrifuged to obtain the cell extract. The expression and activity changes of various enzyme proteins were observed using immunoblot analysis.

[Appendix 2]

Prior Art

1 Introduction

(Page 5)

This study explored potential therapeutic agents for gastric cancer, one of the major cancers in Korea and identified that *Euonymus alatus* and *Zizania latifolia* are widely used in traditional remedies. To evaluate their effects, it aims to investigate the antioxidant and anticancer properties of ethanol extracts from *Euonymus alatus* and *Zizania latifolia*, as well as their mechanisms of action in order to provide foundational data for developing new drugs. To this end, the study analyzed total polyphenol content, total flavonoid content, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity to evaluate the antioxidant properties. Then, to assess anticancer efficacy, it treated four cancer cell lines: gastric cancer cells (AGS), cervical cancer cells (HeLa), liver cancer cells (HepG2), and breast cancer cells (MCF-7) with *Euonymus alatus* and *Zizania latifolia* extracts to measure cell viability (MTT assay), perform Western blot analysis, and observe cells using fluorescence microscopy. Also, to determine whether the activation of caspases induces apoptosis, it studied the effects of *Euonymus alatus* and *Zizania latifolia* extracts on the expression of Bcl-2, Bax, and caspases-3 in cancer cells.

2 Materials and Methods

(Page 7)

2. Sample Extraction

Zizania latifolia and *Euonymus alatus* for use were dried and ground to a 25-mesh size. Each 5 g sample was immersed in 80% ethanol and extracted at room temperature for 3 hours while agitated at 150 rpm using an orbital shaker (VS-201D, Vision Scientific Co.). The extract was filtered using Whatman paper No. 2 and vacuum-concentrated using a rotary vacuum evaporator (N-1000S-W, Tokyo Rikakikai Co.) to evaporate ethanol. Each sample was placed in a 50 mL conical tube, frozen for 24 hours in an ultra-low temperature freezer (MDF-794, Sanyo Electric Co.), and then lyophilized into powder using a freeze-dryer (PVTFD10R, Ilshin Lab Co.). The weight of each sample was measured, and the extraction yield (%) was calculated (28). The resulting extracts were then used as samples for later experiments.

(Page 9)

7. Cell Culture

In this study, the AGS, MCF-7, and Vero cell lines were cultured in RPMI-1640 medium, while the HeLa and HepG2 cell lines were cultured in Minimum Essential Medium (MEM). The culture media were added with 10% FBS and 1% antibiotic-antimycotic reagent and incubated at 37°C in a 5% CO_2 incubator. For the experiments, cells were subcultured and seeded at a concentration of 1×10^5 cells/mL.

3 Results

(Page 17)

6. Anticancer Efficacy of Extracts After 24-Hour Treatment

To evaluate the anticancer efficacy of *Zizania latifolia* and *Euonymus alatus* extracts on gastric cancer cells (AGS), cervical cancer cells (HeLa), liver cancer cells (HepG2), and breast cancer cells (MCF-7), the extracts were treated at varying concentrations (10, 50, and 100 μg/mL) for 24 hours, and anticancer activity was measured using the MTT assay (Fig. 4). Also, to evaluate the cytotoxicity of the extracts on normal cells, Vero, kidney cells, were treated with the same concentrations, and assessed for the extracts' cytotoxicity.

In gastric cancer cells (AGS), *Zizania latifolia* extract showed no anticancer efficacy, but when treated with the *Euonymus alatus* extract at 50 μg/mL, cell viability of AGS was decreased to 21.8% and at 100 μg/mL to 8.4% (Fig. 4A). When treated with *Zizania latifolia* extract, cervical cancer cells (HeLa) showed cell viabilities of 101.0%, 78.1%, and 67.8% at the extract's concentrations of 10, 50, and 100 μg/mL, respectively. When treated with *Euonymus alatus* extract, cell viabilities fell to 85.3%, 11.7%, and 10.0% at the same concentrations (Fig. 4B). When treated with *Zizania latifolia* extract, liver cancer cells (HepG2), decreased in cell viability to 96.8%, 96.7%, and 83.7% at concentrations of 10, 50, and 100 μg/mL, respectively, and when treated with *Euonymus alatus* extract, they showed cell viabilities of 73.9%, 59.7%, and 48.3% at the same concentrations, showing lower anticancer efficacy in HepG2 cells compared to that in gastric cancer and cervical cancer cells (Fig. 4C). *Zizania latifolia* extract decreased cell viability in breast cancer cells (MCF-7) to 94.2%, 88.1%, and 84.1%, while *Euonymus alatus* extract to 99.5%, 99.1%, and 76.1% at the same concentrations. Among the cancer cell lines tested, the lowest anticancer efficacy was observed in breast cancer cells, while the highest was in gastric cancer and cervical cancer cells (Fig. 4D). In normal kidney cells (Vero), both *Zizania latifolia* and *Euonymus alatus* extracts were confirmed to have no cytotoxicity (Fig. 4E).

(Page 19)

7. Anticancer Efficacy of Extracts After 48-Hour Treatment

Cells were treated with *Zizania latifolia* and *Euonymus alatus* extracts at varying concentrations (10, 50, and 100 μg/mL) for 48 hours, and the anticancer activity of the extracts were measured using the MTT assay (Fig. 5). Kidney cells (Vero) were also treated with the extracts at the same concentrations to evaluate their cytotoxicity.

The *Zizania latifolia* extract showed no anticancer inhibitory effect on gastric cancer cells, while the *Euonymus alatus* extract exhibited remarkable efficacy, with cancer cell viabilities of 63.7%, 10.8%, and 6.7% at concentrations of 10, 50, and 100 μg/mL, respectively (Fig. 5A). The *Zizania latifolia* extract did not have anticancer efficacy in cervical cancer cells, which indicates that drug resistance have developed after 24 hours. In contrast, *Euonymus alatus* extract showed sustained anticancer efficacy, with cervical cancer cell viabilities of 86.2%, 7.2%, and 6.0% (Fig. 5B). The anticancer efficacy of the *Zizania latifolia* extract in liver cancer cells was minimal, with a cell viability of 79.2% at a concentration of 100 μg/mL. In contrast, the *Euonymus alatus* extract exhibited concentration-proportional efficacy, with cell viabilities decreasing to 68.0%, 46.3%, and 38.6% at concentrations of 10, 50, and 100 μg/mL, respectively (Fig. 5C). In breast cancer cells, both *Zizania latifolia* and *Euonymus alatus* extracts exhibited only a little anticancer efficacy, but the *Euonymus alatus* extract demonstrated slightly higher anticancer efficacy (Fig. 5D). Both were found to have no cytotoxicity in normal kidney cells (Fig. 5E).

(Page 21)

8. Anticancer Efficacy of Extracts After 72-Hour Treatment

Cells were treated with *Zizania latifolia* and *Euonymus alatus* extracts at varying concentrations (10, 50, and 100 μg/mL) for 72 hours to measure cell viability using the MTT assay (Fig. 6). Vero or normal kidney cells were also treated with the same concentrations for the same duration to evaluate the extracts' cytotoxicity for normal cells.

When gastric cancer cells were treated with the *Zizania latifolia* extract, there was little change in cell viability, indicating that *Zizania latifolia* has low anticancer efficacy. However, when treated with *Euonymus alatus* extract at concentrations of 10, 50, and 100 μg/mL, gastric cancer cell viabilities were 66.7%, 18.7%, and 7.5%, respectively. This means that even with prolonged treatment, the extract consistently inhibited cancer cell proliferation,

demonstrating the remarkable efficacy (Fig. 6A). The *Zizania latifolia* extract did not affect the cell viability of cervical cancer cells, and prolonged treatment beyond 24 hours appeared to result in resistance, as cell viability was similar to that of the untreated control group. On the contrary, the *Euonymus alatus* extract significantly reduced cervical cancer cell viability, with viabilities of 91.2%, 5.2%, and 7.0% at the extract's concentrations of 10, 50, and 100 μg/mL, respectively (Fig. 6B). Liver cancer cells exhibited resistance to the *Zizania latifolia* extract and their cell proliferation significantly increased. Their cell viability was even higher than the untreated control group. In contrast, the *Euonymus alatus* extract demonstrated sustained anticancer efficacy, with liver cancer cell viabilities of 96.4%, 20.7%, and 18.9% at concentrations of 10, 50, and 100 μg/mL, respectively (Fig. 6C). The *Zizania latifolia* extract had weak anticancer efficacy against breast cancer cells, while the *Euonymus alatus* extract showed concentration-dependent anticancer efficacy, decreasing breast cancer cell viabilities to 90.9%, 62.8%, and 33.9% at concentrations of 10, 50, and 100 μg/mL, respectively (Fig. 6D). Both *Zizania latifolia* and *Euonymus alatus* showed slight cytotoxicity in normal kidney cells (Fig. 6E).

Table 2 presents the inhibitory concentration 50% (IC50) values of *Zizania latifolia* and *Euonymus alatus* extracts, indicating the concentrations required to inhibit cancer cell growth by 50% over various durations, which were measured using the MTT assay.

Table 2. The half maximal inhibitory concentration (IC_{50}) values of the extracts at different time periods

(단위 : μg/mL)

Extracts	Time	AGS	HeLa	HepG2	MCF-7
Zizania latifolia	24 hr	324.3±4.7	215.0±3.0	288.9±4.4	411.7±1.5
	48 hr	338.7±1.7	267.2±0.5	264.6±4.9	315.6±2.8
	72 hr	263.3±2.0	297.7±2.9	364.9±2.7	264.3±1.8
Euonymus alatus	24 hr	32.2±1.0	29.2±0.4	92.7±6.7	260.0±9.6
	48 hr	20.2±2.0	28.3±0.3	42.8±2.1	202.5±7.3
	72 hr	23.9±0.4	29.1±0.6	34.5±0.4	72.1±1.3