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ORIGINAL ARTICLE

# Effects RHizoma Gastrodiae Alcohol Extract on Monosacide Composition and Biological Activity of Grifola Frondosa Exopolysacide

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***Abstract:*** The composition and anti-tumor bioactivityRHizoma Gastrodiae alcohol extract to the liquid fermentation system of Grifola frondosa.. HPLC. applied. analyze. monosaccharide composition. exopolysaccharide samples, re-sults showed, crude exopolysaccharide samples. composed. glucoseMannose, A small. galactose.Whereas. mannoseRhamnoseGlucoseGalactoseArabinoseFucose etc. main monosaccharide composition. pure exopolysaccharides.Furthermore. Addition.RHizoma gastrodiae alcohol extract had a significant change. content. monosaccharide component. exopolysaccharideBut did not produce. new monosaccharide component. Polysaccharide.. Sample. pure exopolysaccharides. Monosaccharide molar ratio. mannose: Rhamnose:Glucose: Ga-lactose Arabinose:Fucose. 13. 8:3. 9:5:3. 7/:1:1. 7/While. pure exopolysaccharides fermented.RHizoma gastrodiae monosaccharide mole ratio. 12. 7/:3. 2:5. 6:3. 5:1:1. 6..: About. Biological Activity grifola frondosa exopolysaccharide. mice macrophage(RAW264. 7/)Showed, pure exopolysaccharides fermented.RHizoma gastrodiae had obvious activation. macrophage.. Pure exopolysaccharides, exopolysaccharides obtained by fermentation.RHizoma gastrodiae had certain activation. macrophage.. Activation. macrophages by crude exopolysaccha-rides. not significant.. VitroFour polysaccharide samples showed no inhibitory effect. Human hepatoma HepG 2 cells.

***Keywords:*** RHizoma gastrodiae alcohol extract;Grifola frondosa;Monosaccharide;Composition;

At present most has many kinds of biological activity of polysaccharide are from fungi especially is edible fungi such as mushroom,Grifola frondosa,Leather cover Bacteria Sugar composition analysis found its monosaccharide components for mannose,Glucose and xylose material of than16. 87:1:2. 99.In vivo experimental study show that Grifola frondosa polysaccharide on improve immune and anti-tumor effect explicit With found Grifola frondosaD-Components of cancer cells show growth suppression.Study show that medicinal fungi in fermentation transformation traditional Chinese medicine when traditional Chinese medicine Points not only can stimulating microbial growth at the same time can participate in to microbial Metabolism in increase its secondary metabolism product of Biological Activity.This research group early study results show that in Grifola frondosa liquid fermentation system in add Gastrodia elata alcohol extract and its main components gastrodin,P-hydroxybenzaldehyde,P-hydroxybenzylalcohol can significantly promote Grifola frondosa production cell the more Sugar and mycelium growth its promoting extracellular polysaccharide yield of mechanism and effective improve Grifola frondosa extracellular polysaccharide synthase enzyme live on.In this paper, we hope that through the study of the changes in the Fermentation of gray tree flowers with the ethanol extract of Gastrodia, we can further explore the changes of the extracellular polysaccharide of the metabolites of gray tree flowers.

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# 1. Materials and Methods

## 1.1 Material,Reagent and Medium

Tree, floral fungus, strain(Grifola frondosaStrain Number: 5.404), China General microbial culture preservation management center;Tianma(RHizoma strodiae)Tianma planting base, Dejiang County, Guizhou Province. Dialysis bags,SolarbioCompany;Mannose,Rhamnose,Glucose,Galactose,Arabian sugar,Fucose monosaccharide standard, Shanghai yuanye biotechnology Co., Ltd.; the rest reagents are commercially available analytical pure.

Slope Medium:Potato glucose agar(PDA)Medium.Liquid seed medium (G/l):Glucose30, Peptone2.Yeast Extract6.,$KH_2Po_4$0. 5,$MgSO_4 \cdot 7\ h_2O$ 0. 5.Fermentation medium (G/l):Glucose50, Peptone5.Yeast Extract10,$KH_2Po_4$2.,$MgSO_4 \cdot 7\ h_2O$ 2.

## 1.2 Instruments and equipment

BXM-30RVertical sterilization pot, Shanghai boxun Industrial Co., Ltd. Medical equipment factory;RE-2000ARotary evaporator, Shanghai yarong biochemical instrument factory;SW-CJ-1DModel purifies workbench, Suzhou purifies Equipment limited company;TGL-20MHigh-speed refrigerated centrifuge, long, sand, maijiassen, instrument, device design, preparation Co., Ltd;ZWY-C2112BDouble-deck rotary programmable constant temperature and humidity shaker, Shanghai Zhicheng analytical instrument Manufacturing Co., Ltd;Nicolet is50Type in-situ diffuse reflectance Fourier exchange infrared spectrometer, United States Seymour Fisher Technology Co., Ltd;CTFD-12SQingdao Yonghe chuangxin Electronic Technology Co., Ltd;Agilent 1100Style High Performance Liquid chromatograph and detector,Agilent 5TC-$C_{18}$Style column AmericanAgilentThe company.

1.3Experimental Methods

### 1.3.1 Grifola frondosa Training Methods

1)Grifola frondosa strains Culture.From a test tube in selected soybean grain size mycelium block inoculation inPDACant Central,25Constant Temperature training to mycelium with the whole slope transpose4Save.

2)Grifola frondosa liquid seed culture.With inoculation spoon in Inclined Plane Strain tube take1Spoon small mycelium inoculation in liquid seed medium in,500 mlTriangle bottled liquid of200 mlJoin a little small glass beads in25,150 r/minShaker in training6 d.

3)Grifola frondosa fermentation culture.Sterile conditions under10%The amount of inoculation of the with pipette gun take10 mlSeed liquid in fermentation medium in,250 mlTriangle cone bottled liquid of100 ml150 r/minShake

25Training.

### 1.3.2 Gastrodia elata alcohol extract preparation and add

Gastrodia elata wash,55Drying crush after.80The spare.Accurate said take the above10gOf Gastrodia elata powder join100 mlVolume Fraction For75%Of Ethanol.25Extraction48 hAfter Filter,60Decompression remove ethanol.And25 mLDistilled Water Heavy soluble after filter the get 2.5 mL/gOf Gastrodia elata alcohol extract (RG).To Grifola frondosa fermentation medium in add7/g/LOf Gastrodia elata alcohol extract with the high-pressure high-temperature sterilization cooling after access seed liquid fermentation culture12 d.

### 1.3.3 Grifola frondosa extracellular crude polysaccharide preparation

Take2 mLOf to mycelium fermentation broth join4Times volume 95%Anhydrous ethanol in4Refrigerator alcohol Analysis24 hOut after4 000R/minCentrifugal15 minTake the sediment and volume fraction95%Ethanol precipitation3Times after in60Digital Display blast drying oven in drying the for crude polysaccharide (CEPS).

### 1.3.4 Grifola frondosa extracellular crude polysaccharide purification Processing

The alcohol precipitation of crude polysaccharide with volume fraction3%Trichloroacetic Acid

Off protein Macroporous Adsorption ResinD303Decolorization by water dialysis2 DAgain by distilled water dialysis1 dAfter in Vacuum Frozen dryer in drying get purification after of Grifola frondosa extracellular polysaccharide samples (PEPS).

### 1.3.5 Determination of polysaccharide and protein content in the samples of the extracellular

**polysaccharide of the gray tree**

Take10 mgPolysaccharide sample, with distilled water for constant volume50 mlLee Analysis of polysaccharide and protein content.The linear regression equation of glucose standard curve is:Y = 0. 554 2x-0. 003 5,$R^2$= 0. 998 7.The protein standard curve isY = 8. 740 5x0. 009 3,$R^2$=0.999 2. 0.3.6 Determination of monosaccharide composition of polysaccharides from Grifola frondosa

1.)Acid Hydrolysis of polysaccharide Samples.Take separately30 mgPolysaccharide samples were accurately prepared with distilled water.30 mg/mLSolution, add4 mol/LTrichloroacetic Acid1 mL, Sealed with nitrogen,110Lower Hydrolysis2 hAfter cooling, add methanol2 mLAfter mixing50Rotary steam drying, repeat3.Steamed and dried1 mLDerivatization of deionized water.

2.)Polysaccharide samples and Monosaccharide standards1-Phenyl-3-Methyl-5-Pyridine ketone(PMP)Derivatization.Reference [12], Call for nectar

Sugar,Rhamnose,Glucose,Galactose,Arabian sugar,Fucose standard materials2 mg, Add deionized water in5 mLVolumetric flask for constant volume, the equivalent volume of monosaccharide standard solution with a concentration0. 4 mg/mlStandard mixed liquid of Monosaccharide.

Extract the mixed liquid of polysaccharide hydrolysate and Monosaccharide Standard1 mLYu10 mlCentrifugal, tube, add1 mL 0. 3 mol/LOfNaOHAfter mixing, take the mixture1 mL, Add1 mL 0. 3 mol/LOfPMPMethanol solution, after the vortex70Lower Reaction100

5 mL.Chloroform extraction, shaking,Carefully absorb the aqueous phase (Upper Layer), Repeat3.Sub-netPMP(The chloroform layer is colorless).0. 45 μMFilter Membrane for water-phase reactionHPLCDetection.

3.)Polysaccharide sampleHPLCDetection.Column:Agilent 5tc-$C_{18}$Column.Mobile phase:0. 1 mol/LPhosphate buffer solution (PHValue 6.7.)-Acetonitrile, Volume Ratio83:17;Column temperature30Flow rate is1 ml/s.Injection amount20μL, Wavelength245 nm.

### 1.3.7 Infrared Spectrum experiment

1.~1. 5 mgSample and200~KBR 300 mg(Sample and

KBRQuality ratio1.:200)In agate mortar grinding into a uniform mixture of powder, with a small spoon into the production mold in the Oil Press10

Keep under pressure2 minAfter removing the pressure, the test plate made of the sample shall be transparent and placed on the sample stand for inspection..

### 1.3.8 Detection of the biological activity of the extracellular polysaccharide from the gray tree

Detection Method for screening models of Antitumor Active substances using sulfonyl Rodin protein staining (SRBLaw)Methods: The proliferation model of mouse spleen lymphocytes was established by tetrazolium salt reduction (MTTLaw).The purpose of this study is to determine the biological activity of the extracellular polysaccharide from Grifola frondosa by the Research Center of Pharmacology and biological activity, Key Laboratory of natural product chemistry, Chinese Academy of Sciences, Guizhou Province..

## 1.4 Statistical methods

AdoptedSPSS 17. 0Software analysis experiment data significance,Ori-Gin 7. 5Software Mapping.In the bioassay experiment, each concentration of the sample was parallel3.Repeat2.Times, The resultM±sdExpress.

# 2. Results and Analysis

## 2.1 Analysis of polysaccharide and protein content in polysaccharide Samples

The extracellular polysaccharide samples of Grifola frondosa are mainly composed of polysaccharides (see table1.).After extraction and purification, the polysaccharide was further purified, and the protein was not detected..

## 2.2 Structural characteristics of extracellular polysaccharide from Grifola frondosa

Infrared Spectrum of the extracellular polysaccharide from the leaves of the gray tree1.,Figure2..From

We can speculate about their structural characteristics in the atlas.,Sugar residues and Their Configurations.

3 600~3 200$^{-1}$.There is a wide and strong characteristic absorption peak of polysaccharides.O-HThe stretching vibration of polysaccharides indicates the existence of intramolecular and intermolecular hydrogen bonds..In2 900$^{-1}$.There are polysaccharides in both left and right

Add Gastrodia elata alcohol to wu yu Grifola frondosa fermentation system for experimental Group not add any exogenous of for control group.Add Gastrodia elata alcohol extract after Grifola frondosa extracellular crude polysaccharide not produced new of monosaccharide composition but monosaccharide material of than change significantly see Figure4,Figure5And table3.SamplesCEPS,CEPS-RGMonosaccharide Composition in glucose content most secondly for mannose at the same time detection content relative is less galactose or also containing rhamnose.This also further from monosaccharide composition of angle explain the infrared spectrum in cell the crude polysaccharide not detection the obviousαConfiguration of reason.Lee sugar,Glucose,Galactose,Arabia sugar,Fucose and Monosaccharide components (See figure6,Figure7/).Which add Gastrodia elata alcohol extract of Grifola frondosa extracellular polysaccharide monosaccharide components produce obvious change,PEPSSample middle-upper 6A monosaccharide material of than times13. 8:3. 9:5:3. 7/:1:0.7/AndPEPS-RGSample monosaccharide components material of12. 7/:3.2:5. 6:3. 5:1:1. 6(See table4).By contrast monosaccharide peak area shows that Gastrodia elata alcohol extract of join can promote Grifola frondosa secretion Rhamnus

Sugar,Galactose,Arabia sugar,Fucose and Monosaccharide Composition.At the same time by figure7/The Gastrodia elata alcohol extract of join did not make Grifola frondosa polysaccharide produce new monosaccharide Components.

4Grifola frondosa extracellular fine polysaccharide monosaccharide composition material of

And ginseng extract can improve Ganoderma lucidum polysaccharide on macrophages cells of activation Ability.This may and Gastrodia elata alcohol extract change Grifola frondosa extracellular polysaccharide monosaccharide composition about.By compare the purification and without purification of extracellular polysaccharide on mice macrophage cellsRAW264. 7/The role the by purification after

Of Grifola frondosa extracellular polysaccharide on mice macrophage cells have stronger of activation role this May and polysaccharide by purification after has biological activity of monosaccharide content increase about.Studies have show that polysaccharide of monosaccharide composition also and anti-tumor activity related usually containing glucose and mannose of polysaccharide anti-tumor activity is good, this main because in Human Macrophages cells in has been existing glucose and mannose receptor so such polysaccharide has height

LPSFor positive control.Sample standard weighing according to sample the requirements with physiological saline dissolved.

## 2.6 Grifola frondosa extracellular polysaccharide anti-tumor activity screening experimental results.

Screening Model:People Hepatocellular Carcinoma CellsHepG 2.Screening methods:Tetrazolium salt reduction method (MTTMethod).

Model Principle:Living Cells of mitochondrial in there andNADPRelated of dehydrogenase can will yellowMTTReduction for insoluble the blue-violet,Formanzan;Dead cells this enzyme disappear,MTTDon't be reduction.This model accordingMTTThe reduction degree of to detection samples of tumor cells of role in calculation formula see (2).

Income of Grifola frondosa extracellular polysaccharide samples on Human Hepatocellular Carcinoma CellsHepG 2No in vitro suppression role see table6.Caused by the experimental results of reason on the one hand may because activity polysaccharide of Source,Origin,Extraction Methods Different In Vitro inhibition tumor of effect there will be difference with same Polysaccharide

# 3. Knot On

In this experiment, the monosaccharide composition and anti-tumor biological activity of the extracellular polysaccharide obtained from the ethanol extract of Gastrodia elata was studied after being added to the liquid fermentation medium of gray tree flowers..Fourier transform infrared spectroscopy

# 4. The structure of extracellular polysaccharide samples was analyzed.

In$875^{-1.}$,$805^{-1.}$There is obvious characteristic absorption.This may be the result of purification andAlphaThe increase of monosaccharide Configuration.UtilizationHPLCOkay.

4.The monosaccharide composition of exopolysaccharide samples was analyzed.Sugar,Mannose and a small amount of galactose and other simple sugars.,Rhamnose,Glucose,Galactose,Arabian sugar,Monosaccharide composition such as fucose.At the same time, according to the detection results, the monosaccharide content of the extracellular polysaccharide from the leaves of the trees was significantly changed by the addition of the ethanol extract of the leaves of the trees, but the new monosaccharide component was not produced;PEPs

The antitumor effects of different fractions or sub-fractions of the polysaccharide are different. On the other hand, the anticancer mechanism of the polysaccharide is not to kill tumor cells directly because it is similar to other fungal polysaccharide preparations, by activating the immune system, increasing the phagocytosis of macrophages, promoting the formation of immunoglobulin, improving the transformation rate of lymphocytes and improving the resistance of the body to disease, we can resist the growth of cancer cells and

The purpose of stopping tumor metastasis and preventing normal Cell Canceration.Many animal experiments show that the biological effect of inhibiting tumor metastasis.Galactose:Arabian sugar:Fucose in turn13. 8:3. 9:5.:3. 7:1.:1. 7, AndPEPs-RGMolar ratio of monosaccharide Components12. 7:3. 2:5.6.:3. 5:1.:1. 6.Compared with the peak area of monosaccharide, The results showed that the ethanol extract of Gastrodia can promote the secretion of rhamnose from gray tree flowers.,Galactose,Arabian sugar,Monosaccharide composition such as fucose.Biological Activity of polysaccharides was detected by mouse macrophages.(RAw264.7)The activation experiment showed that,PEPs-RGIt can obviously activate macrophages,PEPs,Ceps-RGThey play a role in the activation of macrophages,CepsNo clear activation of macrophages.Through in vitro experiments4.Seed polysaccharide samples on Human Hepatocellular Carcinoma CellsHepG 2No Inhibitory Effect.

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