Natural Product Radiance

Inhibitory activities of *Phyllanthus amarus* on HIV replication

There is a globally acknowledged demand for a broader, safer and also cheaper repertoire for the treatment of human immunodeficiency virus (HIV) infection. The medicinal plant *Phyllanthus amarus* Schum. & Th. has not only a recorded use in folk medicine for centuries, but has been shown to have multiple beneficial biological effects on a scientific level. *P. amarus* derived preparations were previously shown to inhibit RT inhibitor-resistant HIV variants as efficiently as wild-type strains. The drugs target different steps of the HIV life cycle, thereby presenting multiple antiviral activities. Researchers working at Institute of Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany observed that a water/alcohol extract of *P. amarus* blocks HIV-1 attachment and the HIV-1 enzymes integrase, reverse transcriptase and protease to different degrees. A gallotannin containing fraction and the isolated ellagitannins, geraniin and corilagin were shown to be the most potent mediators of these antiviral activities. The *P. amarus* derived preparations blocked the interaction of HIV-1 gp120 with its primary cellular receptor CD4 at 50% inhibitory concentrations of 2.65 (water/alcohol extract) to 0.48 µg/ml geraniin. Inhibition was also evident for the HIV-1 enzymes integrase (0.48–0.16 µg/ml), reverse transcriptase (8.17–2.53 µg/ml) and protease (21.80–6.28 µg/ml). In order to prove the *in vivo* relevance of these biological activities, plant material was administered orally to volunteers and a potent anti-HIV activity in blood could be demonstrated. Sera at a final concentration of 5% reduced HIV replication by more than 30%. These results support the conclusion that *P. amarus* has inhibitory effects on HIV not only *in vitro* but also *in vivo* [Notka Frank, Meier Georg and Wagner Ralf, Concerted inhibitory activities of *Phyllanthus amarus* on HIV replication *in vitro* and *ex vivo*, Antiviral Res, 2004, 64 (2), 93-102].

Anti-HIV benzylisoquinoline alkaloids and flavonoids from the leaves of *Nelumbo nucifera*

*Nelumbo nucifera* Gaertn. (Hindi — Kamal) is a perennial aquatic crop, grown and consumed throughout Asia. Its parts have been used for various medicinal purposes in oriental medicine. In particular, the leaves are known for diuretic and astringent properties, and are used to treat fever, sweating, and strangury and as a styptic. Kashiwada and others during their search for plant-derived anti-HIV agents from natural products, found that 95% EtOH extract of the leaves of *N. nucifera* displayed significant anti-HIV activity (EC₅₀ < 2 µg/mL, TI > 5). The chemical compounds (+)-1(R)-Coclaurine (1) and (−)-1(S)-norcoclaurine (3), together with quercetin 3-O-β-D-glucuronide (4), were isolated from leaves and identified as anti-HIV principles. Compounds 1 and 3 demonstrated potent anti-HIV activity with EC₅₀ values of 0.8 and <0.8 µg/ml, respectively and therapeutic index (TI) values of >125 and >25, respectively. Compound 4 was less potent (EC₅₀ 2 µg/mL).

In a structure–activity relationship study, other benzylisoquinoline, aporphine, and bisbenzylisoquinoline alkaloids, including liensinine (14), negerine (15) and isoliensinine (16), which were previously isolated from the leaves and embryo of *N. nucifera*, were also evaluated for anti-HIV activity. Compounds 14–16 showed potent anti-HIV activities with EC₅₀ values of <0.8 µg/mL and TI values of >9.9, >8.6 and >6.5, respectively. Nuciferine (12), an aporphine alkaloid, had an EC₅₀ value of 0.8 µg/mL and TI of 36. In addition, synthetic Coclaurine analogs were also evaluated. Compounds 1, 3, 12 and 14–16 can serve as new leads for further development of anti-AIDS agents [Kashiwada Yoshiki, Aoshima Akihiro, Ikeshiro Yasumasa, Chen Yuh-Pan, Furukawa Hiroshi, Itoigawa Masataka, Fujioka Yoshihiro, Mihashi Kunihide, Cosentino L Mark, Morris-Natschke Susan L and Lee Kuo-Hsiung, Anti-HIV benzylisoquinoline alkaloids and flavonoids from the leaves of *Nelumbo nucifera* and structure–activity correlations with related alkaloids, Bioorg Med Chem, 2005, 13 (2), 443-448].
Toxicological studies on *Artemisia* extract used as dietary supplement

Russian Tarragon, *Artemisia dracunculus* Linn. has a traditional Persian history of use as a natural cleanser of the blood and for the treatment of headaches and dizziness. Studies done on rats showed that an extract of this artemisia has anticoagulatory and anti-hyperlipidemic activities. Thus the extract appear potentially useful as agent to help decrease the incidence of coronary disease in humans since a reduction in serum cholesterol of 15% and serum triglycerides of 25% was observed in rats treated with extract and maintained on a hyperlipidemic diet.

TARRALIN™ is an ethanolic extract of Russian Tarragon an ancient herb that has been safely and widely used as a medicine, flavour and fragrance. In addition to historical use documentation, we now have a formal toxicological evaluation of the preparation in animals reinforcing its safe use as a dietary supplement or in functional foods. As an herb, its well-known flavour and fragrance are, in part, attributed to the presence of the compounds, estragole and methyl eugenol, that comprise the major constituents of the herb’s essential oil. Estragole and methyl eugenol are common components of many herbs and as such are widely consumed in foods but do raise serious questions of safety. Estragole is effectively removed from TARRALIN™ during processing.

Researchers from USA and Germany evaluated the safety of TARRALI™, to be used as an herbal preparation to help normalize elevated blood glucose concentrations. Since safety information of *A. dracunculus* and its extract is limited to historical use, TARRALIN™ was examined in a series of toxicological studies. Complete Ames analysis did not reveal any mutagenic activity either with or without metabolic activation. TARRALIN™ was tested in an acute limit test at 5000 mg/kg with no signs of toxicity noted. In a 14 day repeated dose oral toxicity study, rats appeared to well tolerate 1000 mg/kg/day. Subsequently, TARRALIN™ was tested in an oral subchronic 90-day toxicity study (rat) at doses of 10, 100 and 1000 mg/kg/day. No noteworthy signs of toxicity were noted in feeding or body weight, functional observational battery or motor activity. Gross necropsy and clinical chemistry did not reveal any effects on organ mass or blood chemistry and microscopic examinations found no lesions associated with treatment. Therefore, TARRALIN™ appears to be safe and non-toxic in these studies and a no-observed adverse effect level in rats is established at 1000 mg/kg/day. This data suggests that adverse human health effects at lower levels of daily exposure would not be expected.

Anti-HIV-1 agent from Hops

Natural products provide a large reservoir for screening of anti-HIV-1 agents with novel structure and anti-viral mechanism because of their structural diversity. Xanthohumol is a constituent of beer, the major dietary source of prenylated flavonoids and a natural product with multi-biofunctions purified from Hops, *Humulus lupulus* Linn. Scientists of China tested its anti-HIV-1 activity. They observed that xanthohumol inhibited HIV-1 induced cytopathic effects, the production of viral p24 antigen and reverse transcriptase in C8166 lymphocytes at non-cytotoxic concentration. The EC50 values were 0.82, 1.28 and 0.50 μg/ml, respectively. The therapeutic index (TI) was about 10.8. Xanthohumol also inhibited HIV-1 replication in PBMC with EC50 value of 20.74 μg/ml. The activity of recombinant HIV-1 reverse transcriptase and the HIV-1 entry were not inhibited by xanthohumol. The results from this study suggested that xanthohumol is effective against HIV-1 and might serve as an interesting lead compound. It may represent a novel chemotherapeutic agent for HIV-1 infection. However, the mechanism of its anti-HIV-1 effect needs to be further clarified.


Anti-hyperglycaemic activity of *Origanum vulgare*

The effect of an aqueous extract of *Origanum vulgare* Linn. (OV) leaves on blood glucose levels in normal and streptozotocin (STZ) diabetic rats were investigated by scientists at Morocco. In normal rats, the blood glucose levels were slightly decreased 6 hours after a single oral administration (*P* <0.05) as well as 15 days after once daily repeated oral administration of aqueous OV extract (*P* <0.05) (20 mg/kg). After a single dose or 15 daily doses, oral administration of the aqueous extract (20 mg/kg) produced a significant decrease on blood glucose levels in STZ diabetic rats (*P* <0.001). In STZ rats, the blood glucose levels were normalised from the fourth day after daily repeated oral administration of aqueous OV extract (20 mg/kg) (*P* <0.001). However, this effect was less pronounced 2 weeks after daily repeated oral administration of OV extract. In addition, no changes were observed in basal plasma insulin concentrations after treatment in either normal or STZ diabetic rats indicating that the aqueous OV extract acted without changing insulin secretion. Thus, an aqueous extract of OV could be used as an anti-hyperglycaemic agent in STZ rats without affecting basal plasma insulin concentrations [Lemhadri A, Zeggwagh NA, Maghrani M, Jouad H and Eddouks M, Anti-hyperglycaemic activity of the aqueous extract of *Origanum vulgare* growing wild in Tafilalet region, *J Ethnopharmacol*, 2004, 92(2-3), 251-256].

Fenugreek seeds for gastric ulcer

The seeds of fenugreek, *Trigonella foenum-graecum* Linn. (Hindi — *Methi*) are commonly used to treat a number of gastrointestinal disorders, diabetes and hypercholesterolaemia. Scientists at Department of Biochemistry, Annamalai University and Department of Pathology, Rajah Muthiah Medical College, Annamalai University, Tamil Nadu evaluated gastric antiulcer potential of this ancient drug. The protective effect of fenugreek seeds was studied against ethanol-induced gastric damage in rats. Omeprazole, which is a commonly prescribed drug for increased gastric acid secretion and gastric ulcer was used as a reference drug for comparison.

The seeds were cleaned of extraneous matter, dried and ground in to a fine powder. The powder was mixed with distilled water (1g of seed powder per 100 ml of water). After thorough mixing in a vortex cyclomixer the extract was centrifuged at 3000 rpm for 10 minutes. The supernatant was used as the aqueous extract for feeding the animals.

The aqueous extract and a gel fraction isolated from the seeds showed significant ulcer protective effects. The cytoprotective effect of the seeds seemed to be not only due to the anti-secretory action but also to the effects on mucosal glycoproteins. The fenugreek seeds also prevented the rise in lipid peroxidation induced by ethanol presumably by enhancing antioxidant potential of the gastric mucosa thereby lowering mucosal injury. Histological studies revealed that the soluble gel fraction derived from the seeds was more effective than Omeprazole in preventing lesion formation [Pandian R Suja, Anuradha CV and Viswanathan P, Gastroprotective effect of fenugreek seeds (*Trigonella foenum-graecum*) on experimental gastric ulcer in rats, *J Ethnopharmacol*, 2002, 81(3), 393-397].
Tamarind seed coat extract inhibits nitric oxide production

Plant materials have long been used as traditional medicines for the treatment of a wide variety of ailments and diseases. Tamarind, *Tamarindus indica* Linn. (Hindi — *Imli*), a tree indigenous to India and South East Asia, has been used as a spice, food component and snack.

The seed coat extract of tamarind, a polyphenolic flavonoid, has shown to have antioxidant properties. Researchers of Thailand and UK investigated the inhibitory effect of the seed coat extract on nitric oxide (NO) production *in vitro* using a murine macrophage-like cell line, RAW 264.7, and *in vitro* and *in vivo* using freshly isolated B6C3F1 mouse peritoneal macrophages. *In vitro* exposure of RAW 264.7 cells or peritoneal macrophages to 0.2–200 µg/mL of the extract significantly attenuated (as much as 68%) nitric oxide production induced by lipopolysaccharide (LPS) and interferon gamma (IFN-γ) in a concentration-dependent manner. *In vivo* administration of the extract (100-500 mg/kg) to B6C3F1 mice dose-dependently suppressed TPA, LPS and/or IFN-γ induced production of nitric oxide in isolated mouse peritoneal macrophages in the absence of any effect on body weight. Exposure to Tamarind extract had no effect on cell viability as assessed by the MTT assay. In B6C3F1 mice, preliminary safety studies demonstrated a decrease in body weight at only the highest dose tested (1000 mg/kg) without alterations in hematology, serum chemistry or selected organ weights or effects on NK cell activity. A significant decrease in body weight was observed in BALB/c mice exposed to concentrations of extract of 250 mg/kg or higher. Oral exposure of BALB/c mice to Tamarind extract did not modulate the development of T cell-mediated sensitization to DNF or HCA as measured by the local lymph node assay, or dermal irritation to nonanoic acid or DNFB. These studies suggest that in mice, this extract at concentrations up to 500 mg/kg may modulate nitric oxide production in the absence of overt acute toxicity. Excess NO production has been associated with many diseases such as autoimmunity, rheumatoid arthritis, inflammatory bowel disease and septic shock. The results of these acute and *in vitro* studies which demonstrated suppression of NO production at concentrations of the crude seed coat extract of Tamarind while producing no measured adverse effects suggests that further chemical analysis, identification and testing of the active components of the extract is warranted.

Antimicrobial activity of *Datura innoxia* and *Datura stramonium*

The antibacterial activity of the methanol extracts of the aerial parts of the *Datura innoxia* Mill. and *D. stramonium* Linn. has been investigated by researchers of Iran. The extracts showed activity against Gram (+) bacteria in a dose dependent manner. *D. innoxia* extract showed antibacterial activity mostly against *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus*. The activity was dose dependent and was highest at 2.5 mg/ml. *D. stramonium* had slight antibacterial activity against the Gram (+) at 2.5 mg/ml and lower concentrations were not effective. When the results were compared with the antibacterial activity of Ampicillin, the plant extracts showed equal or better antibacterial activity. Little or no antibacterial activity was found against *Escherichia coli* and *Pseudomonas aeruginosa* [Eftekhar Fereshteh, Yousefzadi Morteza and Tafakori V, Antimicrobial activity of *Datura innoxia* and *Datura stramonium*, *Fitoterapia*, 2005, 76 (1), 118-120].
Antiinflammatory and antiulcer activities of Bambusa arundinacea leaves

The extract of dried and powdered leaves of Bambusa arundinacea (Hindi — Bans) have been studied by the scientists at Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai and Department of Microbiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai to evaluate its antiinflammatory and antiulcer activities.

The antiinflammatory effect of the methanol extract of the leaves against carrageenin-induced as well as immunologically induced paw oedema and also its antilulcer activity in albino rats have been studied and found to be significant when compared to the standard drugs. The combination of methanol extract and Phenylbutazone (Non-Steroidal Antiinflammatory Agent, NSAIA) has been found to be the most potent antiinflammatory agent experimentally with least toxic (no ulcerogenic) activity. Thus, the combination of herbal product with modern medicine (NSAIAs) will produce the best antiinflammatory drug and will be useful for long-term treatment of chronic inflammatory conditions like rheumatoid arthritis with peptic ulcer. Only methanol extract was found to be active and significant. The chloroform and petroleum ether extracts were inactive [Muniappan M and Sundararaj T, Antiinflammatory and antiulcer activities of Bambusa arundinacea, J Ethnopharmacol, 2003, 88(2-3), 161-167].

Wound healing potential of Common Purslane

Common Purslane, Portulaca oleracea Linn. (Hindi — Kulfa) is a cosmopolitan plant distributed in Africa, China, India, Australia, Middle East, Europe and United States. It is reported to possess many medicinal properties. In folk medicine a poultice made from the leaves is applied to draw the pus out of infected sores, useful for burns and skin diseases. The scientists working at Faculty of Pharmacy, University of Jordan, and Faculty of Science, Amman, Jordan investigated the claimed medicinal use of this plant as a wound healing promoter that had been cited in folkloric literature. The homogenous mixture of the fresh plant was applied to the excision wound created on the shaven dorsal back of the male albino Swiss mice (Mus musculus JVI-1) weighing 23–26g and the healing effect was observed at 3-day intervals throughout the 15 days of the experiment, which was compared with that of untreated mice. The results indicated that Common Purslane accelerates the wound healing process by decreasing the surface area of the wound and increasing the tensile strength. The greatest contraction was obtained at a single dose of 50mg and the second greatest by two doses of 25mg. Measurements of tensile strength and healed area were in agreement [Rashed AN, Afifi FU and Disi AM, Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. (growing in Jordan) in Mus musculus JVI-1, J Ethnopharmacol, 2003, 88(2-3), 131-136].

Anticaries effect edible mushroom

The Scientists at Department of Microbiology, Nihon University School of Dentistry, Chiba, Japan evaluated caries-inhibiting effect of the extract of shiitake (Lentinus edodes), the most popular edible mushroom in Japan, both in vitro and in vivo. Shiitake extract showed an inhibitory effect on water-insoluble glucan formation from sucrose by crude glucosyltransferases of Streptococcus mutans JC-2 and Streptococcus sobrinus OMZ-176. The firmly adherent plaque in the artificial plaque formation test was strongly inhibited by shiitake extract. The reduction of firmly adherent plaque caused an increase in the incidence of non- and loosely adherent plaque and a decrease in total plaque formation. A significantly lower caries score was observed in specific pathogen-free rats infected with S. mutans JC-2 and fed with a cariogenic diet containing 0.25% shiitake extract as compared with controls fed the cariogenic diet without shiitake extract [Shouji N, Takada K, Fukushima K and Hirasawa M, Anticaries effect of a component from shiitake (an edible mushroom), Caries Res, 2000, 34(1), 94-98].