Abstracts

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Rapid determination of ulti-protoberberine alkaloids in rhizoma coptidis by near-infrared spectroscopy

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A rapid analytical method, near infrared spectroscopy (NIRS), has been developed to determine the content of five individual alkaloids and total alkaloids in Rhizoma Coptidis (*HL*) samples. Five alkaloids, berberine, coptisine, palmatine, epiberberine and jatrorrhizine were analyzed simultaneously by rapid resolution liquid chromatogram – diode array detection (RRLC-DAD) in which, Taguchi design was applied in optimizing the extraction condition for alkaloids components in *HL*. Analytical parameters of the entire method such as sensitivity, linear range, precision and accuracy were also presented. Finally, a genetic algorithm – partial least squares (GA-PLS) regression method was used to build up the correlation models. The results showed that the correlation coefficients of the prediction models are R = 0.937 for the berberine, R = 0.951 for coptisine, R = 0.948 for palmatine, R = 0.931 for epiberberine, R = 0.862 for jatrorrhizine and R = 0.974 for total alkaloids content. The outcome showed that the new developed NIRS method allows rapid and simultaneous determination of five alkaloids for the quality control of *HL* without sample extraction.

A flavan-3-ol compound in *Drynaria fortunei* exerts estrogen-like activities in rat osteoblastic UMR 106 cells

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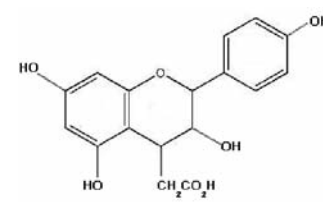
Fi4β-carboxymethyl-(-)-epiafzelechin acid (CEA), is a flavan-3-ol found in traditional Chinese herbal medicine *Drynaria fortunei (Kunze) J. Sm.*. The structure of CEA is shown in Figure 1. This compound was found to be potent in promoting the proliferation of osteoblastic cells. We hypothesize that CEA is a phytoestrogen that stimulates osteoblastic functions through the activation of estrogen receptor (ER). The present study aims to characterize the estrogenic properties of CEA in rat osteoblastic UMR 106 cells. UMR 106 cells were treated with CEA at 10^{-14} to 10^{-6}M, estradiol (10^{-8}M) or its vehicle for 24 h. The effects of CEA on osteoblastic cell proliferation and differentiation were studied by using MTS assay as well as alkaline phosphatase (ALP) activities, respectively. To determine if the activities of CEA were mediated by ER, cells were treated in the presence or absence of a specific ER antagonist, ICI 182780. The ability of CEA to transactivate estrogen response element (ERE)-dependent transcription was determined in UMR 106 cells co-transfected with either ER-α or ER-β construct and ERE-luciferase construct. The ability to bind to ER was assayed by competitive radioligand binding assay using purified ER-α or ER-β protein (Invitrogen). CEA significantly promoted the growth of UMR-106 cells by 52% (10^{-8}M, $p<0.001$ vs. control) and increased ALP activities by 21% (10^{-12}M, $p<0.01$ vs. Control). Co-treatment of UMR 106 cells with ICI 182780 abolished the stimulatory effects of CEA on cell proliferation, suggesting that the effect of CEA on osteoblastic cell growth was ER dependent. Competitive ER binding assay demonstrated that CEA was able to displace [H^3]-labeled E_2 from binding to ERs, suggesting that CEA can bind to ER directly. However, the binding affinity of CEA towards ER-α was higher than that towards ER-β. The relative binding affinity (RBA) of CEA for

Figure 1 - Structure of 4β-carboxymethyl-(-)-epiafzelechin acid (CEA)



ER-α is 41.8% when compared to E_2. Transfection assay indicated that CEA could activate ERE-dependent luciferase activities via ER-α but not ER-β in UMR 106 cells. These results indicate that CEA appears to activate ER selectively in osteoblastic cells. In conclusion, CEA is a natural selective estrogen receptor modulator that selectively activates ER-α but not ER-β in osteoblastic cells. Future experiment will be needed to evaluate its potential use as an osteoprotective drug.

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Activation of TRPV1 channels by dietary capsaicin improves energy metabolism and exercise endurance in mice

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Introduction: Metabolism and endurance exercise, which directly influence cardiometabolic risk factors, are primarily driven by skeletal muscle. Recently, several molecules in skeletal muscle have been shown to improve exercise endurance. The transient receptor potential vanilloid subfamily member 1 (TRPV1) cation channel is expressed in the sarcoplasmic reticulum of rat skeletal muscle, but its role in skeletal muscle function is unclear. In this study we investigated the role of transient receptor potential vanilloid subfamily member 1 (TRPV1) channels in the regulation of exercise endurance and energy metabolism of mice. **Methods:** Male C57 BL/6J wild-type (WT) and TRPV1-null (TRPV1-/-) mice were both randomly fed with regular diet and capsaicin-containing diet. Exercise endurance was determined via treadmill test. When treating for 4 m, six mice per group were subjected to oxygen consumption measurement and sacrificed. Serum lactate and triglycerides were examined. Oxidative muscle fibers were identified by metachromatic ATPase staining. Expression of TRPV1, troponin I for slow skeletal muscle (troponin-ss) and fatty acid transporter (FAT) in gastrocnemius were detected by immunoblotting. Transgenic mice with TRPV1 overexpression (TRPV1-tg) were bred through microinjection. **Results:** *In vitro*, capsaicin caused a Ca^{2+} release from SR stores and an extracellular Ca^{2+} influx in C2C12 myotubes, which was blocked by TRPV1 antagonist and TRPV1 RNA interference. *In vivo*, dietary capsaicin significantly enhanced exercise endurance in WT mice by about 30% when treating for 3 m ($p<0.05$) and this effect was sustained until 12 m. Four months' capsaicin treatment also significantly increased the oxygen consumption and the content of oxidative muscle fibers and reduced serum lactate and triglyceride levels ($p<0.05$). However, these effects were absent in TRPV1-/- mice. The expression of TRPV1, troponin-ss and FAT in gastrocnemius were significantly up-regulated in capsaicin treated WT mice ($p<0.05$), while they were similar in the two groups of TRPV1-/- mice. Furthermore, compared with the WT littermates, exercise endurance and oxygen consumption of TRPV1-tg mice were remarkably improved by about 100% and 50% respectively ($p<0.05$). But there was little difference in body weight and food intake between two groups. **Conclusion:** This study indicated that activation of TRPV1 channels by dietary capsaicin enhanced exercise endurance and energy metabolism in mice.

Study of anti-ageing and radiance-enhancing properties of a food supplement

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Carotenoids, mainly beta carotene, the most prevalent pro vitaminic A carotenoid, are widely used as skin protectors. However, very little is known about other non provitaminic A colored carotenoids such as lycopene and lutein, as well as colorless carotenoids, phytoene UVB filter, and phytofluene UVA filter. This work explores the effect of these non provitaminic carotenoids in a food supplement on mature skin. A double-blind study against placebo was performed to evaluate anti-aging and radiance-enhancing properties of the food supplement in 107 menopausal women, mean age 53 years old. The subjects took onc a day the food supplement during 12 weeks from September to November in Skopje, Macedonia. Several objective standards were used to assess the efficacy of the food supplement. The leading criterion was the skin density measured by ultrasound 25MHz (DERMCUP). Skin elasticity was evaluated by Dermal Torque Meter measurements (skin firmness Ue, skin elasticity Ur/Ue) Skin color variation was evaluated by colorimetry (L*a*b* function) on the forehead and right hand palm; pigment stains visibility on face and hands was assessed by image analysis on front calibrated photographs. Skin relief was measured on silicon prints either by fringe projection print analysis for wrinkles depth reduction or by optical analysis for

superficial skin relief. **Results:** 1°) Enhanced skin density: At T0, both groups were statistically comparable. At T12 weeks, skin density was significantly enhanced +7.8% (p= 0.034) in the food supplement group vs. placebo. 2°) Improvement of skin firmness and elasticity: Firmness significantly increased in the food supplement group (p = 0,012) at T12. Elasticity significantly increased at T6 and at T12 in the food supplement group, (p = 0.005 and p = 0.028) vs. placebo. 3°) Wrinkles depth reduction: The food supplement induced a significant decrease of the maximal depth of the deep wrinkles (of approximately 4%) at T12 while the placebo did not have any significant action on this parameter. 4°) Stains visibility reduction: the food supplement induced a significant decrease of stains visibility at T6 and T12. 5°) Enhanced skin radiance: At 6 weeks the food supplement group experienced significantly longer lasting skin radiance. In conclusion, a food supplement combining non provitaminic colored and colorless carotenoids, was able to reduce skin aging parameters over a 12 weeks period in menopausal women.

Bioassay-guided fractionation of a chinese herb, *Rhodiola sacra* with inhibitory activity on RANKL-induced osteoclastogenesis

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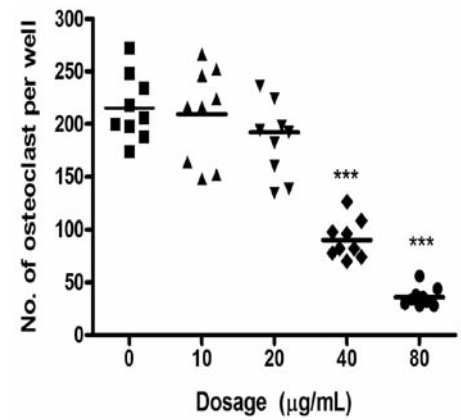
Rhodiola sacra (RS) is a perennial herbaceous plant that grows mainly in Tibet and Qinghai in China at altitudes between 2700m and 5000m about sea level. It is one of the most valuable traditional Chinese medicines for its ability to enhance body's resistance to stress, promote blood circulation and cardiovascular functions. Recently, there is increasing evidence showing that RS or RS-containing formulations can improve the osteoporotic conditions in ovariectomized rat models, as well as in human subjects. However, little is known about the cellular and molecular mechanism of its osteoprotective effects. Hence, our present study aimed at investigating the effects of RS water extract on osteoclast differentiation using mouse macrophage 264.7 cell lines. Our results demonstrated that RS water extract significantly inhibited the osteoclastogenesis in RAW264.7 cell cultures stimulated by receptor activator of NF-kB ligand (RANKL) in the concentrations without cytotoxicity (0-160 μg/mL). RS extract dose-dependently decreased the number of multinuclear tartrate-resistant acid phosphatase (TRAP) positive osteoclasts. At 80μg/mL, RS reduced the number of the osteoclasts by 83% when compared with the respective control group (Figure 1). Further fractionation of RS water extract was performed using n-butanol and subsequent repeated ethanol precipitation to give 3 fractions (n-butanol, ethanol precipitate, and remaining supernatant). Among all fractions, n-butanol soluble fraction was shown to be the most potent in inhibiting the osteoclast formation and also the mRNA expression of TRAP protein. In conclusion, this is the first report of RS water extract can effectively inhibit the osteoclast formation in mouse macrophage RAW264.7 cell cultures. Our results provide scientific evidence to support the potential use of *Rhodiola sacra* water extract in the treatment of osteoporosis.

Figure 1 - Effects of RS water extract on RANKL-induced osteoclastogenesis in macrophage RAW 264.7 cells. At day 4, RS extract significantly inhibited the formation of osteoclast in the concentrations of 40 and 80 μg/ml. Data are the means from 3 independent experiments in triplicate. *** $p < 0.001$ as compared with control group using one-way ANOVA analysis



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Sensitization of T-cell acute lymphoblastic leukemia to trail by flavonoids

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Drug resistance in acute leukemia was first described by Farber et al. in cases of acute lymphoblastic leukemia (ALL) and is still the major cause of death in all types of acute leukemia. Resistance mechanisms of chemotherapy-induced apoptosis were at

least partially due to its insusceptibility to drug-induced mitochondrial alterations. Targeting the alternative death receptor pathway, tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL), shows selective apoptotic activity towards a variety of cancer cells types only, but not in normal cells *in vitro* nor in experimental animals. Among the three T-type acute lymphoblastic leukemia (T-ALL) cell lines, namely, Sup-T1, HSB-2 and MOLT-3, tested for their cytotoxic effect with TRAIL treatment by Trypan blue exclusion assay, only MOLT-3 showed significant decrease in viability. Further, PARP cleavage, caspase-8 activation, truncation of Bid, followed by Pro-caspase-6 and Pro-caspase-3 activation was shown in MOLT-3 treatment cells only. Similarly, markedly increased accumulation of sub-G1 phase cells was only shown in the treatment of TRAIL-sensitive T-ALL, MOLT-3 in cytometric analysis. Sensitization of TRAIL-induced apoptosis was tested with a subtoxic dose of flavonoids, namely, apigenin and genistein in TRAIL-resistant T-ALL, HSB-2. Cell viability was significantly halved at a subtoxic dose of 10 uM and 20 uM respectively prior TRAIL treatment, whereas no significant change was shown in treatment with TRAIL, subtoxic doses of apigenin and genistein alone. All T-ALLs showed a dose-dependent effect upon flavonoid treatment, suggesting flavonoid as a good strategy against chemotherapy- resistant T-ALL. Resistance to TRAIL-induced apoptosis has been shown in many cancers. Here, preliminary studies suggest that flavonoid sensitization might provide a good strategy for the treatment of TRAIL-resistant T-ALL.

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Aryl hydrocarbon receptor-mediated transcription and *CYP1* class gene expression: could it be a possible mode of action of traditional chinese medicine in the management of breast carcinoma?

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Background: Estrogens, in breast tissue, are mainly metabolized by CYP1A1 and CYP1B1 to generate genotoxic quinones. The potent genotoxic quinone and potent estrogenic metabolites are produced by CYP1B1 mediated estrogen metabolism. Besides, CYP1B1 is over-expressed in breast cancer. Thus, inhibiting *CYP1* class gene expression or CYP1 enzyme activity could be the target for prevention and is undergoing study. Traditional Chinese Medicines (TCMs) have been used for many years to treat breast cancer; one of these is *Herba Scutellaria barbata* (SB) which was found to inhibit the CYP1 mediated benzo(a)pyrene metabolism. **Objectives:** In this study, we investigated if the active ingredient of SB, pheophorbide a (PA), affected the CYP1A1 and CYP1B1 gene expression and CYP1A1- and CYP1B1-mediated estrogen metabolism, as well as potential cytotoxic effects on breast cancer cell lines. **Methodology:** We used Real-Time PCR and Western blotting to detect the changes of the amount of mRNA and protein expression of CYP1A1 and CYP1B1 after PA treatment with or without TCDD. The estrogen metabolism of the intact cell upon PA treatment with or without TCDD was investigated by LC/MS. We also performed MTT assay and ^{3}H-thymidine (^{3}H-TdR) Incorporation assay, to study the cytotoxic effect of PA alone and in combination with 17-β estradiol (E_2) and/or tamoxifen citrate (TC). The data are presented as the mean±SD and analyzed by unpaired Student's t-test, one way ANOVA with suitable post hoc test and non-linear regression through the GraphPad Prism® 4. A p-value < 0.05 was considered statistically significant. **Results:** Pheophorbide A inhibited the TCDD-induced CYP1A1 and CYP1B1 mRNA expression. However, the inhibition did not have selectivity on CYP1B1 over CYP1A1. In Western blotting, the inhibition of TCDD induced CYP1A1 and CYP1B1 by PA was observed in both cell lines. The TCDD induced rates of formation of 4-methoxyestradiol (4-$MeOE_2$), 2-methoxyestradiol (2-$MeOE_2$) and 2-hydroxyestradiol (2-OHE_2) were reduced to 33±12%, 44±12% and 44±7% respectively for MCF-7; 28±22%, 25±11% and 30±15% respectively for T-47D upon 10uM PA treatment. The ratio of rate of formation of 4-$MeOE_2$ to 2-$MeOE_2$ (4/2-$MeOE_2$ ratio) in 10uM PA treatment with 10nM TCDD was lower compared to the vehicle control for MCF-7 ($p<0.01$) and T-47D ($p<0.001$). Although the 10uM PA treatment reduced the 4/2-$MeOE_2$ ratio upon 10nM TCDD treatment for MCF-7 and T-47D, it was not statistically significant. Pheophorbide A also lowered the number of viable cells of both cell lines with dose-dependent effect (IC_{50}>50 uM) in the MTT assay. The inhibition of proliferation was observed in ^{3}H-TdR Incorporation assay. The IC_{50} for MCF-7 and T-47D were 12±1 and 26±4 μM. Combined with E_2, PA lowered the viable cells and proliferation of both cell lines in both tests. In the presence of TC, the dose-dependent inhibitory effect of PA was observed in ^{3}H-TdR incorporation assay but not in MTT assay. **Conclusion:** Pheophorbide A inhibited TCDD induced CYP1A1 and CYP1B1 mRNA and protein expression as well as reduced the

rate of formation of the genotoxic estrogen metabolites. It also inhibited the proliferation of MCF-7 and T-47D. PA may be a potential candidate for therapeutic option in the management of breast cancer.

Evaluation of *in vitro* immunomodulatory and anti-proliferative activities of extracts and compounds isolated from *Curcuma longa*

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Various plant species from the *Curcuma* genus (family *Zingiberaceae*) are common ingredients in many health supplements in Asia. Curcumin, a well-known compound of *Curcuma longa* (CL) had previously been shown to possess chemopreventive and immunomodulatory properties. Our present study aimed to evaluate the biological activities of the polar fractions of CL hot water extracts as well as two sesquiterpenoids isolated from CL, namely α-turmerone and aromatic-turmerone (ar-turmerone). The immunomodulatory activities in human peripheral blood mononuclear cells (PBMC) and the anti-proliferative effects in human cancer cells of these fractions and compounds were evaluated. Our results showed that the high polarity fraction of the hot water extract exhibited stimulatory effects on PBMC proliferation and TNF-α production as shown in [methyl-^{3}H]-thymidine incorporation assay and ELISA, respectively. In an attempt to isolate the active components responsible for the activities, sub-fractions were obtained by further sequential partitioned with ethyl-acetate, n-butanol and ethanol. A polysaccharide-enriched fraction showed stimulatory effects on PBMC proliferation and TNF-α, IFN-γ, GM-CSF and IL-12 production. By using antibody-based human cytokine array, several cytokines and chemokine (IL-1α, IL-10, IL-13 and TARC) expressions were also shown to be increased in the fraction-treated PBMC culture supernatant. The composition of monosaccharides and molecular weight of this fraction were also examined. On the other hand, α-turmerone and ar-turmerone were isolated from the non-polar fraction of CL extract. The effects of α-turmerone and ar-turmerone (3.125-100 μg/ml) on human hepatoma cells (HepG2), human breast cancer cells (MCF-7 and MDA-MB-231), as well as in normal human skin fibroblasts (Hs68) were evaluated using MTT assay. The results showed that only α-turmerone significantly inhibited cell proliferation in HepG2, MCF-7 and MDA-MB-231 cells (IC_{50} = 33, 42, 30 μg/ml, respectively), but not Hs68 (IC_{50}>100 μg/ml), suggesting its selective cytotoxic effects. Besides, α-turmerone induced apoptosis in MDA-MB-231 cells, as confirmed by annexin-V & propidium iodide staining, and DNA fragmentation assay. The caspase cascade was activated as shown by a significant decrease of procaspases-3, -8 and -9 in α-turmerone treated MDA-MB-231 cells. In conclusion, our study reported for the first time the immunomodulatory effects of *Curcuma longa* polysaccharides in human peripheral blood mononuclear cells and the anti-proliferative effects of α-turmerone in MDA-MB-231 cells.

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Anti-oxidative properties of post-stroke recovery formula in preventing transient MCAo-induced brain damage

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The recent report of WHO showed that cerebral stroke account for 10% of the total causes of death each year. One-third of the patients have died and the others suffer from permanent disabilities. The disease caused a long-term burden to the countries and of course the families. Cerebral stroke can be classified into hemorrhagic stroke and ischemic stroke. Hemorrhagic stroke is caused by the rupture of a cerebral blood vessel while ischemic stroke is the blockage of a blood vessel, especially the middle cerebral artery (MCA). Due to the high death rate of hemorrhagic stroke, the focus was placed on the post-stroke therapy of ischemic stroke. A rat model mimicking the situation of ischemic stroke, transient middle cerebral artery occlusion (MCAo), was used to demonstrate the protective effect of post-stroke recovery formula (PSR). An intraluminal thread-occlusion surgical method was used in MCA occlusion (1). Brain infarction was induced by inserting a thread into MCA via internal carotid artery (ICA) from external carotid artery (ECA) until blocking the origin of MCA. A 2-h ischemia and 24-h reperfusion could significantly induced more than 50% brain infarct in the contra-lateral hemisphere and around 30% infarct in whole brain, as compared with the sham-operated rat by using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Oral administration of PSR was applied after 2-h ischemia and 2h reperfusion. Dosage of 3 g/kg PSR protected the brain by

decreasing the brain infarction by 13% and by improving the neurological score from 3 to 2.2 as compared with control. By examining the anti-oxidative enzyme (SOD, GPx and catalase) in the brain homogenates, PSR treatment was found to specifically upregulate the SOD by 12.3% per mg of protein while there were no effect on both GPx and catalase protein expression when compared with control. Next, AAPH-induced red blood cell hemolysis (2) was used to examine the direct anti-oxidative/ free-radical scavenging effect of PSR and its individual herbs. The results showed that PSR exhibited a strong anti-oxidative effect in inhibiting hemolysis significantly with IC50 value of 130 μg/ml when compared with vitamin C of 32 μg/ml. Among the seven individual herbs, *Radix Salviae miltiorrhizae* was found to show the strongest anti-oxidative effect with IC50 of 80 μg/ml. In summary, PSR formula was able to reduce the post-stroke brain damage by increasing the anti-oxidative status *in vivo* and *in vitro*. As a supplement, PSR could provide an alternative for better recovery of post-stroke patients.

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Double blind placebo controlled human study of Natural Eggshell Membrane (NEM®) for joint & connective tissue disorders

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Natural Eggshell Membrane (NEM®) is a new dietary supplement that contains naturally occurring glycosaminoglycans and proteins essential for maintaining healthy joint and connective tissues. Two single-center, open-label pilot clinical studies were conducted to evaluate the efficacy and safety of NEM® as a treatment for pain and inflexibility associated with joint and connective tissue disorders, followed by a randomized, multicenter, double-blind, placebo-controlled trial. Subjects received oral NEM® daily for four weeks (open-label) or eight weeks (placebo-controlled). The primary outcome measure for the open-label trials was to evaluate the change in general pain associated with the treatment joints/areas at 7 and 30 days. Range of motion (ROM) and related ROM-associated pain were also evaluated in one of the open-label trials. The primary outcome measure in the double-blind trial was the change in overall Western Ontario and McMasters Universities (WOMAC) Osteoarthritis Index, as well as pain, stiffness, and function WOMAC subscales measured at 10, 30, and 60 days. In the single-arm trial, supplementation with NEM® produced a significant treatment response at 7 days for flexibility (27.8% increase, P = 0.038) and at 30 days for general pain (72.5% reduction, P = 0.007), flexibility (43.7% increase, P = 0.006), and ROM-associated pain (75.9% reduction, P = 0.021). In the double-arm study, supplementation with NEM® produced a significant treatment response for pain at 7 days for both treatment arms (X: 18.4% reduction, P = 0.021, Y: 31.3% reduction, P = 0.014). In the placebo-controlled trial, supplementation with NEM® produced an absolute rate of response that was statistically significant (up to 26.6%) vs. placebo at all time points for both pain and stiffness, and trended toward improvement for function and overall WOMAC scores. Rapid responses were seen for mean pain subscores (15.9% reduction, P = 0.036) and mean stiffness subscores (12.8% reduction, P = 0.024) occurring after only 10 days of supplementation. At 60 days, pain response was maintained (15.4%, P = 0.038), while stiffness had improved further to 26.6% reduction (P = 0.005). Overall mean WOMAC scores resulted in a 15.2% (P = 0.059) absolute improvement versus placebo at 10 days, which was maintained at 60 days (15.1%, P = 0.052). There were no serious adverse events reported during any of the studies and the treatment was reported to be extremely well tolerated by study participants. Thus, Natural Eggshell Membrane (NEM®) may be considered as a possibly new effective and safe therapeutic option for the treatment of pain and inflexibility associated with joint and connective tissue (JCT) disorders, particularly osteoarthritis (OA).

Methods to assess mineral bio availability in animals

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Trace minerals such as zinc, copper, manganese and selenium serve as cofactors for hundreds of enzymes and transcription factors. They therefore play a wide variety of key roles in the cells and tissues of animals, including promoting proper structural development of bones and other tissues, supporting immune system development and response, and combating oxidative stress. As such, many animal diets are supplemented with these minerals. Mineral bioavailability can be defined as the extent to which a mineral in a given source is absorbed by the animal in a utilizable form. Bioavailability of a given mineral source is generally evaluated relative to the bioavailability of a second, or standard, source. Historically, relative bioavailability (RBV) has been assessed in a variety of different ways, including measuring tissue or blood mineral levels, mineral excretion rates, or biomarkers such as the activity of mineral-dependent enzymes.

In recent years, the existence of gene expression markers for mineral status and RBV has become well-recognized. The use of one such a marker, the metallothionen mRNA and protein, as a marker for zinc bioavailability, is presented. Together, these assays demonstrate that chelated, or "organic" trace mineral forms can be more bioavailable to the animal than traditionally-fed trace mineral salts, (e.g., sulfates and oxides). These assays also make it clear, however, that not all organic trace minerals are equally available, and not all are more available than the trace mineral salts. These methods may have implications for evaluating trace mineral sources in human nutrition. Indeed, MT expression has also been used as an indicator of zinc status in humans.

Improvement of penile endothelial function under Elliovir® a dietary supplement rich in arginine and superoxide dismutase. Preliminary results

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Introduction and Objectives: Endothelial dysfunction is considered as a major contributor to erectile dysfunction (ED). Vascular risk factors (VRF) such as hypercholesterolemia, smoking habits, hypertension, diabetes, obesity and metabolic syndrome alter the penile endothelium which surfaces the lacunae of the cavernous bodies. Continuous oxidative stress will end into apoptosis of the endothelial cells leading to untreatable impotence by current medical treatments. Molecularly speaking the decrease expression of NO synthase, either the nNOS (neural) or the eNOS (endothelial), diminishes the production of NO and the ability to relax the cavernous smooth muscle and to achieve and/or maintain an erection. Elliovir® is composed of (1) L-arginine, the precursor to NO. Oral administration improves endothelial NO synthase and restores eNOS activity when reduced by an oxidative stress; (2) High SOD vegetable extract acting as an inductor of eNOS, with antioxidant properties preventing the formation of the free radicals; and (3) Grape extract rich in oligo proanthocyanidins(OPC). These polyphenols protect vascular walls from the negative effects of free radicals. Elliovir® has been designed to protect or improve the quality of the penile endothelium. The primary end point of this work has been to evaluate the improvement of the endothelial response to Elliovir® of patients with various ED aetiologies and correlate it to the improvement of erection achieved by various treatments. **Patients and Methods:** 58 patients aged 54.8 +/-10 y took Elliovir® for 1 to 6 months at a fixed daily dose of L-arginine 2,280 g, SOD 210 units, grape OPCs 0,120 g. 24% had Elliovir alone, 54% had PDE5 inhibitors and 24% Intracavernous Injections in addition to Elliovir. In 23 patients of this group the dietary supplement was given one month prior to surgical or brachy therapy for prostate cancer, and during the further 3-month post-op period. 57% had one or more VRF. Penile endothelial function was evaluated using the PNORT (Penile NO release Test) consisting in the evaluation of the Flow mediated dilation (FMD) of one cavernous artery with the Echo-Doppler (13mHz probe), i.e. the percentage and difference of dilatation after a 5mn total occlusion of the penile arterial inflow. In a population of non ED patients the average index PNORT (iPNORT) has been established at 1,62+/-2. IIEF-5, the erectile domain of the International Index of the erectile function and the personal quoting of each patient measured the evolution of the erections. Statistical calculations were done with *Statistica®* software. Results were considered for improvement or not at a minimum of 3 months, and up to six. **Results:** iPNORT was significantly improved ($p<0.0001$) after an average of 3 months of treatment (1,27 vs. 1.51). In 36% of subjects the increase was >20%, in 29% in between 10 and 20%, and in 22% <10%, thus considered as inconclusive. At one month postoperative, the protective effect was null in the patients undergoing radical prostatectomy. Those who continued the treatment had the same rate of increase than in the series reported. IIEF-5 was improved from 15,9 to 21,1 ($p=0,00019$). 60% of the patients claimed an improvement of their natural erections, and 59% an improvement of the quality of their intercourse. Half the patients using other treatments were able to reduce or stop that former treatment because of improvement of the natural erections. **Conclusions:** A supplementation with L-Arginine, SOD and polyphenols was able to improve the endothelial function of patients with ED and demonstrated endothelial dysfunction of both neural and vascular origin. This improvement helped in the amelioration of natural erections, with or without other more conventional treatments. These preliminary results deserve more studies with a placebo control.

Chinese herb and other plant extracts: from human to fish

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Chinese herbs and other plant extracts have been used for their therapeutic properties for thousands of years, and are still widely prescribed today. Many plant-derived products are regarded as safe by many users, who are mostly Chinese, but are also ridiculed as ineffective by some critics. At present, over half of the Chinese population still relies on herbal prescriptions rather than Western medicines. Due to the proven effectiveness of plant extracts in human health, the same principle is now being

applied to fish health. Recent studies have tested the efficacy of plant extracts, including terrestrial herbs and seaweeds, as replacement for antibiotics and other synthetic drugs in aquaculture. These plant extracts are used as feed additives for enhancement of immune response and growth among commercially important fish and crustacean species. The use of natural, non-pharmaceutical plant-based products is also becoming popular in aquaculture for their other multiple beneficial effects in fish, including: improved feed conversion; inhibition of pathogenic and non-beneficial microbes in the digestive system; and antimicrobial and antioxidant properties. This presentation did highlight some of the Chinese herbs and other plant extracts that were proven to enhance disease resistance in fish and crustaceans.

Rg1 exert estrogen-like effects in the left ventricular muscle, but not in uterus of ovariectomized mice

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Ginsenoside Rg1, an abundant active ingredient in *Panax Ginseng*, is a unique class of phytoestrogens that can activate estrogen-like activities without direct interaction with the estrogen receptor (ER). Our previous study revealed that Rg1 could exert estrogen-like activity *in vitro*. However, whether Rg1 can exert estrogenic effects in uterus and left ventricular tissue is unknown. The present study aimed at investigating the estrogenic effects of chronic administration of Rg1 in different tissues in ovariectomized mice. Six months old C57BL/6J mice were subjected to ovariectomy or sham-operation. OVX mice were administered with Rg1 subcutaneously using mini-osmotic pump (Alzet) at a dosage of 20 mg/kg body weight/day, estradiol (2 mg/kg body weight/day) or its vehicle for 3 months. Upon sacrifice, the uterus and left ventricular muscle tissue were collected and the tissue weight was measured. Real-time RT-PCR was used to detect the expression of estrogen-related genes. Compared with sham group, the uterus weight could be significantly decreased after ovariectomy operation and estradiol could significantly increase the uterus weight in OVX mice. Estradiol could significantly increase the C3 mRNA expression and decrease the progesterone receptor (PR) mRNA expression, but not ERα mRNA expression in OVX mice. Rg1 did not affect uterus weight and had no significant changes on the C3, PR and ERα mRNA expression in the uterus of OVX mice. On the other hand, estradiol and Rg1 did not alter the weight of left ventricular muscle in OVX mice. Rg1 could significantly increase the Bcl-2 mRNA expression in left ventricular muscle of OVX mice. While, estradiol tends to increase the Bcl-2 mRNA expression in left ventricular muscle of OVX mice but the increase was not significant. Based on the results, we conclude that Rg1 exerts estrogen-like effects in the left ventricular muscle, but not in the uterus of OVX mice, suggesting that the estrogenic effects of Rg1 might be tissue selective.

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The *in vivo* therapeutic effect of Xiaoyaosan on an ER negative breast cancer model

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Breast cancer is a malignant growth that begins in the tissues of the breast (1). Patients with breast cancers negative for the nuclear hormone receptor estrogen receptor alpha (ERα) have a particularly poor prognosis. Although advances in screening, surgery, adjuvant radiation, and systemic therapies are in practice, there are still limitations in these therapies (2-4). Alternative approaches with long history of safe use, such as the use of traditional Chinese medicine (TCM), for its prevention and treatment have become highly desirable. In the present study, we aimed at investigating the therapeutic effect of a classical TCM formula, Xiaoyaosan, in a breast cancer mouse model induced with ERa negative breast cancer 4T1 cells. Female Balb/c mice (6-8 weeks) were subjected to ovariectomy to remove the influence of endogenous estrogen. Following a 2-week post-surgical recovery period, the mice were injected into the fourth mammary fat pad with $1x10^5$ viable 4T1 cells in a 10 μl volume in which tumors were palpable 7 days after injection. On the eighth day, primary tumor size was measured using calipers and the mice were divided into 2 groups and tube-fed daily with vehicle, or Xiaoyaosan (13.92 g/kg body weight). Tumor growth and body weight were measured every 3 days upon the start of treatment and the tumor volume was estimated by using the formula (length x width^2 x 0.5). The mice were killed after 28-day treatment. Tumor growth inhibition was most evident in mice treated with Xiaoyaosan in which the percentage of inhibition was 29%. After 28d of treatment, the mice were sacrificed and tumors were removed. The tumor mass were calculated and the average tumor mass of Xiaoyaosan-treated

mice was found to be smaller than that of the untreated group. Western blotting results showed that Xiaoyaosan treatment significantly reduced Bcl-2 and elevated Bax protein expressions in breast cancer tumor. These results were further confirmed by immunohistochemisty. The gene levels of Bcl-2 and Bax remained unchanged in Xiaoyaosan-treated mice. Taken together, these results show that Xiaoyaosan treatment induced apoptosis at protein level and inhibited the tumor growth in 4T1-induced ovariectomized BALB/c female mice, indicating the possibility of its future use in treatment of ERa-negative breast caner.

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A very powerful tool for herbal medicine: QPAR technique

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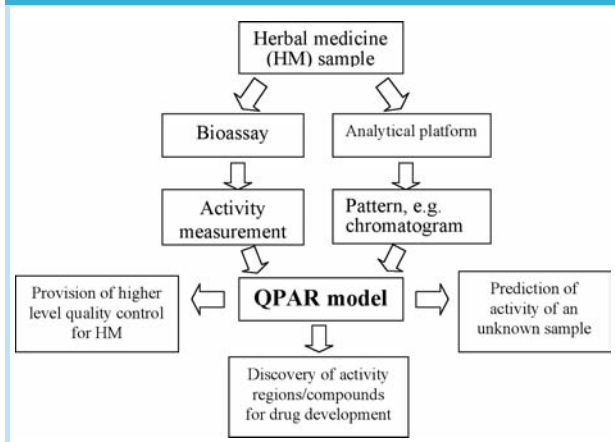
How to relate the bioactivity and chemical composition of herbal medicine (HM) has long been a challenge to scientists in different fields. In most studies, only a few target compounds were considered and they may or may not be bioactive. Recently, we have developed the Quantitative Pattern-Activity Relationship (QPAR) approach and successfully developed a technique to link the bioactivity of a HM to most of its chemical components, through which two pieces of crucial information about the HM were mined. These include (1) a model for predicting total activity from the chromatographic fingerprint and (2) the features in the chromatographic profile responsible for the bioactivity. The QPAR approach and the technique developed as well as its applications are briefly described below. Figure 1 shows the flow chart of QPAR approach, the technique involved, and its applications to HM. In taking the QPAR approach, two kinds of data set are required from the HM concerned. One of them is related to the activity as obtained from, for instance, biological standard study. As for the other one, it provides chemical composition information as represented by a pattern, like chromatogram, acquired via the separation method. The high performance liquid chromatography coupled with a multi-channel UV detector DAD (HPLC-DAD) is an example of this kind. In this way, all the chemical components of the HM as detected by the instrument are utilized in the investigation. Afterward, the QPAR chemometrics data processing methods developed by us are applied to correlate these data sets to build up the QPAR model of the HM (Fig. 1). Our QPAR technique can provide higher level quality control on HM as both the chemical composition and bioactivity are considered at the same time. More than that, it can help to identify the bioactive chromatographic peaks/ regions which correspond to the bioactive components of the HM. This results in finding drug leads from natural source in a much faster, effective and efficient way, as no isolation or fractionation of the HM extracts are required at the very beginning. Of course, when these active components are identified, these procedures are needed. In our study, we obtained the HPLC-UV fingerprints of 78 samples of the HM Radix *Puerariae lobatae* (gegen). At the same time, their antioxidant activity levels were measured by the FRAP method. Then we applied our newly developed chemometrics algorithms to build up the QPAR model of gegen with the use of data sets from 52 samples as the training set. The QPAR model was ap-

Figure 1 - Flow chart of QPAR approach and its applications



plied to predict the FRAP values of the other 26 unknown samples with great success. The correlation coefficient between the predicted and experimental values is up to 0.90. The QPAR approach has also been found working very well in another mixture system. In addition, the bioactive regions in the fingerprint of gegen were indentified and were proven by another set of experiments using preparative liquid chromatography.

Enterprise-ontology-driven TCM (Traditional Chinese Medicine) telemedicine system generation

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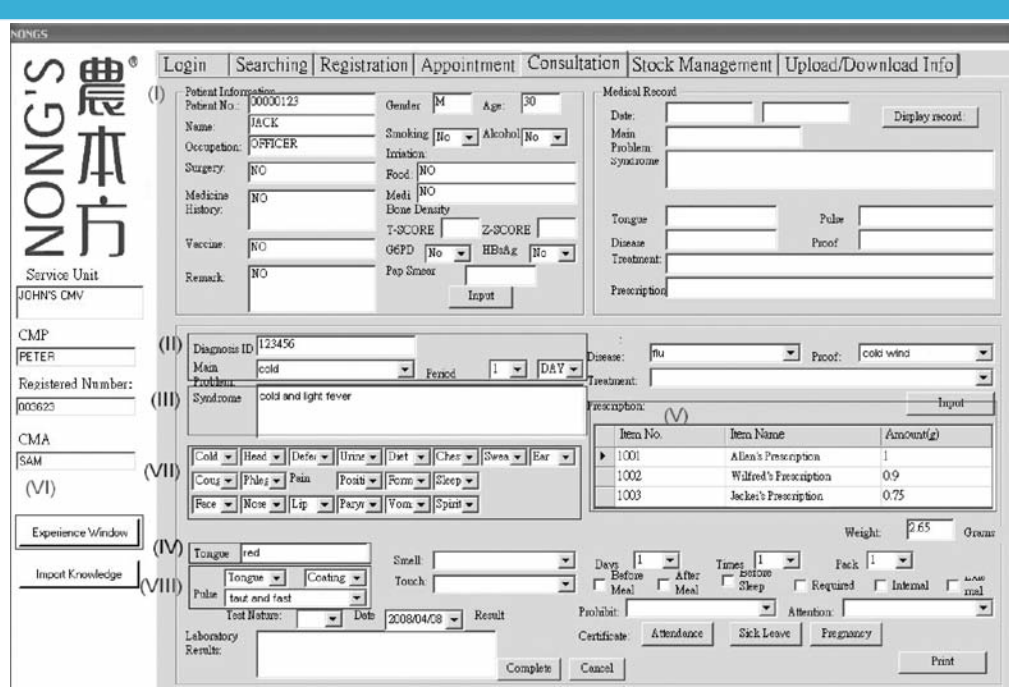
The PuraPharm/Nong's enterprise-ontology-driven information system (IS) development (EOD-ISD) approach is proposed to automate the construction and customization of TCM (Traditional Chinese Medicine) telemedicine systems (1). In this approach the system builder constructs the meta-interface (MI) specification. With the MI, the EOD-ISD generator uses the given enterprise ontology as the basis to generate the corresponding target system. The enterprise ontology is a form of consensus-certified human intelligence with explicit semantics. In the EOD-ISD context the enterprise ontology is locally owned by the establishment that has isolated the desired subset from the global ontology of the knowledge domain (e.g. TCM). From the enterprise ontology, which serves as the standard vocabulary (2) for the establishment, different local/target system variants can be customized anytime and anywhere simply from the MI specifications provided. The PuraPharm/Nong's Diagnosis/Prescription Graphical User Interface has many sections (every section was generated automatically from a specific icon in the MI specification) including: Section (I) – Bar of control functions (e.g. Login). b) Section (II) – Patient/Registration No. (00000123) and Diagnosis ID (i.e. 123456) for treatment; other fields to be filled include: i) Main Problem (i.e. patient's complaint); and ii) Disease and Proof as the diagnostic conclusions. c) Section (III) – Syndrome (i.e. a set of symptoms) obtained by the physician via the standard TCM diagnostic procedure crystallized from accumulated clinical experience. d) Section (IV) – Conclusion for tongue inspection. e) Section (V) –Prescription(s) for the diagnostic conclusion filled in section (II); these prescriptions can be printed and dispensed directly in the MC. f) Section (VI) – Experience window (repository) entrance of the logon TCM physician with unique official medical practice

Figure 1 -



registration number (e.g. 003623 as shown). g) Section (VII) – Questions (by physician) and answers (by patient) (e.g. Do you loathe cold ambience? Do you have a fever?). h) Section (VIII) – Pulse inspection and conclusion.
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Possible immuno-enhancing effects of bilberry in subjects under increased oxidative stress: results of a controlled human intervention study

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Background: Bilberry is a purple-colored berry rich in anthocyanins with powerful antioxidant properties. Increased intake of antioxidant-rich food benefits health, perhaps through opposing oxidative stress. Aging is associated with increased oxidative stress, increased risk of various diseases and decline in immune function. Type 2 diabetes mellitus (DM) is also considered as a state of increased oxidative stress. We explored whether supplementation with an antioxidant rich food (bilberry) benefits immune status in Type 2 DM patients. **Aim:** to investigate effects on immune status (focusing on the main white cell subsets) of dietary supplementation with an extract of bilberry. The target group was Type 2 DM patients (n=20). **Methods:** A double-blinded placebo-controlled human intervention study of cross-over design. Type 2 DM subjects (n=10) took 0.8 g/day (204.8 mg anthocyanin) of bilberry extract and 10 took placebo (starch) for 4 weeks, after which there was a 6-week washout period. Subjects were then crossed-over onto the other treatment for 4 weeks. Fasting blood was collected before and after each treatment and measured (by flow cytometry) for CD3/CD4+ (helper T cells), CD3/CD8+ (cytotoxic cells), CD3/CD19+ (B cells), CD3/CD56+ (natural killer cells) and total white cell count as a marker of immune status. **Results:** Significant bilberry-associated increase ($P<0.05$) was seen in % CD3/CD8+ cells. In addition, an increase (24%) in ratio of T helper to T cytotoxic cells was seen after bilberry, compared to a 7% decrease after placebo treatment, although variation was wide and this effect did not reach statistical significance. **Conclusion:** Some evidence was seen that supplementation with antioxidant-rich bilberry in a group of people under increased oxidative stress resulted in improvement in certain immune parameters. More research into bilberry and other antioxidant-rich foods as potential means to prevent age-related immune decline, which is likely to be accelerated in association with increased oxidative stress, is warranted.

The antiosteoporotic effect of the extract from *Curculigo orchioides*

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Curculigo orchioides (CO) has been safely used for thousands of years in China and was recorded in the literature of past dynasties. It has the properties of "warming kidney, invigorating yang, expelling cold and eliminating dampness". The present study investigated the antiosteoporotic effect of the extract from CO using *in vivo* and *in vitro* models. The rhizomes of CO (5 Kg) were chopped and refluxed 3 times for 2 hours with 8 times volume 60% ethanol. Twelve weeks old sham-operated or ovariectomized (OVX) C57 BL/6J mice were orally fed with either the extracts (low: 500, high: 1000 mg/Kg/day) of CO, 17b-estradiol (3.2 mg/Kg/day) or its vehicle for 12 weeks. Treatment of OVX mice with CO extract had no significant effect on increasing uterus weight at both doses. The CO extract at both doses significantly increased bone mineral density (BMD) and bone volume/ total volume ratio (BV/TV) of femur and tibia in OVX mice. The *in vitro* effects of the extract (0.1-100 mg/mL) on bone cell proliferation and differentiation were determined by MTS and alkaline phosphatase activity assay using UMR 106 cell, a rat osteoblastic-like cell line. The cell proliferation was significantly increased by CO extract at 100 mg/mL at both 24 and 48 hours. The CO extract at 0.1, 1 and 10 mg/mL had significant effect on osteoblastic cell differentiation. Our study demonstrated that CO might be an effective therapeutic agent for treatment of osteoporosis.
Acknowledgement: The study was supported by the Central Research Fund (GU256, GU324) as well as the Niche Area Funding Scheme (1-BB8N) of the Hong Kong Polytechnic University.

Polysaccharides from Cordyceps sinensis mycelial cultures can protect human skin cells against ultraviolet B radiation

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Background: *Cordyceps sinensis* (Berk.) Sacc., also known as Chinese caterpillar fungus, is a fungus that parasitizes the caterpillar of the *Hepialus armoricanus*. Cordyceps is a well regarded 'herb' in Chinese traditional medicine, and there is some evidence of a UV protecting effect by polysaccharides from Cordyceps mycelia. The adverse effects of Ultraviolet (UV) radiation on skin are well documented, including skin cancers and photoaging. UVB is a relatively small component (~5%) of the UV radiation that humans are exposed to, and only 10-20% of UVB can penetrate the epidermal layer. However, it is important because it is high in energy and can directly interact with DNA in outer skin cells, transferring energy to DNA, and creating different types of DNA damage. **Aim:** To investigate the protective effect of polysaccharides from Cordyceps mycelia against UVB-induced DNA damage in a human skin fibroblast model. **Methods:** BJ fibroblasts were incubated with known concentrations (0, 50, 100, 200 μg/ml) of hot water extract of Cordyceps mycelia ('HWECMyc') or extracellular polysaccharides (molecular weight <500kDa) from Cordyceps mycelia ('<500EPSCM') in complete medium for 30 min and, separately, for 24 hours. Complete medium was used as control. Viability of the cells after 24h incubation in Cordyceps extracts was assessed using the trypan blue test. To test for UV protective effects, Cordyceps-incubated and control cells were embedded on a microscope slide in low melting point agarose before being irradiated on ice for 10 sec with UVB (302 nm), with the lamp fixed at a distance of 165 cm. The cells were lysed immediately and T4 endonuclease V was overlaid onto the gels for 15min at 37°C. This enzyme creates DNA strand breaks predominantly at DNA lesions that are induced specifically by UVB radiation. The % DNA tail content was measured as the index of DNA damage using the comet assay. Data were analyzed using one way ANOVA with Dunnett's post-test. Test for linear trend was used to investigate dose response. Cordyceps extracts were also scanned through the UV range to check for direct UV absorption. **Results:** The viability of BJ cells was >92% after 24h incubation in both Cordyceps extracts. Neither of the extracts absorbed UVB directly. Some effect was seen at 30 min incubation but this did not reach statistical significance. There was significant protective effect of each extract ($P<0.05$) after 24 hr incubation, with ~34% and ~27% less DNA damage in cells pre-treated with <500EPSCM and HWECMyc, respectively, at 200 μg dose compared to control cells. Significant linear dose-response was seen with <500EPSCM ($r^2 = 0.84$, $P<0.0001$) and with HWECMyc ($r^2 =0.64$, $p<0.001$). **Conclusion:** A clear protective effect against UVB-induced DNA damage was seen after 24h incubation with both Cordyceps extracts. Since the cells were lysed immediately after UV irradiation, the protective effect was unlikely to be related to upregulating DNA repair. Further study is needed to determine the mechanism behind the genoprotective effects of polysaccharides from Cordyceps mycelia.

Anti-fatigue and endurance enhancement properties of CordyMax® in Humans

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Cordyceps sinensis and a standardized mycelial fermentation product of *C. sinensis*, CordyMax®, are known as medicinal herbal products for invigoration, health preservation, anti-aging, and anti-fatigue. Their anti-fatigue and endurance enhancement functions have been reported. By use of a traditional symptom-analysis method in a double-blind clinical trial, elderly patients with senescence-related symptoms reported improvement of fatigue, dizziness, intolerance to cold temperature, sexual dysfunction, etc. by CordyMax supplementation in majority of patients (J Appl Tradit Chin Med 1993;1:32). Animal studies demonstrated that CordyMax improved steady state bio-energy ATP levels in mouse liver by use of *in vivo* serial ^{31}P NMR spectroscopy (J Alternat Compl Med 2001, 7:231), and promoted efficient use of limited oxygen supply to support body's essential physiological activities and greater tolerance to hypoxia-induced acidosis (Chin Tradit Herbal Drugs 1986;17: 209). We examined the anti-fatigue and endurance enhancement properties of CordyMax with use of sports physiology methods. In double-blind clinical trials with use of an incremental work rate protocol on a cycle ergometer and/or treadmill, we found that CordyMax increased VO_2max by 7.0%, anaerobic threshold by 12.6%, maximal ventilation by 10.4% and maximal work rate by 5.9%, indicating improvement of aerobic exercise capacity in healthy sedentary adults of advance ages. In healthy young athletes and by use of a constant work rate protocol, CordyMax therapy increased O_2 pulse by 7.6% and reduced heart rate (HR) by 2.2%, RER by 2.5%, and lactic acid by 10.5% during endurance exercise, indicating improvement of cardiovascular and metabolism functions during endurance exercise. CordyMax also accelerated

HR recovery 3 min post maximal exercise by 6.3%. In summary, CordyMax supplementation influences favorably aerobic capacity and cardiovascular, pulmonary, and metabolic functions during maximal and endurance exercise, improves fatigue and endurance performance, and facilitates recovery from exercise.

Enhanced bio-energy state in mice liver after administration of CordyMax®

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Natural *Cordyceps sinensis* and its mycelial fermentation product CordyMax® have been advocated for centuries to enhance human vitality. This study evaluated the effect of CordyMax on tissue energetics of male C57-BL/6 mice using non-invasive ^{31}P NMR spectroscopy. Mice were divided into 3 groups. Groups A and B (n=5 each) received an extract of CordyMax, 200 or 400 mg/kg/day, and Group C (n=6) received placebo. All treatments were given by gavage for 7 days and then discontinued. Hepatic -ATP and inorganic phosphate were measured using a ^{31}P NMR spectroscope [Bruker], after mice had been anesthetized with pentobarbital (55 mg/kg, i.p.) and immobilized on shielding belt. An MDPA reference was placed on the back of the coil. Measurements were made at baseline, after 7 days of treatment, and 7 days after discontinuing treatment (washout phase). Tissue pH was calculated from chemical shift differences between -ATP and Pi. At the end of the treatment phase, -ATP was increased in relation to the MDPA reference in mice receiving CordyMax: Group A 3.81±0.03 (+12.3% on average); Group B 4.00±0.04 (+18.4%); compared with Group C 3.36±0.04 ($p<0.001$). Inorganic phosphate was decreased in Groups A and B, but not in Group C ($p<0.001$). Consequently, the ratio -ATP/Pi was also significantly increased in mice receiving CordyMax: Group A 4.81±0.05 (+47.7% on average); Group B 4.50±0.09 (+41.4%); compared with Group C 3.10±0.04 ($p<0.001$). At the end of the washout phase, -ATP had returned to baseline in Groups A and B. Hepatic tissue pH was unchanged throughout the study. We conclude that CordyMax increased steady state levels of hepatic bio-energy when administered to mice for 7 days. Our findings may explain the reported energizing effect of CordyMax in human subjects.

CordyMax® improves glucose metabolisms in animals and in humans

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Preliminary reports demonstrated that supplementation with a mycelia fermentation product of *Cordyceps sinensis* (CordyMax®) increased maximal O_2 uptake and anaerobic threshold in mid-age to elderly humans, and enhanced *in vivo* bio-energy metabolisms in animals (J Alternat Complement Med 2001;7:231; Chin J Integrat Med 2004;10:187; Shanghai J Prevent Med 2008; 20: 367). We further studied in a randomized, double-blind clinical trial the effect of CordyMax (4.5 g/day, 6 weeks) on glucose metabolism in highly-fit athletes. Male adventure racers and multi-sport endurance athletes (age 32±4 yrs; VO_2peak 63±8 ml/kg/min) were assigned to either a CordyMax or a control group (n=15 each). We found a 7% decrease in fast blood glucose within normal ranges after the CordyMax therapy (92±1 to 87±2 mg/dL; $p<0.01$), but no change in placebo controls. During prolonged sub-maximal exercise (70% VO_2peak, 60 min), reductions of respiratory exchange ratio were found in the CordyMax *vs.* control group ($p=0.02$). In mice given CordyMax for 4 weeks, responses of serum insulin and C-peptide to an oral glucose load were diminished and recovered to the pre-load levels quickly *vs.* control group ($p<0.01$ or 0.05) with no change in the glucose tolerant curve. The glucose-insulin index was lower in the CordyMax (7±1 x10^5 units) *vs.* control group (10±1 x10^5) ($p<0.01$). Our data suggest that CordyMax (1) safely lowers basal glucose in normal humans, (2) improves glucose metabolism by enhancing insulin receptor sensitivity, and (3) enhances fat mobilization and beta-oxidation thereby sparing glycogen expenditure during prolonged endurance exercise.

CordyMax® increases serum HDL-cholesterol and reduces oxidized LDL-cholestrol in humans with reduced serum HDL-cholesterol

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Literature has reported that CordyMax®, a mycelial fermentation product of *Cordyceps sinensis*, regulates the blood lipids in hyperlipidemic patients and prevents the formation of atherosclerosis in animals and humans. (*Administ Tradit Chin Med* 1995;5:14-18). We tested the effect of CordyMax (3.0 g/day) in 133 dyslipidemic subjects on increasing HDL cholesterol (HDL-c) and reducing oxidized LDL cholesterol (ox-LDL). Subjects with reduced serum HDL-c (<40 mg/dL for males or <45 mg/dL for females) were randomized to a CordyMax or placebo group (double-blind). Eight weeks of CordyMax did not change total cholesterol and triglycerides significantly. At Week 8, LDL-c was reduced with CordyMax significantly by 5.4% (p=0.002). Most dramatically, ox-LDL was reduced by 22.9% in CordyMax group (p=0.001). Atherosclerosis Index [= (TC – HDL-c)/HDL-c] reduced with CordyMax by 29% (p<0.001). HDL-c was increased with CordyMax by 31.1% (p<0.001) in females and by 12.3% (p=0.003) in males. Apolipoprotein A1 was increased with CordyMax by 34.7% (p=0.005). The ratio of ox-LDL/HDL-c was reduced by 10.5% (p=0.003). **In conclusion**, CordyMax® significantly lowers LDL-c, ox-LDL and Atherosclerosis Index, and increases HDL-c and ApoA1, reducing the risk of atherosclerosis and cardio- and cerebro-vascular diseases.

CordyMax® extends the lifespan of mice - A preliminary report

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Cordyceps sinensis and its mycelia fermentation product CordyMax® have been used for centuries as anti-fatigue and for endurance enhancement. We reported anti-fatigue and endurance enhancement properties, and improvement of glucose, lipid and energy metabolisms by CordyMax in animals and humans in previous studies. In this study, we explored possible anti-aging effects of CordyMax in mice. A total of 192 healthy ICR mice (12 months of age, half males and half females) were randomized into 4 groups, receiving either vehicle or CordyMax at a dose of 500, 1000, or 1500 mg/kg.bw. All mice were fed with regular forage or forages containing CordyMax at different concentrations. Body weight was monitored once a week. Calorie intake was monitored twice per week and adjusted carefully to match the average calorie intake levels for vehicle controls, males and females respectively. CordyMax administration continued until all mice died. Mice have been treated for 64 weeks thus far. The preliminary results show: (1) No significant differences in body weight and calorie intake were observed amongst 4 groups. (2) Compared to controls, the survival time of 75% animals in the CordyMax groups (500, 1000, and 1500 mg/kg.bw) extends over 14, 14, and 16 weeks respectively, and the survival time of 50% animals in the CordyMax groups extends over 9, 1, and 5 weeks respectively. Analysis with use of Kaplan-Meier Cumuli Survivor Plot showed significantly extended lifespan of the mice and reduced death risks by CordyMax: p=0.049 (Week 36); p=0.036 (Week 40); p=0.059 (Week 48); p=0.004 (Week 60); p=0.027 (Week 64). The low-dose CordyMax treatment (equivalent to the human dose) appeared to show the best survivor curve. This study demonstrates the lifespan-extending effects of CordyMax in mice, while the experiment is still ongoing. At the meanwhile, additional studies demonstrated that CordyMax has anti-oxidation activity, and improved glucose, lipid and energy metabolisms and aerobic exercise capacity in animals and in humans (reported separately), all of which support the general anti-aging function of CordyMax.

Anti-oxidation activities of CordyMax®, a mechanism of its anti-aging property

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Cordyceps sinensis and its mycelia fermentation product CordyMax® have been used for centuries for their anti-fatigue properties, and for endurance enhancement. We reported anti-fatigue and endurance enhancement properties, and improvement of glucose, lipid and energy metabolisms by CordyMax in animals and humans in previous studies. We also demonstrated the anti-aging effect of CordyMax in mice. To explore the mechanism of the anti-aging actions, we tested anti-oxidation activity of CordyMax in mouse models with oxidative damage. Mice were randomized into 5 groups, receiving vehicle or CordyMax at a dose of 500, 1000, or 1500 mg/kg.bw for 60 days. Mice in vehicle and 3 CordyMax groups were given a single dose of 11 Gry ^{60}Co γ-rays radiation, and sacrificed at Day 4 after irradiation. We found that plasma glutathione (GSH) and the thiol groups, and liver superoxide dismutase (SOD) and catalase (CAT) were significantly reduced by 29.0%, 22.6%, 6.0% and 24.7%, and liver protein carbonyl groups were significantly increased by 37.3% in vehicle controls, compared to normal mice. As compared to vehicle controls, CordyMax therapy at a dose of 500, 1000, or 1500 mg/kg.bw increased plasma thiol groups by 22.9%, 20.8%, and 25% respectively (p=0.001, 0.001, and <0.001), and liver CAT by 16%, 14.8%, and 16.4% respectively (p=0.001, 0.002, and<0.001). CordyMax at a dose of 1000 or 1500 mg/kg.bw reduced liver protein carbonyl groups by 8.7%

and 13.5% respectively (p=0.035 and 0.001), and increased plasma GSH by 26.3% and 26.5% (p=0.023 and 0.026). Liver SOD was increased with CordyMax (1000 and 1500 mg/kg.bw) by 9.4% and 5.7% (both p<0.05). Liver GSH-reductase was increased with CordyMax (500mg/kg) by 10.7% (p=0.041). The results indicate that CordyMax improves antioxidant capacity in mice with radiation-induced oxidative injury, representing one of the mechanisms of anti-aging functions of CordyMax.

Maturation of *Cordyceps sinensis* associates with co-existence of *Hirsutella sinensis* and *Paecilomyces hepiali* DNA

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A decades-long debate has not resulted in a consensus on the anamorph-telemorph connection for *Cordyceps sinensis* (Cs). Literature reported isolations of *Paecilomyces hepiali* and *Hirsutella sinensis* from natural *Cordyceps sinensis*, and evidence of molecular existence of *H. sinensis* in *C. sinensis*. We tested a hypothesis in this study that *P. hepiali* and *H. sinensis* co-exist in natural *C. sinensis*, and their proliferation predominance changes during maturation of *C. sinensis*. Mycological and molecular approaches were employed to examine the growth of *P. hepiali* and *H. sinensis* and their genes in freshly collected *C. sinensis* after thorough prior cleaning and surface sterilization. A nested PCR method with use of a touch-down program was used to identify the genes of *P. hepiali* and *H. sinensis* in the caterpillar body and stroma of natural *C. sinensis*. We found that *P. hepiali* and *H. sinensis* were detected simultaneously in freshly collected *C. sinensis* by mycological and molecular examinations. Maturation of C. sinensis after it is visible above ground associates with a large decrease in the ability of competitive growth of *H. sinensis* (p<0.001). *P. hepiali* and *H. sinensis* genes were found in both caterpillar body and stroma of natural C. sinensis. We conclude that *C. sinensis* is a complex traditional Chinese herb with multiple fungi living in its caterpillar and stroma. Its maturation from early May to late June is associated with dynamic changes in proliferation predominance of the fungi.

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Maturation of *Cordyceps sinensis* associates with changes in profiles of proteins and small molecular weight organic chemicals

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Background: Literature reported differential expressions of *Paecilomyces hepiali* and *Hirsutella sinensis* DNA in the caterpillar body and stroma of natural *Cordyceps sinensis* during *C. sinensis* maturation. **Hypothesis:** Differential expressions of *P. hepiali* and *H. sinensis* in natural *C. sinensis* may alter the profiles of proteins and other organic chemicals during maturation of *C. sinensis*. **Design and Methods:** SELDI-TOF MS and HPLC analyses were used to profile proteins and small molecular weight organic chemicals in caterpillar body and stroma of natural *C. sinensis* and mycelia of *P. hepiali* and *H. sinensis*. **Results:** Maturation of *C. sinensis* after it is visible above ground associates with altered profiles of proteins and small organic chemicals. The profiles for the mycelia of fungi do not completely match to those for the stroma or caterpillar body of natural *C. sinensis* during its maturation. The profiles for *H. sinensis* mycelia appear to be more distinct from those for natural *C. sinensis*, than the profiles for *P. hepiali* mycelia. **Conclusions:** The maturation of *C. sinensis* is associated with significant changes in the component organic chemicals. The compounds from all component fungi may contribute jointly to the overall pharmacological functions of natural C. sinensis.

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Cordyceps sinensis. Acta Pharmaceut Sinica 2008; 43 (4): 421-6. 3) Jiang Y, Yao YJ. Names related to *Cordyceps sinensis* anamorph. Mycotaxon 2002; 84: 245-54. 4) Jiang Y, Yao YJ. A review for the debating studies on the anamorph of *Cordyceps sinensis*. Mycosistema 2003; 22(1): 161-76. 5) Guo YL, Zhu JS. Existence of multiple-fungi in *Cordyceps sinensis*: Simultaneous isolation of *Hirsutella sinensis* and *Paecilomyces hepiali*. FASEB J. 2005; 19 (5): A1033. 6) Yao YS, Zhou YJ, Chen W, et al. Maturation of *Cordyceps sinensis* associates with increased expression of *Paecilomyces hepiali* gene and altered differential expressions of 2 forms of *Hirsutella sinensis* genes. Proc 2008 Symposium Chin Assoc Med Mycol. 2008, Oct 7, Nanchang, Jiangxi. Chin Assoc Med Mycol Publishing, pp 53-72

Maturational alterations of differential expressions of CG:AT-biased *Cordyceps sinensis* mutants and *Paecilomyces hepiali* in natural *Cordyceps sinensis*

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A decades-long debate has not given a consensus on the anamorph-telemorph connection for *Cordyceps sinensis* (Cs). Literature reported simultaneous detections of *Paecilomyces hepiali* (Ph) and *Hirsutella sinensis* (Hs) and their DNA in freshly collected natural Cs and a >50% decline of colony-forming ability of caterpillar Hs along with Cs maturation under competitive proliferation conditions. In this study, we tested the expressions of Ph and multiple Hs-related Cs genes during Cs maturation. Southern blotting analysis revealed dramatic increases in expressions of Ph and Hs genes with Cs maturation, with use of Ph ($ITS_{5\text{-}179}$) and Hs ($ITS_{5\text{-}162}$) specific probes. Two Hs-related species on Hs blot were seen after the genomic Cs DNA was digested with *Eco*RI, probably representing the GC:AT-biased mutants. They expressed differentially in stroma and caterpillar body at different stages of Cs maturation. The AT bias does not express in premature Cs caterpillar, but highly predominately in premature stroma; while the GC bias expresses oppositely in the premature Cs compartments. The differential expressions altered non-proportionally in the caterpillar body and stroma when Cs matures. The expression pattern of the AT-bias species is highly similar to that for Ph. *Eco*RI digestions of PCR products in ITS1/5.8S rDNA regions when Cs stroma genomic DNA used as the template showed increased quantity of digestible species in proportion to the increased length of stroma during Cs maturation. In conclusion, Cs maturation associates with augmented expressions of both Ph and Hs genes and altered differential expressions of GC:AT-biased Cs mutants. These maturation-related changes in the expressions of Cs-associated fungi represent the important elements in Cs life cycle, opening an avenue to unmask the anamorph-telemorph connection of this precious Chinese herb.

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Edible coating as carrier of antimicrobial agents to extend the shelf life of fresh-cut apples

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Edible coatings with antimicrobial agents can extend shelf-life of fresh-cut fruits. The effect of lemongrass, oregano oil and vanillin incorporated in apple puree-alginate edible coatings, on shelf-life of fresh-cut 'Fuji' apples, was investigated. Coated apples were packed in air filled polypropylene trays and wrapped with polypropylene film. Changes in headspace atmosphere, color, firmness, sensory quality and microbial growth were measured during 21 days storage at 4ºC. A significant reduction in the rates of O_2 depletion and CO_2 production was observed in samples containing high concentrations of essential oils. Ethylene production in coated apples remained below 50 $\mu l\ l^{-1}$, while production of this gas increased continuously in uncoated apples and those coated without essential oils during storage. Apples coated with apple puree-alginate exhibited ethanol and acetaldehyde formation in the first week. Coatings with calcium chlo-

ride and *N*-acetylcysteine helped to maintain firmness and color, while lemongrass containing coatings induced severe texture softening. Vanillin containing coatings (0.3% w/w) were the most effective in terms of sensory quality after 2 weeks storage. All antimicrobial coatings significantly inhibited the growth of psychrophilic aerobes, yeasts and molds. The antimicrobial effect of essential oils against *Listeria innocua* inoculated into apple pieces before coating was also examined. Lemongrass (1.0 and 1.5% w/w) and oregano oil containing coatings (0.5% w/w) exhibited the strongest antimicrobial activity against *L. innocua* (4 log reduction). Alginate-apple puree edible coatings were successfully formulated with the addition of essential oils and resulted in a variety of beneficial effects on the shelf-life of fresh-cut 'Fuji' apples.

Dehydration of pollock skins prior to gelatin production

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Alaska pollock (*Theragra chalcogramma*) is the USA's largest commercial fishery, with an annual catch of over one million tons. During pollock processing, the skins are discarded or made into fish meal, despite their value for gelatin production. The absence of gelatin processing facilities in Alaska necessitates drying of the skins before transport to decrease the moisture content, but conventional hot-air drying is expensive. This study evaluated a less energy-intensive technology, the use of desiccants for reducing water weight in pollock skins prior to shipment. To ensure that the functional properties of gelatin obtained from dried pollock skins were not affected during desiccation, gelatins were prepared from each skin-drying treatment and compared with gelatin extracted from air-dried pollock skins. None of the desiccation treatments decreased the gel strength of pollock skin gelatin, nor were there major differences in gelling temperature or viscosity among the gelatin solutions. This suggests that pollock skins can be economically stabilized for transport to a gelatin-processing facility through the use of regenerable desiccants that are already common in the food industry.

Physicochemical properties of apple puree-alginate films containing plant essential oils and oil compounds active against *Escherichia coli* O157:H7

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The use of edible films as carriers of antimicrobial plant essential oils and other phytochemicals constitutes an approach for external protection of food systems to reduce surface microbial populations and to enhance oxygen-barrier properties, thus enhancing food safety as well as shelf life. To demonstrate this possibility, we investigated the antimicrobial effects of 0.1-0.5% suspensions in apple puree with alginate used to prepare edible films of the following essential oil/oil compounds against the foodborne pathogen *Escherichia coli* O157:H7: oregano oil/carvacrol; cinnamon oil/cinnamaldehyde; lemon grass oil/citral, as well as their effect on physical and barrier properties of apple puree-alginate based films. Bactericidal activities (BA_{50} values, defined as the % of sample that induced a 50% decrease in colony forming units) against *E. coli* was evaluated after incubation for 3, 30 and 60 min at 21°C. Water vapor and oxygen permeability, and tensile properties of films were also evaluated. BA_{50} values against *E. coli* after incubation for 60 min at 21°C ranged from 0.011% for carvacrol to 0.087% for cinnamon oil. The data also show that the approximate order of antimicrobial activities was as follows: carvacrol > oregano oil > citral > lemon grass oil > cinnamaldehyde > cinnamon oil. Addition of plant essential oils/oil compounds into the apple puree-alginate films decreased water vapor permeability, increased oxygen permeability, but did not significantly alter tensile properties. These results show that the plant-derived substances can be used to prepare apple puree-alginate based antimicrobial edible films or coatings to protect foods against pathogenic microorganisms.

Allspice, garlic and oregano plant essential oils in tomato films inactivate the foodbrone pathogens *Escherichia coli* O157:h7, *Salmonella enterica* and *Listeria monocytogenes*

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Edible films containing plant essential oils are gaining importance as potential antibacterial formulations to extend product shelf life and reduce risk of pathogen growth on food surfaces. An evaluation of both antimicrobial and physicochemical prop-

erties of edible films is important for applications to food systems. The main objective of the present study was to evaluate physical properties and antimicrobial activities against *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* of tomato films with added allspice, garlic, and oregano oils (0.5-3.0% w/w in film-forming solutions). Antimicrobial activities were determined by overlay of the film on top of the bacteria and by vapor phase diffusion of the antimicrobial from the film onto the bacteria. Activities against *E. coli* O157:H7 and *S. enterica* were in the following order: oregano > allspice > garlic oils. Garlic oil was the most effective against *L. monocytogenes*, even at a concentration of 0.5%. *L. monocytogenes* bacteria were less resistant to inactivation than were *E. coli*. The presence of plant essential oil antimicrobials reduced the water vapor permeability of the tomato films. The results of the present study show that some essential oils in a tomato film matrix possess good physical and antimicrobial properties for food applications.

Hedonic evaluation of cooked chicken wrapped with apple and tomato films formulated with cinnamaldehyde and carvacrol

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Edible films and coatings can be used as carriers of plant essential oils and their active antibacterial components to protect food against bacterial pathogens and spoilage, while potentially enhancing sensory properties of the wrapped foods. To demonstrate this possibility, this study evaluated the effect of adding (0%, 0.5%, 0.75%, 1.0%) of carvacrol (the active ingredient of oregano essential oil) and of cinnamaldehyde (the active ingredient of cinnamon oil) to apple- and tomato-based film-forming solutions on sensory properties of the resulting films. Paired-comparison preference tests performed by 55-65 untrained human volunteers indicated that baked chicken wrapped with tomato and apple films containing 0.5% carvacrol or cinnamaldehyde were equally preferred over chicken wrapped with tomato or apple films without the plant antimicrobials. The consumers preferred carvacrol-containing tomato film chicken wraps over the corresponding apple film wraps. Statistical analysis of the sensory data also indicates that the cinnamaldehyde (0.5% and 0.75%)-containing apple films were preferred over the corresponding carvacrol-containing films. The data suggest that films and coatings containing antibacterial essential oils can be used to protect raw chicken pieces against bacterial contamination without adversely affecting sensory preferences of cooked wrapped chicken pieces.

Effect of plant essential oils on antimicrobial and physical properties of apple puree edible films and coatings

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The use of edible films as carriers of antimicrobial plant essential oils and other phytochemicals constitutes an approach for external protection of food systems to reduce surface microbial populations and to enhance oxygen-barrier properties, thus enhancing food safety as well as shelf life. The objective of this study was to investigate the antimicrobial effects of 0.1-0.5% suspensions in apple puree and pectin used to prepare edible films of the following essential oil/oil compounds against the foodborne pathogens *Escherichia coli* O157:H7 and *Salmonella enterica*: oregano oil/ carvacrol; cinnamon oil/cinnamaldehyde; lemon grass oil/citral, as well as their effect on physical and barrier properties of apple puree-pectin based films. Bactericidal activity of apple puree based film forming solutions was tested against the foodborne pathogen *Escherichia coli* O157:H7 and *Salmonella enterica* in terms of the achievement of a 50% decrease in the initial number of bacteria (BA_{50}). Water vapor permeability (WVP), oxygen permeability (O_2P) and tensile properties of apple puree-pectin edible films, with and without plant essential oils and oil compounds, were also compared. BA_{50} values against *E. coli* after incubation for 60 min at 21°C ranged from 0.011% for carvacrol to 0.094% for cinnamon oil. The corresponding range against *Salmonella* was from 0.0052% for carvacrol to 0.041% for cinnamaldehyde. The data also show that (a) the test samples were 2-3 times more effective against *Salmonella* than against *E. coli*; (b) the approximate order of antimicrobial activities were as follows: carvacrol > oregano oil > citral > cinnamaldehyde > lemon grass oil> cinnamon oil; and (c) addition of plant essential oils/oil compounds into the apple puree films decreased water vapor permeability, increased oxygen permeability, but did not significantly alter tensile properties. These results show that the plant-derived substances can be used to prepare apple-based antimicrobial edible films or coatings to protect foods against pathogenic microorganisms.

Allspice, cinnamon and clove bud plant essential oils in edible apple films inactivate the foodbrone pathogens *Escherichia coli* O157:h7, *Salmonella enterica* and *Listeria monocytogenes*

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Plant essential oils (EOs) are rich sources of volatile terpenoids and phenolic compounds. Such compounds have the potential to inactivate pathogenic bacteria in the vapor phase. Edible films made from fruits or vegetables containing EOs can be used commercially to protect food against contamination by pathogenic bacteria. EOs from cinnamon, allspice and clove bud are compatible with the sensory characteristics of apple-based edible films, and these films could extend product shelf life and reduce risk of pathogen growth on food surfaces. The main objective of this study was to evaluate physical properties as well as antimicrobial activities against Escherichia coli O157:H7, *Salmonella enterica* and *Listeria monocytogenes* of allspice, cinnamon, and clove bud EOs in apple puree film-forming solutions formulated into edible films at 0.5-3.0% (w/w) concentrations. Antimicrobial activities were determined by two independent methods: overlay of the film on top of the bacteria and vapor phase diffusion of the antimicrobial from the film to the bacteria. The antimicrobial activities against the three pathogens were in the following order: cinnamon oil > clove bud oil > allspice. The antimicrobial films were more effective against *L. monocytogenes* than against the *S. enterica*. The presence of the EOs reduced the viscosity of the film-forming apple solutions at higher shear rates, but did not affect water vapor permeability of the films. The oils also increased elongation and darkened the colors of the films. The results of the present study show that some volatile EOs can be used to prepare apple-based antimicrobial edible films with good physical properties, and that the films are effective against major foodborne pathogens when evaluated by both direct contact and indirectly by vapors emanating from the films. Application of the antimicrobial apple films to contaminated produce and other foods merits study.