OVA aerosol inhalation challenge. Histopathological changes were examined to study the effects of *Perilla* consumption.
Results The rat model of bronchial asthma was successfully established. Compared with model group, large, medium and small doses of *Perilla* seed significantly increased the weight of bronchial asthma rats ($p < 0.01$) and significantly decreased serum levels of NO and IL-6 ($p < 0.01$).
Conclusions *Perilla frutescens* seed has a protective effect in the rat model of bronchial asthma.
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Biochemical Pharmacy

13 THE EFFECTS OF BIO SUGAR INFLUENCE ON TOAD SCIATIC NERVE-TRUNK ACTION POTENTIAL

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Objectives MSD is a type of bio sugar made by an MS bio sugar research institute in South Korea. The bio sugar has a glycan structure with a functional group on the monosaccharide. Our objective was to observe the effects of different concentrations of MSD on toad sciatic nerve-trunk action potential, and whether MSD can improve the inhibitory effect on toad sciatic nerve-trunk action potential of hypertonic glucose solution.
Methods Toad sciatic nerve trunks were randomly divided into three groups, namely a control group, an MSD group and a hypertonic glucose group. Following soaking with different concentrations of MSD, toad sciatic nerve-trunk action potential amplitudes and conduction velocities were determined using a BL-420 biological function experimental system.
Results Compared with the control group, 1% MS inhibited toad sciatic nerve-trunk action potential amplitude and conduction velocity but the effects disappeared after 10 min, while 0.5% MSD decreased action potential amplitude and increased conduction velocity. Soaking in 10% hypertonic glucose with 0.5% MSD after processing permanently inhibited toad sciatic nerve-trunk action potential. MSD can inhibit toad sciatic nerve-trunk action potential amplitude and conduction velocity.
Conclusions The activity of 0.5% MSD is better than that of 1% MSD. MSD can improve the inhibitory effects of 10% hypertonic glucose solution on toad sciatic nerve-trunk action potential.
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14 EFFECTS OF SAMPLE EXTRACT METHODS ON THE PRODUCTION AND ANTIOXIDANT ACTIVITY OF *LEPISTA SORDIDA* POLYSACCHARIDES

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Background Polysaccharides, one of the main bioactive compounds in natural resources such as fungi, plants and algae, have attracted lots of attention in biomedical and functional food science due to their significant pharmaceutical activity. In this study, four extraction methods were compared regarding the production, physicochemical properties and antioxidant activity of polysaccharides from *Lepista sordida* using hot water, an alkaline solution, a multiplex- enzyme method and an ultrasonic-assisted method.
Methods Bioactive polysaccharides from *L. sordida* were extracted using hot water, an alkaline solution, a multiplex-enzyme method and an ultrasonic-assisted method. The anthrone-sulfuric acid method was applied to measure the crude polysaccharide content and Coomassie Brilliant Blue (CBB) G-250 staining was used to detect protein content. The scavenging effect of the hydrolysates on α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical was measured.
Results Polysaccharides extracted using the multiplex-enzyme method had the highest DPPH scavenging efficiency of 91.62%. The extraction efficiency of the multiplex-enzyme method was 19.61 ± 0.60%, only 1.49% lower than that of the alkaline solution method, but the polysaccharide content was 94.47 ± 3.84%, which was much higher than the 74.93 ± 5.98% obtained using the alkaline solution. The polysaccharides extracted using the multiplex-enzyme method had a low protein content.
Conclusions The multiplex-enzyme method was selected to extract the antioxidant polysaccharides of *L. sordida* due to a higher extraction efficiency, better purity and powerful DPPH radical scavenging ability, and lower energy consumption than the other methods. The results are of great significant for developing functional food from *L. sordida* bioactive polysaccharides in the future.
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15 ANTITUMOUR ACTIVITY OF GLUCOSAMINE HYDROCHLORIDE IN VITRO

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Background Glucosamine hydrochloride, a natural biopolymer present in the daily diet, has various biological activities including antitumour properties and protective effects against pathogens. Early studies showed that daily administration of a derivative of glucosamine induced proliferation of leukemia cells and prolonged overall survival in mice; importantly, no toxicity was associated with the glucosamine treatment. However, the potential mechanism of the antitumour effect is unknown. This study aimed to investigate the inhibitory mechanism and effect of glucosamine on human gastric carcinoma cells in vitro.
Methods Gastric carcinoma MKN-45 cells were exposed to 0, 100, 500 and 1000 μg/mL glucosamine hydrochloride for 72 hours, and then the viability and proliferation of gastric carcinoma cells in vitro was measured using the MTT (3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) assay. Quantitative gene expression of MMP-2 and -3 was determined by real-time polymerase chain reaction. Protein level was analyzed using an enzyme-linked immunosorbent assay (ELISA).

Results Glucosamine hydrochloride has a significant inhibitory antitumour effect on MKN-45 cells in vitro. The cell viability of MKN-45 cells treated with different concentrations of glucosamine hydrochloride rose continuously from 24 to 72 hours compared with the untreated control. MKN-45 cells were inhibited by 54% by 500 μg/mL glucosamine hydrochloride and by 85% by 1000 μg/mL glucosamine hydrochloride. Administration of 500 μg/mL glucosamine hydrochloride resulted in a significant decrease in MMP-2 and MMP-3 expression of about 79% and 70%, respectively, in MKN-45 cells.
Conclusions In this study, we showed that the antitumour activity of glucosamine hydrochloride significantly suppresses MKN-45 cells in vitro. This effect was shown by inhibitory gene expression of MMP-2 and -3.
Acknowledgments This research was financially supported by Zhuhai College of Jilin University.

16 DISCRIMINATION OF BRAZILIAN GREEN PROPOLIS AND CHINESE PROPOLIS BASED ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC FINGERPRINTS AND MULTIVARIATE STATISTICAL ANALYSIS

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Objectives The determination of chemical components is usually used in the quality control of propolis. However, chemical components from different types of propolis are similar. The objective of this investigation was to establish a method based on a specific chemical fingerprint profile and a multivariate mixed model statistical analysis which could easily distinguish propolis of different origins and promote the quality control of propolis.
Methods A novel approach using high performance liquid chromatography (HPLC) coupled with multivariate statistical analysis was established for profiling and distinguishing Chinese and Brazilian green propolis. A batch of 22 propolis samples was analyzed, and the datasets on retention time, peak area and sample codes were subjected to mixed multivariate statistical analysis consisting of principal component analysis (PCA) and a self-organization mapping net (SOM).
Results The fingerprints were profiled. PCA score plots showed Chinese and Brazilian green propolis clearly classified into two groups. The visualized SOM results showed data from the two groups projected to the adjacent neurons clearly separated from each other. Artepillin C, which contributed greatly to the differentiation, was screened out and identified as the reference compound. Artepillin C is the characteristic component in Brazilian propolis which can be used as chemical marker to distinguish propolis of different origins.
Conclusions In this study, fingerprints coupled with multivariate statistical analysis have been successfully applied to distinguish Chinese from Brazilian green propolis. The research identified a chemical marker, and thus helps to investigate and promote the quality control of propolis.

17 DELIVERY OF BETULINIC ACID LIPID NANOPARTICLES ASSEMBLED BY A MICROFLUIDIC DEVICE

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Background Microfluidics chip-based approaches (MF) can achieve unique transport properties through laminar flows and vastly increased surface-to-volume ratios, which have been extensively utilized to prepare smaller and homogeneous lipid nanoparticles (LNPs). LNPs have shown potential to carry highly insoluble medicines, especially betulinic acid (BA) which has significant antitumour activity but is difficult to administer for cancer therapy due to its poor water solubility. In this study, we investigated the parameters involved in the continuous production of LNPs encapsulating BA (BA-LNPs) by MF, and the possibility of improving antitumour efficacy and reducing toxicities was described.
Methods Briefly, a three-inlet MF system was developed and used to produce the LNPs. EggPC, cholesterol and BA (45:37:18% molar) were dissolved in ethanol. The lipid solution was then loaded into a 1 mL glass syringe and injected into the centre inlet channel, while phosphate-buffered saline (PBS; pH 6.5) was loaded into two 5 mL glass syringes and introduced into the two side inlet channels to establish hydrodynamic focusing. To investigate the physicochemical and biological properties of fabricated liposome at different shear forces, the total flow rate (TFR) is varied from 0.3 to 0.8 mL/min.
Results The TFR has a very small effect on particle size distribution, but an increase in TFR causes a progressive decrease in liposome size. The percentage of encapsulating efficiency (EE) was 77–92% and there were no significant changes when BA-LNPs were stored at 4° C. The inhibitory rate of BA-LNPs was significantly higher compared to free BA in vitro. Immunohistochemical analysis showed many damaged tumor cells after BA-LNPs were injected for 15 days. The survival of mice treated with BA-LNPs was apparently prolonged compared to mice treated with free BA.
Conclusions This result indicates that much stronger antitumour effects were induced by BA-LNP administration, which is most likely due to the relatively small particle sizes produced by MF and which are suitable for intracellular transportation.

Pharmaceutical Analysis

18 NATURAL MEDICINES TO TREAT LIVER PROBLEMS

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Objectives Liver disease, especially the chronic diseases, is a serious health problem worldwide. Natural medicines have been used for thousands of years to treat liver problems. Patients with liver problems sometimes try natural medicines, especially