

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO			
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DEP:	2020 DIRECCION DE NUEVAS CREACIONES	EVE:	378 FASE NACIONAL INCLUIDO CAPITULO I
TRA:	11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS:	28
ACT:	519 PRESENTACION		

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS REINALDO OLARTE GARCIA

Asunto: Radicación: 13-111842- -12
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 10477
 Folios: 28

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2011/059197				
Fecha de Presentación Internacional	03/11/2011				

1. Documentos estudiados

Descripción:	Radicado N°13-111842-00000-00000	03/Mayo/2013
Reivindicaciones:	Radicado N°13-111842-00000-00000	03/Mayo/2013
	Radicado N°13-111842-00011-00000	03/Mayo/2013
Prioridad:	US 61/410,279	04/Noviembre/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10)

reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Para el caso de la presente solicitud, al verificar la tasa pagada, todo el capítulo reivindicatorio presentado se tendrá en cuenta para realizar el examen de patentabilidad

Título de la solicitud: “Composiciones que contienen derivados de antraquinona como promotores de crecimiento y agentes antifúngicos”.

El problema planteado en esta solicitud es la necesidad desarrollar composiciones y métodos que promuevan el crecimiento de la planta.

Para resolver este problema técnico la solicitud en estudio proporciona un método para modular el crecimiento de una planta, debidamente definido y composiciones (radicado N°13-111842-00000-00000, Págs. 3 y 4).

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 01 N 35/06 // 65/30 // P 21/00 // 3/00.

4. No es una invención (Art 15 D 486 literal (b))

El objeto de las reivindicaciones 13 y 14 no se considera una invención, de acuerdo con el Art citado, por cuanto la composición se está caracterizando por un extracto a partir de material vegetal de la planta *Reynoutria sachalinensis* que comprende derivados de antraquinona sin especificar el compuesto marcador que caracteriza el extracto y su huella cromatográfica, lo cual, corresponde a material biológico tal y como se encuentra en la naturaleza o a aquel que pueda ser aislado, ya que no existe evidencia del diseño de una composición que incluya las fracciones y extractos caracterizados por el compuesto marcador, por lo anterior se concluye que la composición no puede ser objeto de patente de conformidad con lo dispuesto en el artículo 15 lit. b) de la referida Decisión 486.

Por otra parte, se recuerda a la solicitante que cuando se habla de un ingrediente botánico extraído se puede tratar de un compuesto puro, o una mezcla de compuestos conocidos y plenamente identificados por diferentes métodos de elucidación estructural, situación que se omite en el caso de la solicitud en estudio. Asimismo, es necesario aclarar que la obtención de compuestos biológicamente activos a partir de fuentes naturales incluye procesos de secado, extracción, purificación, evaluación biológica, elucidación química y estructural, elementos que no se encuentran dentro de la divulgación de la solicitud. Y por otra parte, cuando se habla de un extracto es necesario hacer alusión al tipo de extracto, parte de la planta de donde se obtiene y método de obtención. Seguido de al menos el perfil cromatográfico donde se indique al menos el compuesto marcador y el diseño de composiciones farmacéuticas, nutraceuticas o alimenticias a partir el mismo, sin embargo, dicha información tampoco se encuentra reportada en la reivindicación independiente.

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El método de las reivindicación 1 – 12 corresponden al uso del extracto que contiene un derivado de antraquinona para modular el crecimiento de la planta. Es evidente que las llamadas etapas de “aplicar por inmersión...”, “aplicar por irrigación...”, “atomizar el surco” corresponden a la forma de usar el extracto, aunque en su preámbulo se reclame como método. Se le aclara al solicitante que un método cuya única etapa corresponde a una aplicación, dicha aplicación es un uso. Aunque se menciona trasplantar la planta y la vía de irrigación por goteo o inmersión son acciones típicas en el momento de sembrar y aplicar plaguicidas o controladores de crecimiento¹, que si

¹ Bordoli J.M et AL. Aplicación de fertilizantes. Curso de fertilizantes. Facultad de agronomía –

pueden llegar a tener una ventaja, como se ha comprobado en algunos estudios², pero para la solicitud no se evidencia una ventaja sino por el contrario se evidencia que puede ser uno u otro.

Por lo tanto dicho método no es patentable de acuerdo con el artículo 14.

6. De la Solicitud de Patente

La solicitud en trámite, que presento el procedimiento acelerado de exámenes de patente (PPH) no puede ser concedida tal como fue en Estados Unidos, ya que como se mencionó en los anteriores ítems, no cumple con los requisitos necesarios contemplados en Colombia de acuerdo a lo establecido en la Decisión 486. Adicionalmente, la solicitante no presenta el capítulo reivindicatorio tal como fue concedido en los Estados Unidos tal como se evidencia en la tabla comparativa del radicado N°13-111842-00009-00000; por lo tanto es procedente a realizar el requerimiento de 45.

Título

El título no corresponde con el objeto de la solicitud porque se menciona como eje central las composiciones que como ya se mencionó no es una invención, sin mencionar el método que abarca la mayoría de reivindicaciones, por lo cual se sugiere al Solicitante hacer la corrección necesaria.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no se encuentra debidamente sustentado puesto que claramente evidencia que el método no es más que la aplicación de un extracto y observar el crecimiento de ciertas partes de la panta, lo cual se corrobora con las realizaciones preferidas que el supuesto método consiste en la aplicación a diferentes proporciones de un extracto, que no se encuentra caracterizado por un cromatograma o una composición clara de los compuestos del extracto, tal como se evidencia en la tabal 1, que muestra en que parte de la planta se da la aplicación, la proporción y la composición que solo es extracto de la planta.

Tabla 1. Ejemplos del método

Tipo de aplicación	Parte de la planta	Proporción	Etapas	Composición
Inmersión	Toda la planta	0.25, 0.5 y 1.0%	Aplicar	Extracto de <i>Reynoutria sachalinensis</i> (REGALIA®ME)
Recubrimiento	Semillas	5% (p/v)	Aplicar	Extracto seco de <i>Reynoutria sachalinensis</i> (REGALIA®ME) en etanol
Remojar	Transplantes / plantas de raíces desnudas	0,5; 1% (v/v)	Aplicar	Extracto de <i>Reynoutria sachalinensis</i> (REGALIA®ME)
Riego por goteo	planta	0.25; 0.5; 1% (v/v); 2, 4 qt/a	Aplicar	Extracto de <i>Reynoutria sachalinensis</i> (REGALIA®ME)
Sumergir	Tomate	0,5; 1% (v/v)	Aplicar	Extracto de <i>Reynoutria sachalinensis</i> (REGALIA®ME)

Universidad de la Republica uruguay, 2010.
 2 Uremis Ilhan et Al (2004). Studies on different herbicide. Application methods in second-crop maize fields. Crop protection, 23, Pág. 1137-1144

El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción.

El capítulo reivindicatorio no se encuentra sustentado porque se habla que el extracto proviene de diferentes especies de plantas y según las realizaciones preferidas solo proviene de *Reynoutria sachalinensis*, por lo tanto el resto de especies no estaría debidamente sustentado y puede ser que los efectos sean diferentes. El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción.

El capítulo reivindicatorio no se encuentra debidamente sustentado porque menciona un extracto de *Reynoutria sachalinensis* que ya es un producto comercial y ya es conocido que extractos de esas plantas presentes antraquinonas por lo tanto llegar a decir que se esta caracterizando el extracto por la presencia de antraquinonas no es posible porque ya es conocido en el estado de la tecnica (ver D1)³.

Las reivindicaciones 4 y 5 contienen expresiones que corresponde a resultados a alcanzar tales como

-“... modulación de crecimiento es la modulación de la germinación”

-“... modulación de la iniciación de las raíces o la extensión de las raíces de una planta”

Lo cual no define el método porque la invención no queda definida por el resultado a alcanzar, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado.

Las reivindicaciones 1 y 12 no son concisas porque los términos “disolvente”, “vehículo sólido”, “agente de recubrimiento” es muy general puesto que solo se estaría refiriendo por la función de dicho compuesto y no se estaría definiendo por denominación común de dicha sustancia o por nombre IUPAC. Se recuerda al solicitante que las composiciones se deben definir por ingredientes activos específicos y sus proporciones.

Descripción (Art 28 D 486)

En el capítulo descriptivo (radicado N°13-127979-00000-00000, Págs. 15 – 24) se mencionan marcas registradas, tales como, REGALLA®ME, ALIETTE®, entre otros se sugiere modificar de tal manera que se establezca de manera inequívoca su estructura o características químicas para que pueda ser reproducible la invención.

A lo largo de la descripción se describe claramente que no es un método con una serie de etapas con condiciones definidas y tampoco un método donde se aplique una composición que contiene un extracto con más componentes, sino un método de aplicación de un extracto de una planta de la familia Polygonaceae (Pág 3, líneas 26 a Pág. 4, línea 4; Pág. 7, líneas 24 – 25; Págs. 13 y 14)

7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad: Junio 04 de 2010 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	Inoue M. et Al, Journal of Chemical Ecology,	“Allelochemicals from <i>Polygonum sachalinense</i> FR.Schm. (polygonaceae)”	1992

³ Regalia® SC, características, composición, peligros y cuidados, USA, URL:<http://strawberry.ifas.ufl.edu/plant/pathfiles/Fung%20label/fung%202009/regalia%20SC.pdf> (anexo1)

	vol. 18		
D2	JP 08-099813	“Water-Based liquid agent for plant growth regulation”	16/abril/1996

El documento D1 ^[4] divulga que extractos de raíces de *Polygonaceae sachalinense* en un sistema de recirculación, inhibió el crecimiento de las plántulas de lechuga. Los rizomas y raíces de *Polygonaceae sachalinense* se extrajeron con acetona al 80%. Del extracto se identificaron compuestos de antraquinona: emodin y fiscion, también se aislaron compuestos glucosidados, además se determinó la composición de todos los compuestos (abstract, Págs. 1833 – 1834)

El documento D2 ^[5] divulga un agente líquido a base de agua para la regulación del crecimiento de plantas que comprende un componente (A) antraquinona, entre otras, (B) ácido orgánico; los derivados de antraquinona provienen de diferentes plantas de la familia Polygonaceae (abstract, Párrs. [0001], [0005] – [0008], [0020] – [0025])

8. Examen de Patentabilidad (Art 14 D486)

A pesar de que la solicitud se sigue considerando un uso por ser un método de aplicación, se realiza un análisis de nivel inventivo para evidenciar aún más que es posible del estado de la técnica derivar la aplicación de un extracto de plantas de la familia Polygonaceae.

Nivel Inventivo (Art18 D486)

La **reivindicación 1** consiste en un detergente debidamente definida (Rad. N°13-111842-00011-00000, Pág. 9)

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Método que comprende:	Método que comprende:	Composición que comprende:
Aplicar por inmersión a la raíz de una planta o a toda la planta del 0,25 a 1,5% de una composición que comprende derivados de antraquinona derivado de plantas Polygonaceae, Rhamnaceae, y un disolvente o vehículo	Planta de lechuga se siembran en una olla aceptor y la solución de nutrientes a partir de Polygonaceae sachalinense se levanta a través de una tubería en el pozo aceptor (aplicación en el surco), el extracto de P. sachalinense tienen antraquinonas tales como Emdoin y fisción	Un derivado de antraquinona proveniente de Polygonaceae, azúcar, agua, ácido orgánico

El documento D1 es considerado el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga que extractos de raíces de *Polygonaceae sachalinense* en un sistema de recirculación, inhibió el crecimiento de las plántulas de lechuga. Los rizomas y raíces de *Polygonaceae sachalinense* se extrajeron con acetona al 80%. Del extracto se identificaron compuestos de antraquinona: emodin y fiscion, también se aislaron compuestos glucosidados, además se determinó la composición de todos los compuestos. Planta de lechuga se siembran en una olla aceptor y la solución de nutrientes a partir de Polygonaceae sachalinense se levanta a través de una tubería en el pozo aceptor (aplicación en el surco) (abstract, Págs. 1833 – 1834)

La diferencia entre la invención, definida en la reivindicación 1 y el método de D1 consiste en que el compuesto se aplica por inmersión y en una cantidad de 0,25 –

⁴anexo 2
⁵URL:http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=19960416&CC=JP&NR=H0899813A&KC=A y anexo 3 (traducción a ingles)

1,5%, mientras que en D1 no se especifica cómo es dicha aplicación.

El efecto que lograría las diferencias antes mencionada en el método de la reivindicación 1, en cuanto a mejorar el crecimiento, no puede ser determinado que la mejora del crecimiento se deba al tipo de aplicación o a la cantidad de la composición aplicada.

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa al el método conocido según D1 para que sea un método y no una simple aplicación.

Sin embargo ya es conocida en el estado de la técnica que una composición que contiene antraquinona y otros componentes ayuda en el crecimiento de plantas, D2 divulga un agente líquido a base de agua para la regulación del crecimiento de plantas que comprende un componente (A) antraquinona, entre otras, (B) ácido orgánico; los derivados de antraquinona provienen de diferentes plantas de la familia Polygonaceae. Además la composición tiene un efecto sinérgico (abstract, Párrs. [0001], [0005] – [0008], [0020] – [0025])

Como consecuencia de los hechos mencionados en los párrafos anteriores la persona medianamente versada en la materia habría derivado de manera obvia y sin mucho esfuerzo de su parte el método descrito en la reivindicación 1 a partir de incluir las enseñanzas de D2 en cuanto a emplear antraquinonas en el crecimiento de plantas y en una composición, junto con las enseñanzas del método en el que se aplica el extracto de Poligonaceae a plantas del documento D1 toda vez que el método sigue siendo la aplicación del extracto de plantas de la familia de Poligonaceae . Por lo cual al combinar D1 con D2 la reivindicación se considera obvia

Puesto que las reivindicaciones dependientes 2 – 12 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

Conclusión:

Las reivindicaciones 1 – 12, cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Inoue M. et Al, Journal of Chemical Ecology, vol. 18	1 – 12	nivel inventivo
D2	JP 08-099813	1 – 12	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-08-26

Elaboró: Mayra Alejandra Quintero
Revisó: Gloria Jacqueline Alfonso Roza

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
CONSULTA DE DOCUMENTOS

ALLELOCHEMICALS FROM *Polygonum sachalinense* FR. SCHM. (POLYGONACEAE)

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Abstract—The root exudates from *Polygonum sachalinense* in a recirculating system significantly inhibited lettuce seedling growth. The rhizomes and roots of *P. sachalinense* were extracted with 80% acetone. Bioassay of the neutral-acidic fraction on the TLC agar plate showed the inhibitory activity corresponded to the two yellow pigment bands. Two orange needles were isolated and identified as anthraquinone compounds: emodin and physcion. Both compounds exhibited inhibitory activities against the seedling growth of several testing plant species. Glucosides were isolated from *P. sachalinense* and were identified as emodin-1-*O*- β -D-glucoside and physcion-1-*O*- β -D-glucoside, respectively. On plant growth bioassay, these glucosides showed no phytotoxic activity against lettuce seedlings. The concentrations of emodin, physcion, and their glucosides from rhizome with roots, aerial parts, fallen leaves, and soil were determined. The rhizome with roots and fallen leaves contained emodin and physcion at relatively high concentrations. Emodin also occurs in the soil of this plant community with effective concentrations in the fall. The results indicate that these anthraquinones are responsible for the observed interference and are potent allelopathic substances.

Key Words—*Polygonum sachalinense*, allelopathy, allelochemicals, anthraquinone, emodin, emodin-1-*O*-D-glucoside, physcion, physcion-1-*O*-D-glucoside, root exudates.

INTRODUCTION

Chemical interactions among plants have long been recognized. Many scientists have shown that certain plants produce secondary compounds that have the

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ability to inhibit growth and germination of other plants (Rice, 1984; Harborne, 1988). The genus *Polygonum* has about 300 species and is distributed all over the world. AlSaadawi and coworkers demonstrated that *Polygonum aviculare* was allelopathic, and several phenolic compounds and long-chain fatty acids appeared to be responsible for this activity (AlSaadawi and Rice, 1982a,b; AlSaadawi et al., 1983).

P. sachalinense (Sachaline giant knotweed), or "Ohitadori" in Japanese, is a perennial grass found in Honshu and Hokkaido. Its rhizome has been used as an herbal medicine for laxative and diuretic purposes. This grass forms colonies along roadsides and river banks in gravelly soils. Judging from its ability to rapidly colonize, we hypothesize that allelopathy contributes to its aggressiveness.

This paper deals with the isolation and identification of allelochemicals in *P. sachalinense* and the evaluation of these allelochemicals in the ecosystem.

METHODS AND MATERIALS

Root Exudate Recirculating Systems. The evaluation of the effect of the root exudates as allelochemicals was carried out according to the root exudate recirculating system of Stevens and Tang (1985). Lettuce seedlings were planted in the acceptor pot, while *P. sachalinense* was grown in the donor pot. Nutrient solution (Tadano and Tanaka, 1976) from the donor pot was lifted through 8-mm-diameter glass tubing to the acceptor pot using an air pump. Nutrient levels were maintained, and pH was kept 5.5 with H_2SO_4 or NaOH. Recirculating solution was not changed during the experiment except for replenishing water and nutrient solution. After 10–14 days, the growing condition of lettuce (*Lactuca sativa* L.) seedlings was compared with that of the control growing under the same conditions without the donor plant. The experiment was carried out in a greenhouse.

Plant Materials and Extraction. Rhizomes with roots, aerial parts, and fallen leaves of *P. sachalinense* were collected at several wild places near the campus of Hokkaido Tokai University. Excavated rhizomes were rinsed with tap water and brushed lightly to remove soil, and air-dried. The rhizome (5.1 kg) and aerial part (3.8 kg) were sliced and extracted in separate containers with 80% acetone for about two months at room temperature. The extracts were filtered and concentrated under reduced pressure. In another experiment, 100 g of fallen leaves were extracted with 300 ml of ether for 24 hr, and the residues were removed by filtration.

Fractionation. The acetone extract of rhizome with roots was divided into ether-soluble and water-soluble fractions. The ether-soluble fraction was further divided into neutral, acidic, and basic fractions in an usual manner. The water-

soluble fraction was evaporated to dryness for bioassay; it did not show any significant activity against the growth of lettuce seedlings.

TLC Agar Plate Bioassay. Thin-layer chromatography was carried out on a 20 × 7-cm silica gel glass plate (Merck, Kieselgel 60 F254) 0.25 mm thick using hexane-CHCl₃-MeOH (4:10:1 v/v/v) as developing solvents. The chromatogram was covered with a 0.5% agar layer (2 mm), and the seeds of green amaranth (*Amaranthus viridis* L.), timothy grass (*Phleum pratense* L.), crabgrass (*Digitaria adscendens* Henr.), and Chinese cabbage (*Brassica campestris* L.) were sown in a row perpendicular to the bands on the chromatogram. The seeds were incubated in a moisture saturated growth chamber at 24–26°C with 14 hr light and 10 hr dark. After four to seven days, seed germination and plant growth were examined.

Seedling Growth Bioassay. In a 25-mm-high × 40-mm-ID glass Petri dish 10 germinated seeds were placed on 0.5% agar gel with a known concentration of the test sample. Lettuce, green amaranth, and timothy grass were used for bioassay. The assay was done in duplicate. Incubation was for seven days in a growth chamber at 24–26°C with a 14-hr light and 10-hr dark cycle. The average length of hypocotyl or leaf sheath and the longest roots of the testing plants were measured and compared with that of the control growing on 0.5% agar gel only.

Isolation and Identification of Allelochemicals. The inhibitory substances against seedling growth were fractionated by SiO₂ column chromatography using a hexane-CHCl₃-MeOH gradient as an eluent, and the desired products were recrystallized from hexane-CHCl₃ to give two orange-needle crystals. The isolated compounds were identified from IR, UV, mass, and NMR spectra.

Emodin (1): IR (KBr) $\nu_{\max}$ 3400 (H)(OH), 1630 (C=O) cm⁻¹; UV (MeOH) $\lambda_{\max}$ 221, 253, 265, 289, 437 nm; [¹H]NMR [(CD₃)₂CO] δ 2.46 (3H, s, Ar-CH₃), 6.65 (1H, d, *J* = 2.5 Hz, H-2), 7.12 (1 H, br s, H-7), 7.24 (1H, d, *J* = 2.5 Hz, H-4), 7.55 (1H, br s, H-5), 12.05 (1H, s, OH), 12.18 (1H, s, OH); HR-EI-MS *m/z* 270.0515 (C₁₅H₁₀O₅).

Physcion (2): IR (KBr); $\nu_{\max}$ 3400 (OH), 1630 (C=O) cm⁻¹; UV (MeOH) $\lambda_{\max}$ 223, 254, 265, 287, 434 nm; [¹H]NMR (CDCl₃) δ 2.45 (3H, s, Ar-CH₃), 3.94 (3H, s, Ar-OCH₃), 6.68 (1H, d, *J* = 2.5 Hz, H-2), 7.08 (1H, br s, H-7), 7.36 (1H, d, *J* = 2.5 Hz, H-4), 7.63 (1H, br s, H-5), 12.12 (1H, s, OH), 12.31 (1H, s, OH); HR-EI-MS *m/z* 284.0704 (C₁₆H₁₂O₅).

Isolation and Identification of Anthraquinone Glycoside. The rhizomes with roots and the aerial part of *P. sachalinense* were collected in June 1991. Immediately after cutting, they were extracted in EtOH by refluxing for 5 hr and the EtOH solution concentrated to one third of the original volume. The aqueous concentrate was extracted with ether to remove free anthraquinones and other ether-soluble constituents. Ba(OH)₂ solution (5%) was added to precipitate the organic acids. After filtration, the filtrate was adjusted to pH 3 and was extracted

with *n*-BuOH. The *n*-BuOH layer was separated and evaporated in vacuo to give the crude glycoside fraction, which was further fractionated by SiO₂ column chromatography using a hexane—CHCl₃—MeOH as an eluent to obtain anthraquinone glycosides. Rechromatography and further recrystallization from MeOH gave two glycosides as orange-needle crystals. Identification was based on the comparison of the melting point, *R_f* values, and spectrometric data with those in Kang and Woo (1982) and Kato and Morita (1987).

Emodin-1-*O*-β-D-glucoside (**3**): mp 193°C; IR (KBr) $\nu_{\max}$ 3400 (OH), 1630 (free C=O), 1600 (chelated C=O) cm⁻¹; UV (EtOH) $\lambda_{\max}$ 221, 253, 265, 286, 421 nm; [¹H]NMR (CD₃OD) δ 2.40 (3H, s, Ar-CH₃), 4.96 (1H, d, *J* = 7.5 Hz, anomeric H), 7.04 (1H, br s, H-7), 7.10 (1H, d, *J* = 2.5 Hz, H-2), 7.32 (1H, d, *J* = 2.5 Hz, H-4), 7.48 (1H, br s, H-5); FAB-MS (DMSO) *m/z* (NaCl addition) 455 (*M* + Na)⁺, 478 (*M* + 2Na)⁺.

Physcion-1-*O*-β-D-glucoside (**4**): mp 240°C; IR (KBr); $\nu_{\max}$ 3400 (OH), 1640 (free C=O), 1600 (chelated C=O) cm⁻¹; UV (MeOH) $\lambda_{\max}$ 226, 273, 417 nm; [¹H]NMR (CD₃OD) δ 2.46 (3H, s, Ar-CH₃), 3.97 (3H, s, Ar-OCH₃), 4.96 (1H, d, *J* = 7.5 Hz, anomeric H), 7.11 (1H, br s, H-7), 7.30 (1H, d, *J* = 2.5 Hz, H-2), 7.57 (1H, d, *J* = 2.5 Hz, H-4), 7.60 (1H, br s, H-5).

Quantitation of Anthraquinones and Anthraquinone Glycosides. Plant samples were collected at several wild places near the campus of Hokkaido Tokai University. Fresh aerial parts, rhizome with roots, and fallen leaves were extracted with 80% acetone. After concentration, the aqueous residues were extracted with ether. The ether-soluble fractions were quantitatively analyzed by HPLC (JASCO System-800) and compared with standard emodin and physcion (purchased from Funakoshi Co. Ltd.). The analytical conditions are as follows: column—YMC-Pack ODS-AM (30 cm × 10 mm ID); solvent—MeOH/H₂O (80:20, v/v); flow rate—4 ml/min, wavelength of detection—245 nm.

Soil samples were collected in November 1990 from the center of a *P. sachalinense* community near the campus of Hokkaido Tokai University at the depth of about 10 cm. Litter was removed as completely as possible by sieving and the soil was dried at room temperature overnight. A 500-g soil sample was stirred in 2 liters of acetone–1 N HCl (2:1, v/v) for 1 hr at 50°C and further treated with ultrasonic energy for 1 min. After leaving overnight at room temperature, it was filtered. The filtrate was evaporated to remove acetone and extracted three times with ether. The ether extract was evaporated in vacuo and fractionated by SiO₂ column chromatography. The contents of emodin and physcion were determined by HPLC. The amounts of anthraquinone glycosides were determined by weighing the isolated crystals of emodin-1-*O*-β-D-glucoside and physcion-1-*O*-β-D-glucoside.

Spectrometers. IR spectra were recorded on a JASCO IR-810 as KBr disk for crystals. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. NMR spectra were measured on a Bruker AM-400 (400 MHz) spectrometer

using TMS as an internal standard. Mass spectra were recorded using JEOL JMS-DX303HF GC-MS spectrometer.

RESULTS AND DISCUSSION

The root exudates from *P. sachalinense* showed an inhibition on lettuce seedling growth using the recirculating system. It is well known that organic compounds are exuded from healthy undamaged plant roots (Rovira, 1969), and we expect that allelochemicals or their precursors are present in the plant tissue and also released from the rhizome and roots.

From the results of the TLC agar plate bioassay, growth inhibitory activity was shown in the two bands of yellow pigments against green amaranth and timothy grass on the chromatogram of the neutral-acid fraction (Figure 1). Two corresponding orange needles were isolated and identified as anthraquinone compounds: emodin (**1**) and physcion (**2**) (Figure 2).

Emodin and physcion exhibited inhibitory activities against the seedling growth of several test species (Figure 3). Emodin inhibited root and hypocotyl or leaf sheath growth over 100 ppm (3.7×10^{-4} M). It severely inhibited the growth of lettuce seedlings at 50 ppm (1.85×10^{-4} M). Physcion was less active and inhibited root and hypocotyl growth at 200 ppm (7.0×10^{-4} M).

The presence of anthraquinone glucosides was expected since they are common for transportation and storage and are less toxic in the plant. As a result, two glucosides were isolated from both the rhizome and aerial part of *P.*

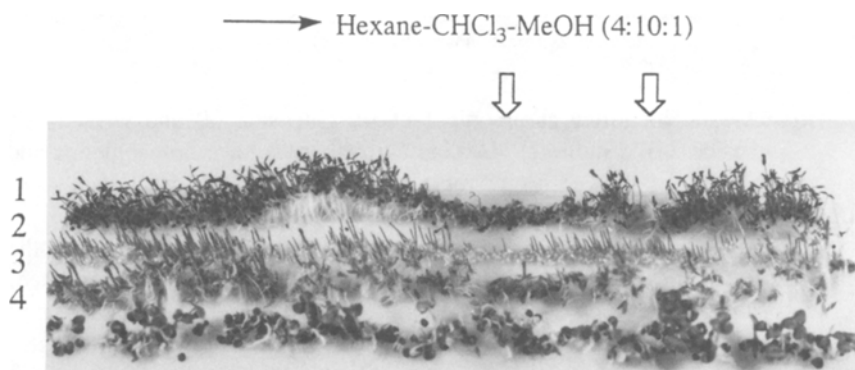


FIG. 1. Bioassay of neutral and acid substances from *Polygonum sachalinense* by TLC agar plate method (5 days). Arrows point to areas of inhibited plant activity. (1) green amaranth, (2) timothy grass, (3) crab grass, (4) Chinese cabbage.

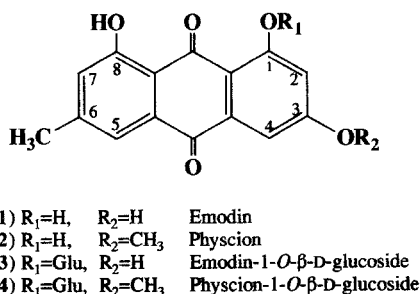


FIG. 2. Chemical structures of allelochemical candidates from *Polygonum sachalinense*.

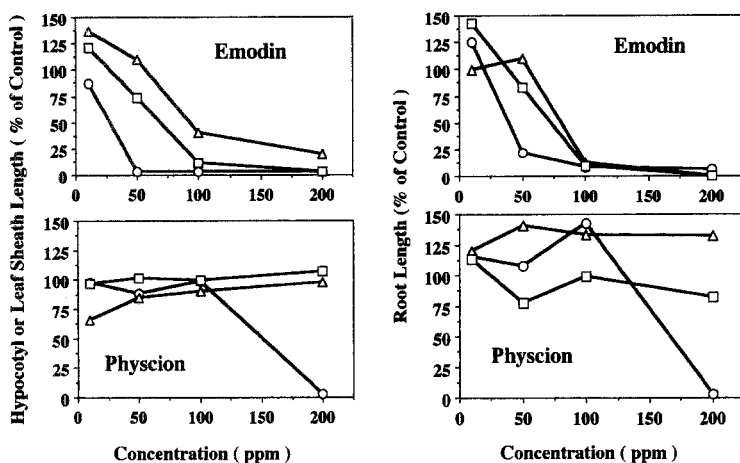


FIG. 3. Growth inhibitory activities of emodin and physcion against plant seedlings. ○: lettuce, △: green amaranth, □: timothy grass.

sachalinense and identified as emodin-1-*O*- β -D-glucoside (3) and physcion-1-*O*- β -D-glucoside (4) (Figure 2). In plant growth bioassay, both emodin and physcion glucosides showed no phytotoxicity at 200 ppm against lettuce seedlings.

The concentrations of emodin and physcion, and their glucosides in the rhizome, aerial part, fallen leaves, and soil were quantitatively determined. Contents of emodin and physcion in the fresh rhizome were ca. 158 and 32 mg/kg fresh weight, and in the aerial part, ca. 72 and 22 mg/kg fresh weight, respectively. Large amounts of anthraquinones were still detected in dry fallen leaves more than four months after defoliation: emodin, 213 mg/kg dry weight; physcion, 180 mg/kg dry weight. In addition, contents of emodin and physcion in the soil were 55 and 30 mg/kg dry weight, respectively; these concentrations

are enough to inhibit plant seedlings. These facts indicate that the anthraquinones are very stable in the ecosystem.

Allelopathic substances are released from plants in four ways (Tukey, 1969): (1) Litter of leaves and stems decomposes by physical or biological processes and the substance is released. (2) The active substance is released to the soil from the root directly by exudation or through the decay of dead roots. (3) Volatile material is vaporized from plants and acts on other plants through the air. (4) Rain and fog drips transfer toxic compounds from leaves to the soil.

We consider that the first two items above are associated with the allelopathy of *P. sachalinense*. It has anthraquinone glucosides in the plant tissue. We propose that either these glucosides or the aglycons are released from the rhizome. The glucosides are then decomposed to active forms—emodin and physcion—and subsequently exhibit plant growth inhibitory activities against other plant species. To confirm this hypothesis, isolating glucosides and aglycons from the root exudate solution is currently under investigation. Furthermore, anthraquinones exuded from the fallen leaves of *P. sachalinense* accumulate on the soil surface and affects early seedling growth of nearby plants of the community.

There are reports about antimicrobial activities of emodin and physcion (Podojil et al., 1978; Anke et al., 1980; Kitanaka and Takido, 1986). Some antimicrobial activity by emodin and physcion may directly or indirectly affect the growth of other plants. Rice (1964, 1984) and AlSaadawi et al. (1983) pointed out that some weeds produce allelopathic chemicals that inhibited soil bacteria and changed the soil environment to their own advantage. While these anthraquinone compounds are potent allelopathic substances, the question of the extent of their impact against the flora in *P. sachalinense* is yet to be fully evaluated.

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KODA KATSUMI

(54) WATER-BASED LIQUID AGENT FOR PLANT GROWTH REGULATION
(57)Abstract:
PURPOSE: To provide a water-based liquid agent for plant growth regulation, exhibiting a defense action on disease and insect damage, usable without imparting the pathogen with chemicals resistance and having high safety in the decomposition in soil.
CONSTITUTION: This water-based liquid agent for plant growth regulation contains (A) a component composed of (i) at least one compound selected from berberine, matrine, an anthraquinone derivative, a flavonoid compound, glycyrrhizin, an essential oil, an alkylphthalide, a phthalide derivative and capsaicin and (ii) at least one compound selected from starch, isoflavone compound and sugar and containing berberine as an essential component and (B) a C-6-C-8 linear carboxylic acid.

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WATER-BASED LIQUID AGENT FOR PLANT GROWTH REGULATION

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application]the plant growth adjustment with high safety [in addition to a vegetable growth promotion operation, the present invention has a defense operation on a damage by blight and harmful insects, and moreover do not make a disease germ produce tolerance, and] at the time of decomposition in soil -- service water -- it is related with a sex drug solution.

[0002]

[Description of the Prior Art]Until now, synthetic organic agricultural chemicals etc. have been applied as a direct control means against a pest. Manure etc. have been used about vegetable growth promotion.

[0003]

[Problem to be solved by the invention]However, about use of synthetic organic agricultural chemicals, there is a problem of delay of vegetable growth, the deterioration of a resistance force to a damage by blight and harmful insects, a noxious insect and a disease germ acquiring tolerance further. Destruction of the environmental pollution by use of synthetic organic agricultural chemicals or an ecosystem poses a problem. On the other hand, about manure, it must be embedded in vegetable soil etc., the complicated work of the embedding of digging up of soil, dressing, and soil, etc. is required, and the reduction of work is called for.

[0004]the plant growth adjustment with high safety [this invention was made in view of such a situation, in addition to a vegetable growth promotion operation, has a defense operation on a damage by blight and harmful insects, and moreover do not make a disease germ produce tolerance, and] at the time of decomposition in soil -- service water -- offer of a sex drug solution is set as that purpose.

[0005]

[Means for solving problem]in order to attain the above-mentioned purpose -- plant growth adjustment of this invention -- service water -- a sex drug solution takes the composition of containing the following (A) component and the (B) component.

(A) Berberine, sophocarpidine, the Anthraquinone derivative, flavonoids, glycyrrhizin, oil refinement, alkyl phthalides, a phthalide derivative, and the group which consists of capsaicin, And it is a component containing the compound chosen at least one [at a time] from each group of the group which consists of starch, isoflavone, and sugars, respectively, and berberine is made indispensable.

(B) With a carbon number of four or less low-grade organic acid.

[0006]

[Function]Namely, the place which was asking for what can control a damage by blight and harmful insects without the inventors using synthetic organic agricultural chemicals, The low-grade organic acid which is a common gender anaerobic fermentation thing of a certain kind of crude drug, the berberine which is the crude drug ingredient, etc., It found out that it had a remarkable effect that you make a natural state recover the rhizosphere microorganism (the vital force declines according to environmental aggravation) adhering to a vegetable root etc., or make it activated, and it was effective in not using synthetic organic agricultural chemicals together, but ** making agricultural products harvest by high yield, and this invention was reached.

[0007]First, it describes about the raw material used by this invention.

[0008]An operation of the (A) component which constitutes this invention, the effect, etc. are as follows. The following component used as such human being's drugs finds out that it is effective also in vegetable growth, and this invention is the characteristics of the maximum [this]. In the following, the operation on a plant is shown with the parenthesis.

- berberine -- the main ingredients of the bark (cork tree bark) except the periderm of a yellowfin tuna (*Phellodendron amurense* Ruprecht) (Rutaceae) or its species of the same genus. Stomachic medicine for intestinal disorders. [Inhibition of the inhibition effect and Acari to an antibacterial action, bacillus subtilis, etc. to *Staphylococcus aureus* etc., inhibition of mold.]

- the root of sophocarpidine Clara (*Sophra flavescens* Aiton) (Leguminosae) -- the main ingredients of the thing [remain as it is or] (*sophorae radix*) except the great portion of periderm. Stomachic, urination, and an antipyretic analgesic. [The growth stop of antiprotozoal action, an antifungal action, and *Trichophyton*, the necrosis reaction of TMV, the eradication activity of a virus.]

- The main ingredients of the usually rhizome (*rhei rhizoma*) of Anthraquinone derivative *Rheum palmatum* Linn., *R.tangticum* Maxim., *R.officinale* Baillon, and *R.coreanum* Nakai (Polygonaceae). Stomachic. [The synthesis depressant action of bacterial nucleic acid and protein, the antibacterial action to *Staphylococcus aureus*, an antiviral action.]

- Flavonoids *Scutellaria baicalensis* (*Scutellaria*) The root (*Scutellaria root*) or Satsuma orange (*Citrus unshiu* Markovich) except periderm of *baicalensis* Georgi (*Labiatae*) (*C. aurantium*Linn. subsp.) The main ingredients of the fruits (*Aurantii nobllis pericarpium*) in which nobilis Markino (*Rutaceae*) or close species matured. Aroma stomachic. [The strong antibacterial action to an antibacterial action, a dysentery bacillus, a *staphylococcus*, etc. to gram positive bacteria.]

- The glycyrrhizin *Glycyrrhiza*. The liquorice with a hide by the root and stolon (liquorice) of *glabra* Linn. var. *glandulifera* Regel et Herder, *G.uralensis* Fischer (*Leguminosae*), or

these congeners as it is, The main ingredients of the thing excluding [leather **** liquorice] periderm. Detoxication, resolution, and antitussive. [Antiviral action.]

- The main ingredients of the rhizome (ginger) of oil-refinement ginger (*Zingiber officinale* Roscoe) (*Zingiberaceae*). It is contained also in an *angericae radix* and a *cnidium rhizome*. Aroma stomachic. [Antibacterial properties and antiprotozoal action.]

- The main ingredients of what (*angericae radix*) passed the root of alkyl phthalide *Angelica acutiloba* Kitagawa (*Umbelliferae*) or allied species through hot water usually. A drug for female use and the tonic. [The antibacterial action to a dysentery bacillus, *Escherichia coli*, etc.]

- The main ingredients of what (*cnidium rhizome*) passed the rhizome of the phthalide derivative *cnidium rhizome* (*Cnidium Officinale* Makino) (*Umbelliferae*) through hot water usually. A drug for female use and the tonic. [Depressant action to gram-negative disease germs, such as a dysentery bacillus and *Salmonella typhi*.]

- Capsaicin *capsici fructus* (*Capsicum annuum* Linn.) (*Solanaceae*) or the main ingredients of the fruits (*capsici fructus*) of the variety. Circulation sthenia agent. [Antibacterial properties and an insecticidal action.]

- Starch, isoflavone kudzu (*Pueraria*) The main ingredients of the root (*pueraria root*) of the root except the periderm of *lobata Ohwi* (*P. pseudo-hirusta* Tang et Wang) or *P.lobata Ohwi* var. *chinensis*(Benth.) *Ohwi* (*Leguminosae*). Perspiration and an antipyretic. [The nutrient of wild type yeast.]

- A sugars jujube tree (*Zizyphus jujuba* Miller var. *inermis* Rebder) (*Rhamnaceae*) (*Z. vulgaris*Lamark var. *inermis* Bunge) or the main ingredients of the fruits (*zizyphi fructus*) of the close species. Relaxation, urination, and tonic. [The nutrient of the wild type yeast of *sophorae radix* origin.]

[0009]The mutual using rate of the component contained in groups, such as berberine in the above (A) component, and the component contained in groups, such as starch, is a weight ratio, and group =1:1.5-1.5:1, such as group:starch, such as berberine, is preferable. If antibacterial properties, anti-mold, and an antiviral action become will be hard to exert if the ratio of groups, such as berberine, becomes small and the ratio of groups, such as starch, becomes small, The nutrients of useful microorganisms will run short and the alimentation power, natural healing energy, and enemy control power of the plant which should be activated through activity of useful microorganisms will be deteriorated.

[0010]As the operation and effect over the plant of the low-grade organic acid (acetic acid, propionic acid, succinic acid, etc.) which is the (B) components which constitute this invention, there are the hormone Mr. effect by use, vegetable spindly growth inhibition, a rooting facilitatory effect, etc. in proper quantity.

[0011]As for the mutual content ratio of the above-mentioned (A) component and the (B) component, 1:4-1:2 are preferable. If a defense operation on the damage by blight and harmful insects by a crude drug ingredient if the ratio of (A) becomes small is no longer exerted sufficiently and becomes largely, the effect for hormone by low-grade organic acid, vegetable spindly growth inhibition, a rooting facilitatory effect, etc. will become are hard to exert.

[0012]the next -- plant growth adjustment of this invention -- service water -- it describes about the process of a sex drug solution.

[0013]plant growth adjustment of this invention -- service water -- the process of a sex drug solution, ** There are a case where a crude drug is fermented on a common gender aversion target by the wild type yeast of *sophorae radix* origin, ** common gender

anaerobic yeast, and a case where prepare independently a common gender ** (A) component and the (B) component, and it mixes with the case where it is made to ferment anaerobically, when using it, about a crude drug by the above-mentioned microorganism preferably.

[0014]First, when fermenting a crude drug on a common gender aversion target by the wild type yeast of ** sophorae radix origin, it prepares as follows. A cork tree bark, a sophorae radix, rhei rhizoma, a pueraria root, a Scutellaria root, zizyphi fructus, a ginger, liquorice, Aurantii nobllis pericarpium, an angericae radix, a cnidium rhizome, and 12 kinds of crude drugs of capsici fructus, It is supplied to about 20-40 degrees C warm water, and on a common gender aversion target's, i.e., the sometimes stirred degree, conditions, in ordinary temperature, it does not aerate specially, but the degree of 30 - 60 schedule is period-neglected, and it ferments. During the above-mentioned period, it is long, and in a summer, it is adjusted by temperature at winter as short. Under the present circumstances, auxiliary ingredients, such as a compost, chicken droppings, and an oil cake, may be added in order to promote fermentation. While helping vegetable nutrient uptake, the animal protein which has a repellent action to a noxious insect may be added. Since the evasion effect over small harmful animals, such as a rat and a mole, is increased, hop may be added. The surface of the liquid after the end of fermentation is covered by the white flora by the wild type yeast of sophorae radix origin, etc. This bacillus acts on the autonomic nervous system of an insect or a mite, excites [this is paralyzed, or] it, and carries out activation of the plant cell further at the same time it inhibits generating of a fusarium bacillus and other plant pathogenic microbes in cooperation with a crude drug ingredient which is a subject.

On the other hand, it has a good operation of inhibiting abnormal growth.

That is, an important operation is synergistically performed on the invention purpose with the above-mentioned crude drug ingredient. Therefore, this flora is also used as it is as a component of the crude drug liquid of the invention in this application. Even if it keeps fermented material in a container as it is, there is no possibility of decomposing over a long period of time. however -- making it dry at low temperature, if it is with necessity -- powder -- or it can also granulate.

[0015]Next, ** common gender anaerobic yeast and when fermenting a crude drug on a common gender aversion target by the above-mentioned microorganism preferably, it prepares as follows. With the above-mentioned yeast, the above-mentioned crude drug is thrown in in about 20-40 degrees C warm water, on a common gender aversion target, i.e., the bottom of the condition of the degree which is not aerated specially but is sometimes stirred, the 30 to 60th [about (short / in a summer / In winter. /)] day, is neglected and will ferment. Under the present circumstances, a compost, chicken droppings, an oil cake, an animal protein, hop, etc. are added by request like the case of **. This fermentation form is organic acid fermentation intrinsically. Therefore, although water, a raw material crude drug, and other sterilization are not usually required, in order to avoid the influence of the wild type yeast accompanied to a sophorae radix, it is necessary to sterilize on the sterilization conditions of a common microorganism, for example, 120 degrees C, and the conditions for about 15 to 30 minutes. in addition -- making it for there to be no possibility of decomposing over a long period of time, even if it keeps this fermented mash in a container as it is like the case of **, but dry at low temperature, if it is with necessity -- powder -- or it can also granulate. As long as the yeast which is used for the common gender anaerobic fermentation of the above-mentioned crude drugs and in which it deals exists in a nature and a common gender anaerobic fermentation operation is played in itself,

there is no restriction in the kind exceptionally, but each following publicly known fungi are used especially preferably.

- Kluyveromyces MARUKISHI anus (Zygosaccharomyces MARUKISHI anus)
(Kluyveromyces marxianus (Zygosaccharomyces marxianus)) GRIF--C.Wehmer. IFO 0219, BURETTANO Mrs. KUSUTERUSHIANUSU (Brettanomyces custersianus) vander Walt IFO 1585, and BURETTANO Mrs. knurl DENENSHISU (B. naardenensis). Kolfshoten & Yarrow. IFO 1588 - Dekkera BURUKU selenium cis- (Dekkera bruxellensis) van der Walt IFO1590 and Dekkera Intel Medea (D. intermedia) van der. Walt (torulopsis silyne DORIKA (Torulopsis cylindrica) IFO 1591 and Hansenula ANOMARA (Hansenula anomala) IFO1760) [0016] When preparing ** (A) component and the (B) component independently and mixing when using it, it prepares as follows, for example. First, commercial starch, such as berberine, etc. are purchased, and mixing preparation of the component of both groups is carried out so that both percentage may be set to 1:1. On the other hand, low-grade organic acid (acetic acid, propionic acid, succinic acid, etc.) is added suitably, and the (B) component is prepared. blending next, so that the above-mentioned (A) component and the (B) component may be set to 1:3, and adding water, such as distilled water, to this -- plant growth adjustment of this invention -- service water -- a sex drug solution is prepared. Water etc. are added by the plant etc. to be used and concentration is prepared.

[0017] thus, the obtained plant growth adjustment -- service water -- in a sex drug solution, As a component, (A) Berberine, sophocarpidine, the Anthraquinone derivative, flavonoids, glycyrrhizin, oil refinement, alkyl phthalides, a phthalide derivative, and the group which consists of capsaicin, And it is a component containing at least one or more compounds chosen from each group of the group which consists of starch, isoflavone, and sugars, respectively, and the component which makes berberine indispensable is contained and low-grade organic acid (acetic acid, propionic acid, succinic acid, etc.) is contained as a (B) component. For this reason, berberine, sophocarpidine, the Anthraquinone derivative, flavonoids, glycyrrhizin, oil refinement, alkyl phthalides, a phthalide derivative and the antibacterial properties by the component of the group which consists of capsaicin, anti-mold, an antiviral action, etc. work. Starch, isoflavone, and sugars serve as a nutrient of yeast. There are the hormone Mr. effect by low-grade organic acid (acetic acid, propionic acid, succinic acid, etc.), vegetable spindly growth inhibition, a rooting facilitatory effect, etc. in addition, the above-mentioned plant growth adjustment -- service water -- in a sex drug solution, very a variety of substances, such as emission matter outside a fungus body from the derivative, the decomposition product, yeast, and the bacterial flora of a crude drug ingredient based on fermentation, are included. As these substances, vitamins, such as amino acid, such as aniline and proline, niacin, and biotin, lactic acid bacteria, etc. are contained, for example. Lactic acid bacteria activate vegetable alimentation power, natural healing energy, and enemy control power as a useful rhizosphere microorganism, and amino acid and vitamins are used as a nutrient of lactic acid bacteria.

[0018]

[Effect of the Invention] as mentioned above, plant growth adjustment of this invention -- service water -- a sex drug solution, As a component, (A) Berberine, sophocarpidine, the Anthraquinone derivative, flavonoids, glycyrrhizin, oil refinement, alkyl phthalides, a phthalide derivative, and the group which consists of capsaicin, And are a component containing at least one or more compounds chosen from each group of the group which consists of starch, isoflavone, and sugars, respectively, contain the component which makes

berberine indispensable, and as a (B) component, Since low-grade organic acid (acetic acid, propionic acid, succinic acid, etc.) is contained, the dramatically outstanding cultivation results are acquired as synergistic effects, such as a growth promotion operation of the plant by these components, and a defense operation on a damage by blight and harmful insects. Since the above-mentioned component is a crude drug ingredient etc., it does not make a disease germ produce tolerance, and its safety at the time of decomposition in soil is high. Thus, it can contribute to the productivity drive of agriculture and horticulture, laborsaving, and an environmental clean-up by providing stably safe agricultural chemicals without a possibility of destroying a natural ecosystem.

[0019]Next, this invention is described in detail based on an working example.

[0020]

[Work example 1]35 kg of cork tree barks, 6 kg of rhei rhizoma, and 29 kg of sophorae radixes are added to 1000 liters of water, Add 50 parts by weight (it omits the following "part") of pueraria roots, 50 copies of Scutellaria roots, 50 copies of zizyphi fructus, 15 copies of gingers, five copies of liquorices, 30 kg of mixtures which consists of five copies of Aurantii nobllis pericarpium and 50 copies of angericae radixes, 50 copies of cnidium rhizomes, and 5 kg of mixtures which consists of five copies of capsici fructuses to this, and After mixing, When it allowed to stand for two months at the room temperature, stirring 2 to 3 times every day, the fermented material covered by the bacterial flora with the white surface was obtained. thus, the obtained plant growth adjustment -- service water -- the result about the main constituents produced by high performance chromatography etc. analyzing a sex drug solution is shown in Table 1. As for the weight ratio of berberine:all sugar, Table 1 shows that the weight ratio of an abbreviated 1:1 and (A) component:(B) component serves as the abbreviation 1:3.

[0021]

[Table 1]

組 成 物	結 果	分 析 方 法
ベルベリン	2 7 0 m g / 1 0 0 g	高速液体クロマトグラフ法
全糖*	0 . 2 9 (組成%)	アンスロン硫酸法
有機酸	1 . 3 2 (組成%)	高速液体クロマトグラフ法
酢酸	0 . 6 6	
乳酸	0 . 5 6	
プロピオン酸	0 . 0 8	
コハク酸	0 . 0 2	
遊離アミノ酸	3 2 m g / 1 0 0 g	アミノ酸自動分析法
アラニン	8 m g	
プロリン	8 m g	
その他	1 6 m g	
ビタミン類	3 5 2 . 5 μ g / 1 0 0 g	微生物定量法
ナイアシン	3 5 0 μ g	
ビオチン	2 . 5 μ g	
乳酸菌	7 . 6 $\times 10^6$ / g (菌数)	MRS 寒天平板嫌気培養法

* Compute a sample by using it as the grape sugar produced by carrying out acidolysis processing. [0022]the above-mentioned plant growth adjustment -- service water -- the result of having used the sex drug solution for bentgrass is shown in Table 2. per [1 m of bentgrass ²] -- the above-mentioned plant growth adjustment -- service water -- sprinkling 1 liter of 500 dispensing solutions of a sex drug solution -- as contrast -- plant growth adjustment -- service water -- tales doses of water was sprinkled instead of the sex drug solution. Comparison testing of the thing on Heisei 6(1994) April 11, April 26, May 9, and May 23 which was sprinkled 4 times, was sampled on June 6, and was air-dried after rinsing was carried out on June 13. the effect that a leave and stem root promotes growth capable is accepted -- plant growth adjustment -- service water -- although the bentgrass which sprinkled the sex drug solution became vivid green till the checking date and the goodness of **** was accepted, the bentgrass which sprinkled water had shrunk.

[0023]

[Table 2]

	全 重		茎 葉 重		マット部重		根 重	
	g	%	g	%	g	%	g	%
薬液散布	1.07	155	0.16	229	0.66	150	0.25	139
水散布	0.69	100	0.07	100	0.44	100	0.18	100

* Weight is a dry matter weight. [0024]

[Work example 2]The sophorae radix is beforehand sterilized on 120 degrees C and the conditions for about 15 to 30 minutes. 35 g of cork tree barks, 6g of rhei rhizoma, and 29 g of sophorae radices are added to 1000 ml of distilled water, 50 parts by weight (it is below the same) of pueraria roots, 50 copies of Scutellaria roots, 50 copies of zizyphi fructus, 15 copies of gingers, five copies of liquorices, the mixture 30g which consists of five copies of Aurantii nobllis pericarpium and 50 copies of angericae radices, 50 copies of cnidium rhizomes, and the mixture 5g which consists of five copies of capsici fructuses are added to this, 2 ml of pure culture things (a 10⁶ - 10⁷ individual / ml) of Kluyveromyces MARUKISHI anus IFO 0219 were added, and it was neglected for two months in 28 degrees C humidistat. thus, the obtained plant growth adjustment -- service water -- the result about the main constituents produced by high performance chromatography etc. analyzing a sex drug solution is shown in Table 3. As for the weight ratio of berberine:all sugar, Table 3 shows that the weight ratio of an abbreviated 1:1 and (A) component:(B) component serves as the abbreviation 1:3.

[0025]

[Table 3]

組 成 物	結 果	分 析 方 法
ベルベリン	275mg/100g	高速液体クロマトグラフ法
全糖*	0.26 (組成%)	アンスロン硫酸法
有機酸	1.41 (組成%)	高速液体クロマトグラフ法
酢酸	0.67	
乳酸	0.58	
プロピオン酸	0.11	
コハク酸	0.05	
遊離アミノ酸	30mg/100g	アミノ酸自動分析法
アラニン	7mg	
プロリン	9mg	
その他	14mg	
ビタミン類	346.3μg/100g	微生物定量法
ナイアシン	344μg	
ビオチン	2.3μg	
乳酸菌	7.2×10 ⁶ /g (菌数)	MRS 寒天平板嫌気培養法

* Compute a sample by using it as the grape sugar produced by carrying out acidolysis processing.

[Translation done.]

TECHNICAL FIELD

[Industrial Application]the plant growth adjustment with high safety [in addition to a vegetable growth promotion operation, the present invention has a defense operation on a damage by blight and harmful insects, and moreover do not make a disease germ produce tolerance, and] at the time of decomposition in soil -- service water -- it is related with a sex drug solution.

CLAIMS

[Claim(s)]

[Claim 1]plant growth adjustment currently containing the following (A) component and the (B) component -- service water -- a sex drug solution.

(A) Berberine, sophocarpidine, the Anthraquinone derivative, flavonoids, glycyrrhizin, oil refinement, alkyl phthalides, a phthalide derivative, and a group which consists of capsaicin, And it is a component containing a compound chosen at least one [at a time] from each group of a group which consists of starch, isoflavone, and sugars, respectively, and berberine is made indispensable.

(B) With a carbon number of four or less low-grade organic acid.

[Claim 2]wild type yeast which low-grade organic acid of the above (B) accompanies to a sophorae radix -- a crude drug -- common gender -- the plant growth control according to claim 1 produced by fermenting anaerobically -- service water -- a sex drug solution.