Abstracts of some researchers papers of Seabuckthorn

Below are abstracts of a sample of papers which UKSA thought worth bringing to attention. However in selecting these, we claim no specific expertise; we have merely looked for papers that have a degree of verifiability.
Hopefully all searches for paper, institute and lead researchers are accurate but as many have been translated, we cannot guarantee it. Should any papers be of interest please contact the originators directly as UKSA has limited resources.

Since hundreds of millions of dollars are now pouring into research we expect to add to this list on regular basis. We would greatly appreciate any help in bringing to attention research we have not yet presented.

Abstract titles for search.

1 Adaptogenic evaluation of Seabuckthorn (Ilippophae rhainnoides) fruit extracts on rats using C-H-R animal model

2 In vitro and in vivo seed germination of Leh Berry (Ilippophae rhanmoides); seedling establishment and their utilities

3 Seabuckthorn (Hippophae rhamnoides L.) - Frankia : A novel association below the ground

4 As a Potential Radio protector
Madhu bala, Radiation Biology Division, Institute of Nuclear Medicines and Allied Sciences Brig. SK Mazumdar Marg, Delhi 110054

5 Seahuckthorti pulp oil ameliora Its isoproterenol induced myocardial damage in rats

6 Identification and characterization of Immunomodulatory potential of flavones of Seabuckthorn fruitberry.

7 Phytochemical studies on Hippaphae silicifolia from Sikkim Himalayas Chinchu

8 Supercritical CO2 extraction of fruit oil

9 Proteome analysis of Seabnekthorn, the Himalayan gold bush to fish executors of cold tolerance for crop improvement

10 Enhancing Vaccine Efficacy with Immmunostimulatory herbal Adjuvant

11 Supercritical carbon dioxide (SC CO2) extraction of leaves of Seabuckthorn (Hippophae rhamnoides)

12 Quality Indicators In Seabuckthorn

13 Comparative studies towards extraction, quantitative determination and antioxidant activity of bioactive phenolics

14 Anti-atherogenic potential of Hippophae

15 Dermal wound healing efficacy of Hippophae rhamnoides L.

16 Development of SSR markers for assessing genetic diversity in Seabuckthorn

17 Prevention of Gamma radiation induced Conditioned Taste Aversion (('TA) in Sprague-Dawley rats by Hippophae leaves

18 Cytoprotective effect of Seabucthorn on hypoxic stress in ghat cells: Mod illation of gain ill a glutamylcysteine synthetase

19 Application of Seabuckthorn products as traditional medicine in Trans-Himalayas of Nepal

20 Protective role of Scabuckthorn pomace against cadmium induced oxidative stress in hepatic and renal tissues of the poultry

21 Anti-microbial activity of flavonoid rich fraction of Sea buckthorn leaves

22 Adaptogenie evaluation of Seabuckthorn (Ilippophae rhanmohles) fruit extracts on rats using C-H-R animal model

23 Antioxidant effects of Seabuckthorn leaf based herbal formulation

24 Development and evaluation of Seabuckthorn leaves based herbal food formulation and baked foods

25 Immunomodulatory and hemolytic activities of Seabuckthorn glycoside

26 Antioxidant activity of phenolic rich fraction of Seahnckthorn (Ilippophae rhanmoides) leaves in vitro and in vivo.

27 Ultrasonic cavitation technology for Seabuckthorn processing- A Review

28 Genetic variability in economic traits of Seabuckthorn (Hippaphae D.Don)

29 Isolation of sex-linked genes in gender specific DNA marker in Hippophae rhanntoides

30 Cytoprotective and antioxidant activities of Supercritical CO2 extract of Seabuckthorn (Hippophae rhamnoides) leaves.

31 Optimization of protein extraction procedures from Hippophae rhontnoides for dissecting its cold tolerance

32 Antioxidant activity of tiavonoid rich fraction of Seabuckthorn (Hippuphae rhatnnoides) leaves; Quantitative analysis of its major components by RP-HPLC.

33 Development of green extraction process for Seabucktorn bioactives

34 Transcriptome analysis and development of microsatellite markers in seabuckthorn

35 Study on the morphological variations in seabuckthorn (Hippophae rhainnoides ssp. turkestanica) populations growing in Lahaul-Spiti, dry temperate Himalayas

36 Influence of origin, harvesting time, and growth location on contents of inositols and methylinositols in sea buckthorn berries

37 Trace elements accumulation in sea-buckthorn fruits

38 Trace elements accumulation in sea-bucktliorn root tubercles

39 Trace elements accumulation in sea-buckthorn leaves

40 Detection of 5-hydroxytryptamine hydrochloride of Hippophae rhumnoides

41 Cleavage of f3-carotene to flavor compounds by the microorganism from seabuckthorn juice

42 Determination of glucose, fructose and sucrose in seabuckthorn fruit honey by HPLC-ELSD

43 Selective and sensitive determination of fatty acids and amino acids in Hippophae rhamnoides L. fruit using pre-column derivatization HPLC method

44 Seabuckthorn for protection against high altitude stress

45 Health effects of sea buckthorn berries:

46 Isolation and synthesis bioaetive flavonols from Indian seabukthorn.

47 Cosmetic use of Hippophae rhanmoides winter twigs extracts to lighten skin pigmentation.

48 Evaluation of effect of Seabuckthorn extract on cognitive impairment

49 Possible mechanism of sea buckthorn fruit extract as a functional food in restraint-induced behavioral deficits and brain serotonin metabolism: Focus on 5-HT-IA receptors in depression

50 Effect of feeding seabuckthorn leaves for milk production in cross bred animals

51 Experimental study of proanthocyanidins extract from seabuckthorn seed on the effect of immune regulation in mice

52 Prophylactic efficacy of seabuckthorn oil and omcproazole in gastric erosions and ulcerations in dogs

53 Effects of seabuckthorn polysaccharide on blood glucose of Normal Mice and Diabetic Mice

54 Immunomodulation by dietary seabuckthorn

55 Experimental study on effect of Tiangui Gengnian Soft Capsule on the mitochondrial functiong inflluence in aged female rats

56 Investigation of antibacterial properties of seabuckthorn (1-lippophae rhanmoides L.) leaf extracts against common skin and wound microbial pathogens

57 Studies on effects of scabuckthorn (Hipphophae L.) leaf extract and seed oil on infected cutaneous wound healing process in rabbit experimental model

58 Enhanced cAMP/PKA pathway by seabuckthorn fatty acids in aged rats

59 Modulation of Hypoxia-Induced Pulmonary Vascular Leakage in Rats by Seabuckthorn (Hippophae rhamnoides L.)

Abstracts

1 Adaptogenic evaluation of Seabuckthorn (Ilippophae rhainnoides) fruit extracts on rats using C-H-R animal model

Priyanka Sharma Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, 11 0 054.

NCSBT 2010

Seabuckthom (Hippophae rhainnoides) is a well-known high-altitude shrub (2500-4000 m), native to Europe an.d Asia. Seabuekthom has many nutritional and medicinal benefits and all the parts of the plant are a good source of bioactive substances. Seabuckthom fruits are rich source of vitamin C and E, folic acid, carotenoids and various saturated and unsaturated fatty acids. The beneficial effects of seab.uckthorn are well reported against cardiovascular diseases, mucosal injuries, and skin disorders. The present study was conducted to evaluate adaptogenic potential of various extracts of seabuckthorn fruit using Cold-Hypoxia-Restraint (C-H-R) animal model. C-H-R is a unique model in which the animal can be exposed to three different stresses-cold (5°C), Hypoxia (428 mm Hg) and restraint conditions. Thus adaptogenicity of any compound can be well established using this modef. Both dry

and fresh fruit-pulp was collected and vacuum-dried aqueous and alcoholic extracts were prepared. The different extracts of fruits were evaluated for their dose dependent adaptogenic activity on rats using C-H-R animal model. Various doses of the extract were administered in rats orally 30 minutes prior to C-H-R exposure. Out of these extracts the potent adaptogenic activity were found in seabuckthorn fresh fruit serial alcoholic extract (100 mg/Kg body wt.) and seabuckthorn dry .fruit aqueous extract (75mg/ Kg body wt.) .The HPLC analysis of the -fruit extract showed presence of ascorbic acid and rutin which are potent antioxidants. I lence these antioxidmits may he reTowilIlc For Ilie iidaptogenic activity rotind vx(i.;10.

2 In vitro and in vivo seed germination of Leh Berry (Ilippophae rhanmoides); seedling establishment and their utilities

Sudipta Shekhar Das Bhowmik. & Lingraj Sahoo Department of Biotechnology, Indian Institute of Technology, Guwahati, Assam-781039

Hippophaerhainnoides'L. commonly known as Seabuekthorn or Leh Berry is a unique plant of cold desert of Ladakh region having enormous neutraceutical, pharmaceutical and environmental usage. The plant is also a store house of many important cold tolerant genes. As the plant grows in far-flung areas of Ladakh any biochemical, molecular biological and genetic engineering research on this plant demands easy access of the living plant. This is possible only when the plant can be easily grown under both in vitro and in vivo conditions at distant places. Seed germination and seedling establishment of this plant is the primary requisite in this direction. Seeds of Hippophae rhamnoides L. collected from Leh were germinated aseptically.under in vitro condition in various medium supplemented With MS (Murashige and Skoog) and without MS nutrients. Around 80 — 90 % seed germination was recorded in both nutrient rich and non-nutrient medium under controlled in vitro condition within 5-7 days. However seedling growth and development was better in MS medium as compared to non-nutrient medium. Seed germination was also tried under in vivo conditions at various ratios of soilrite, vermicompost, sand and soil composition. A maximum of 64v/ci:sted germination was recorded within a week under in vivo condition containing only soitrite at 16 C and 60 — 70 % R.H and 40.5p.moles rn s' light intensity. Healthy seedlings of around 4 5 cm were raised within a month after proper irrigation with water every alternate day. These seedlings would be nurtured and raised to maturity under controlled green house condition at Indian Institute of Technology, Guwahati.

3 Seabuckthorn (Hippophae rhamnoides L.) - Frankia : A novel association below the ground

Janardan Yadav Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Chemistry, Banaras Hindu University, Varanasi-221005.

Seabuckthom (Hippophae rhamnoicies L.) of the family Elacagnaceae is a hardy, angiospermic, deciduous, dioecious, drought-and cold-tolerant shrub whose berries pulp contains essential minerals, antioxidants, vitamins A, B-complex and C, and seed

oil is rich in vitamin E. Below the ground, though its roots bear nodules and fix atmospheric N2 symbiotically by Frankia, an-endophytic actinornycefe. The actinorhizal plants are classified into 8 families and 25 genera comprising more than 220 species. About 80of the total N, accumulated in the plants, estimated to about 200-350 kgha-lyr-', can be fixed by Franicia-actinorhizal root nodules. Frankia is a sporulating, gram +ve, filamentous bacteria of the Actinomycetales (family-Frankiaceae). Isolation of pure culture from root nodule can be obtained by isolating single — spore — derived colonies on BPA medium with some modifications in carbon sources. Four main subdivisions (i) a large group mainly including Frankia aim" and other typical nitrogen-fixing strains belonging to the Alnus and the Casuarina host infection groups, respectively, (ii) uncultured endophytes of Dryas, Coriaria, and Datisca species, (iii) strains of the Eleagnus host infection group, and (iv) atypical nonnitrogen-fixing strains have been established using a comparative sequence analySis of PCR-amplified 16S ribosomal DNA. Phenotypic properties like susceptibility to antibiotics, production of pigments and isoenzymez are used to differentiate between Frankia strains. Fankia may be considered as plant growth promoting rhizobacteria (PGPR) because it possesses not only endophytic symbiotic N, fixation but also the other mechanisms of plant growth stimulation by secretion of Indole acetic acid like hormones methanol, Indole-3-ethanol and Indole-3-lactic acd), nutrient acquisition, particularly Fe, by production of Fe-chelating siderophores, and prevention of soil born phytopathogans like oomycete Phytophthora sp. and the fungal Botrytis cinema, Fusarium culinorum, Rhizoctonia solani and Heterobasidion alMOS11112 Frankia has synergistic interaction with Gigaspom margarita (an arbuscular mycorrhizal fungus) and Pseudomonas put/c/a and P. aerugenop.
A novel antibiotic, demethyl (C-11) cezomycin, produced by the Frankia is of major attraction because it belongs to calcimycin class of antibiotics and structurally close to cezomycin..
It is strongly active against Gram+ve pathogenic Clavihacter inichiganensis subsp. Sepecionicus bacteria and several other plants pathogenic fungal strains similar to the antimicrobial activities of calcimycin metabolites isolated from Streptomvces and Dactvlo,sporangium strains. Its novel effectiveness against growth of Streptococcus pyog&ws, Staphylococcus aureus and methicillin- resistant gaureus strains have shown that it is not affected by the resistance mechanisms of the antibiotic — resistant bacterial strains. Also, this enzyme may be a better alternative as a Ca' channel antagonist to revolutionize new drug therapies for cardiovascular diseases without any side effect.
- NCSBT 2010

In conclusion Seabuckthorn .Frankia association has great importance for rejuvenation of eroded, less fertile, undulated and unutilized barren lands by seabuckthom plantations under harassed conditions. Further researches are needed on Frankia for its utilization as PGPR as well as for the development of novel antibiotics in benefit of humankind.

4 As a Potential Radio protector

Madhu bala, Radiation Biology Division, Institute of Nuclear Medicines and Allied Sciences Brig. SK Mazumdar Marg, Delhi 110054

The whole body exposure to ionizing radiation 'results in multi-organ dysfunction syndrome(MODS). Development of a prophylactic drug (radioprotector) to prevent MODS caused by lethal doses of irradiation, has remained a challenge to the scientific community till date. The molecular or synthetic drugs (comprising of single molecule or a small group pf molecules) have not yielded desirable results. Such drugs have either lacked the required efficacy or have exhibited unacceptable level of toxicity to one or more vital body systems at the concentrations very close to the effective concentration. Therefore, the worldwide efforts are still in progress to develop safe, non-toxic yet effective radi.oprotector for human use.
Hippophae rhainnoide,.s. L. (common name Sea buckthorn) is a natural bio-factory of a large number of protective molecules having medicinal properties viz. polyphenols and flavonoids, vitamin E, C, and K, metallothioneins, superoxide dismutase (SOD) and phytosterols. A preparation from Hippophae leaves, developed in our laboratoz, offered protection to > 90% population of whole body irradiated ("Co-gamma-ray, 10 Ely) mice, in comparison to the 100% lethality in untreated, irradiated (10 Ely) control population. A single dose of our drug, when administered intra-peritoneally, could rend4,-T 94% radioprotection. Our radioprotective formulation, developed from Hippophae leaves, offered radioprotection to Eli tract and haemopoietie system; activated proliferation *of haemopoietic stem cells; scavenged superoxide radicals and hydroxyl radicals. It countelied the radiation induced inflammation, haemolysis of RBCs and was non-mutagenic and non; recombinogenic. These studies suggested that our drug promoted radioprotection by more than one intracellular and systemic mechanism. This study has implications in development of an herbal rad ioprotective drug from Hippophae leaves.

5 Seahuckthorti pulp oil ameliora Its isoproterenol induced myocardial damage in rats

Saurabh Blurt?, Sameer Goya, Sachin Aroran, Ruma Rayb. Dharamvir Singh Aryan Dcpartrncnlp of Pharmacology' and Pathology': All India Institt c of Medical Sciences, New Delhi-29.
Present study was aimed to ,aluate the cardioproteetive potential of seabuckthom (SBT) pulp oil on hemodynamie, c -diac injury markers. enzymatic and non enzymatic antioxidants, lipid peroxidation, 1 .stopathological and ultrastructural changes in isoproterenol (ISO) induced cardiotox ity in rats,, Subcutaneous injection of ISO (85 mg/ kg) administered for 2 days at an interval of 24h was used for induction of cardiotoxicity. ISO administration showed cardiac dysfunction discernible by decrease in arterial pressure indices, maximal positive and negative rate of developed left ventricular pressure (-±LVdP/ dt , a marker of myocardial contraction and relaxation respectively) and an increase in left max ventricular end-diastolic pressure (LVEDR a marker of pre-load). Additionally, significant enhanced lipid peroxidation and depletion of cardiac injury marker enzymes, superoxide dismutase, catalase and glutathione level were observed in myoeytes of ISO treated rats. Oral pretreatment with SBT pulp oil (5, 10 and 20 ml/kg/day) favorably modulated the studied parameters in dose dependent manner. However, the effect was more pronounced at 20 ml/kg/day than that of other two doses. Histopathology and ultrastructural stuaies of myocardium further validated the protective effect of SBT pulp oil in ISO treated rats. Thus. the present study revealed that SBT pulp oil mitigates myocardial damage in ISO-induced cardiac injury by maintaining

hemodynamic, biochemical, histopathological and ultrastructural perturbations owing to its free radical scavenging and antioxidant activities. Diet containing SBT pulp oil may be beneficial for the patients who are at a higher risk of developing myocardial injury.

6 Identification and characterization of Immunomodulatory potential of flavones of Seabuckthorn fruitberry.

KP Mishra, Sudipta Chanda, RC Sawhney and Lilly Ganjti Defence Institute of Physiology and Allied Sciences. Lucknow Road, Timarpur, Delhi -110054
Consumption of food rich in flavonoids is associated ith a lower incidence of certain degenerative diseases, including cardiovascular disease. Flavones of Seabuckthom (SBT) (Hippophae rhomnoides L.) fruits can modulate the production and level of several signaling molecules associated with immune function and inflammation in vitro, including several eytokines. We have evaluated the immundpodulatory activity of ethanolic solution of SBT flavone in human peripheral blood mononuclear cells (PBMCs). The SBT flavone was found to stimulate production of interleukin-6 (IL-6) and tumor necrosis factor-a (TNF-a) in PBMCs. However, increased expressions of p-IK. NE-KB. and p-p38 were found in fhtvone-treated human PBMCs with significantly suppressed expression of CD25 (IL-2R). There was no alteration found in the nitric oxide (NO) production in mouse macrophage cell line RAW 264.7. These observations suggest that stimulation of IL-6 and TNF-alpha secretion may contribute to the putative beneficial effects of dietary tlavone against microbial infection.

7 Phytochemical studies on Hippaphae silicifolia from Sikkim Himalayas Chinchu

Bose, N Pandttrangan, Asoke Banerji, Sushen Pracilian*, Rat-. Kumari Basnett*. B C Basistha* School of Biotechnology, Amrita Visliwa Vidyapcethain. Ku IIi1 'State Council of Science &Technology for Sikkini.Gangiolr
In addition to nutraceuticals, seabuckthorn (SBT) is also a source of high-value products of clinical importance. There is increasing interest in India on the multi Carious use of this under utilized plant. Hippophoe rhomnoides (111?) is the most dominant species of SBT in India. Logistically, collection of FIR presents many problems such as its occurrence in difficulty accessible places, presenie of thorns etc. Bioprospection for SBT shows that another species of sal-, namely H. so/kilt:ilia (HS) occurs in substantial quantities in Himachal Pradesh, Uttanchal and Sikkim in more accessible locations and also has less thorns. Compared to HR. much less is known on the agrotechnology. nutraceuticals and bioactive principles of HS. In order to expand the biosource base for SBT, studies on the nutraceutical and bioactive principles from HS has been undertaken. A comparative study of HR and HS will lead to a more realistic commercial potential of these species. Compared to HR, 'US leaves contain more 1%-carotene. The sterols (sitosterol) and triterpenoids (ursolic acid) contents of both the plants were more or less similar. The polar fraction of HS contained free gallic acid and glycosides. Hydrolysis of the extract gave quercetin, gallic acid and trace quantity of isorhamnetin and glucose. This is in sharp contrast with HR where isorhamnetin is the major iiavonoid with less amount of quercetin and kaempfcrol. A detailed analysis of seed oil of HS (yield 7.0%) by N MIZ, UV and IR was carried

out. The results will be discussed. This presentation deals with the study on leaves of HS from Lachen, Sikkim.

8 Supercritical CO2 extraction of fruit oil

Prabel K Gosh, Ramachandran Gobal, Alphonsus Utioh
Food Development Centre, Manitoba R1N 3J9

Seabuckthorn (Hippophae rhatnnoides L.) fruit (puree) contains 10-20% Oth Fruit oil is rich in palrnitoleic acid (omega-7), palmitic acid, linoleic acid (omega-6), carotene compounds and beta-sitosterol. Seabuckthom fruit (cit. Indian SIMIttla variety) was used in the extraction of the oil Seatmekthorn fruit puree was prepared by mechanically removing the seeds and skin using a pulper finisher. The resulting puree was freeze-dried to produce. the dry matter which was used for oil extraction. Supercritical carbon dioxide extraction (SC-0O2) was used to extract oil from the dried Seabuckthorn fruit puree. SC-0O2 extraction offers a low temperature, environmentally-friendly and high yielding process versus conventional solvent and cold press extraction methods. Extractions were carried out for three hours at four different pressure levels: 200, 250, 300 and 350 bars with constant temperature and flow rate of CO, at 40°C and 100gitnin, respectively. Oils obtained at different pressures were analyzed for fatty acid profile and other important hioactives such as sterols and tocopherols, as well as carotenoids. Up to 37% of palmitoleic acid, 34% pahnitic acid, 13% linoleic acid, 545 mg/100g beta-sitosterol, 253 mg/100g alpha-tocopherol, and 103 ppm total carotenoids were achieved. Defatted cakes obtained at different pressures were analyzed for moisture content, carbohydrate, protein and residual oil; and they showed good potential for applications in the food industry.

9 Proteome analysis of Seabnekthorn, the Himalayan gold bush to fish executors of cold tolerance for crop improvement

Ravi Gupta and Renu Deswal Molecular Plain Physiology and Proteornics Laboratory, Department of Botany. University of Delhi, Delhi.

In spite of extensive gene expression analysis in abiotic stress, such studies have limited scope in functional genomics due to presence of many genes with unknown functions ataalso due to absence of a I: 1 relationship between gene and protein expression. Proteins are the real executors as well as the final reflectors of aerie expression. Therefore, it is important to know the protein complement or proteome. Moreover, Seabuckthorn genome is not sequenced or annotated yet therefore, it is prudent to analyze its proteorne. Unfortunately, proteomics studies are hardly reported for Seabuckthorn. As it is cold tolerant a comparative proteome analysis could provide beneficial targets Coy crop improvement. Our group is trying to decipher the targets and mechanism of cold stress signaling in H. rhanmoides. Protcome of cold/freeze treated, lab grown seedlings was analyzed by ID and 2D gel electrophoresis. Differentially expressed proteins were resolved on 2D-gels and were

identified by MALDI-TOF and LC-MS after tryptic digestion. During freezing conditions, proteins called anti-freeze proteins (APPs) are secreted in the apoplast to prevent freeze induced damage. Therefore, apo-plastome was analysed to detect freeze induced differential expression. Ice Adsorption Chromatograph (IAC) was used to purify these proteins. Antifreeze activity, as measured by splat assay and phase contrast microscopy showed significant antifreeze activity in cold stress treated apoplast. Identification of cold/freeze induced differentially expressed apoplastome by Mass spectrometry showed three putative AFP's. Interestingly, 40% of the up-regulated proteins were associated with signalling and stress. IAC Purification of apolastic extract showed 3 polypetides of 52, 48, and 41 kDa, while berry extracts five polypeptides of 43,41,38,30 and 8 kDa. Primers were designed for all putative AFP's. PCR amplification of C repeat binding factor (CBF), a cold induced transcription factor yielded an amplicon of 655 bp. Over expression of this gene in E Coli is underway. Freeze induced AFP's activity was detected. Some putative AFP's are purified and a gene is cloned suggesting involvement of these in cold tolerance mechanism in Seabuckthorn.

10 Enhancing Vaccine Efficacy with Immmunostimulatory herbal Adjuvant

Lilly Ganju, Monika Jain, Bindhya Jayshankar, Sudipta. Chanda, Divya Singh, KP Mishra, MS Yogendra Kumar and Kshipra Misra Defence Institute of Physiology and Allied Sciences. Lucknow Road, Timarpur, Delhi -110054

)1' Adjuvant is the substance which when co-administered with an antigen enhances the immUne response. A herbal adjuvant DIP-HIP developed by DIMS is - derived from a medicinal plant which is safe, effective and comparable with commercially available adjuvants. Animals administered with DIP4H1P in formulation with different types of antigens - recombinant, conjugated or native proteins like TT, DT, OVA, l-ISP-DME, BSA etc., significantly enhanced the antigen specific antibody levels with minimum amount of antigen and single booster and the antibody sustenance is about four months. A relative contribution of Thl/Th2 type of immune response is indicated by higher titers of IgGI and Ig G2a antibody subtypes. The cytokine profile correlated well with the Th 1 /Th2 types, supported by higher DTH response, indicating thereby the overall magnitude of Immoral and CMI response generated by DIP-HIP and its ability to evoke both the arms of immunity. Interestingly, using different. strains and species of animals, D1P-HIP responded well. Immunization of animals through different routes like i.m or i.p did not show any variation nor caused any muscular damage or granulomatous reaction. The shelf life of DIP-HIP in the extract form is for four years and as antigen formulation for three months at 4"C. There is no haemolysis caused on treatment of both humans and animal erythrocytes with W-HIP. The extract is in crude form and is being fractionated into various components using Supercritical CO2extraction procedure. The bioactive fractions are being analysed for their adjuvant activity. An Indian patent has been filed for the extract; and CNDA , MTA have been signed with various pharmaceutical companies, Indian as well as 1VINCs. Herbal adjuvant DIP-HIP, Technology No. 086, has qualified amongst 220 technologies selected by DRDO for accelerated global commercialisation, under DRDO-FICCI-ATA.0 program, in collaboration with IC'. University of Texas, Austin.

11 Supercritical carbon dioxide (SC CO2) extraction of leaves of Seabuckthorn (Hippophae rhamnoides)

K Udaya Sankar4, Swapana Sonale R, Yogendra Kumar' and B Manohar - Department of Food Engineering. Central Food Technological Research Insi note (CS1R . Mysore 570020; Defence Institute of Physiology and Allied Sciences. DRDO. Timarpurs Delhi 110054

Leaves of Sea buckthorn were extracted using SCCO2 at a pressure of' 200 bar and 50°C with ethanol as entrainer at 1,3 and 5% of CO2 and resulted in extract yields of 5.1% and 4.3% for 15 hours of extraction. A parametric R.SM study to optimise the extract yields with pressure (180, 280 and 380 bar) and temperature (40,50 and 60°C) and for time periods (5,10 and 15hr) was conducted based on an statistical experimental design using 3% of' ethanol with SCCO, resulted in yields of extract ranging from 1.70 to 6.20%. The RSM contour graphs showed the extract yields increased with time, with temperature and with pressure at other extractions remaining constant. The best yield of 6.4-6.8% of total extract was observed at 280 bar, 60°C and 15 hours of extraction. The extracts were assayed for ABSTS (35.7312.15 - 45.10±2.05mg of Trolox/g of extract) , Total phenolics (16.84-29.12 mg of GAE per grain of extract). DPPH (37.24±2.1)- 52.63+2.10 mg of Trolox per g of extract), FRAP (35.22±2.12 - 45.82±3.10 mg of Trolox per g of extract, the flavanoid content was estimated as Isorhamnetin (0.037±0.0051- 0.068±0.0049 mg per g of extract) by HPLC. The best results of the above assays were observed for extracts obtained at 350 bar and 60°C of SCCO.,. The particle size of the clusters of the extract were observed on a phase contrast microscope showed a more uniform particle size range of less than 10 Am when compared to the ethanol extracted product, providing for the smaller molecular clusters in the extract that may easily lock in with biological receptors more effectively. The extract was subjected to column chromatography and the compounds were fingerprinted for ilavanoids by using LC, LC-MS -ad the extract fractions were subjected to column chromatography and the compounds were fingerprinted for flavanoids using LC, LC-MS and the extract fractions were subjected to NMR

12 Quality Indicators In Seabuckthorn

Comparative studies towards extraction, quantitative determination and antioxidant activity of bioactive phenolics

Thornas Morsel UBF GmbH, CEO An der Muelilc I. 15345 Altiandsberg, Germany

Seabuckthorn is a highly valuable plant. Products from Seabuckthorn have high prices. Especially in eastern countries one of the most important quality parameters is the content on carotenoides. Prices are sometimes directly coupled to content of f3-carotene. In European Community use of some carotcnoides as food colorant is allowed. Thus 13-caroten and capsanthin are allowed for application in foods generally. These colorants arc relatively cheap compared to seabuckthom pulp oil. We

observed that capsanthin has been found in seabuckthom oil as well as cosmetics produced from Seabuckthorn oil. From literature is well known that different carotenoids occur beside (3 -caroten as then main carotenoid. Neither capsanthin has been reported. The aim of this work was to collect data on SET carotcnoids composition. Especially dependence on varieties and occurrence of capsan thin was on focus. Samples were taken from 13 Russian varieties in Barnaul directly from orchard of Lisavenkow institute. 4 German varieties were in addition also investigated. Oil was extracted by method of Blyer and Dyer after smashing of fruits and removing of seeds. Oil was investigated by GC for determination of fatty acid and sterol composition. Carotenoids were analysed by HPLC as well as HPTLC. Fatty acid composition varies between different brands investigated. Also differences in sterols were observed. Never the less the main sterol is P-Sitosterol. Carotenoid composition agrees with findings of Russian scientists, e.g. Novruzew et al. We cannot conclude that capsanthin is a typical component in Scabuckthorn pulp oil. We conclude that capsanthin found in Setibuckthorn pulp Oil or products containing this is an indicator of alteration,

13 Comparative studies towards extraction, quantitative determination and antioxidant activity of bioactive phenolics

Arun K Sinha, Rajesh Kumar, Nandini Sharma, Upendra K Sharma Natural Plant Products Division, Institute of Himalayan Biorcsource Technology (('SIlt). Post Box No. 6, Palampur-176061, H.P.

Nature has provided a fascinating array of chemical structures in the form of bioactive secondary metabolites. Among these, phenolic compounds are one of the most widely occurring groups of phytochemicals present in plants. Owing to the immense importance and several health promoting activities of phenolics, particularly their role as antioxidants, has intensified the research on these molecules. The extraction of phenolics requires special care, because of their susceptibility to oxidation and photo degradation. In this perspective, the emergence of rapid and selective techniques green methodologies such as ultrasound assisted extraction (UAE) and microwave-assisted extraction (MAE) have provided a fresh stimulus. Another area of importance in the utilization of plant nutraceuticals is the regulation of levels of active ingredients in these products for which dependable and validated analytical methods are needed. The antioxidant property of any plant is related to its polyphenol content which generally differs with the source of origin, packaging and storage. For this, reliable data composition based on a rapid test that is able to quantify total antioxidant activity of single compound and/or complex mixtures might serve as a useful tool. Seabuckthorn (Hippophae rhanmoides) is a popular medicinal plant rich in various phenolics including flavonoids and their derivatives. This plant has been recognized as a versatile nutraceutical crop with diverse uses, from controlling soil erosion to being a source of horse fodder, nutritious foods, drugs, and skin-care products. Different parts of this plant are used in traditional medicine for the treatment of diseases, such as flu, cardiovascular diseases, mucosa] injuries, and skin disorders. Keeping in view the importance of phenolics, our efforts towards extraction, isolation and quantitative determination a fb leach ve phenolics from Flippophae rhamnoides will be discussed. In addition, an account of evaluation of antioxidant activity of its different parts will be summoned up during presentation.

14 Anti-atherogenic potential of Hippophae

Basu. K Pal, AS.Malhotra, P Jayamurthy. K Prasad, D Ghost). C Arumughan. RC Sawhney Defence Institute of Physiology and Allied Sciences. Lucknow Road. Titnarpur, Delhi -110054

HYpercholesterolemia is a predominant risk factor for atherosclerosis and associated with coronary and cerebrovascular diseases. Elevated levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) have been established as risk factors for atherosclerosis, which is the primary cause of cardiovascular disease. Control of cholesterol levels through therapeutic drugs has significantly reduced the risk for developing atherosclerosis. However, adverse effects are associated with therapeutic drugs. Herbal remedies have increasingly become attractive alternatives to prevent or treat hypercholesterolemia. Plants growing under extremes of environmental conditions max' develop mechanisms or biomolecules which can provide protection against environmental induced dyslipidemia. In the present investigation, anti-atheronenie potential of Seabuckthorn (SBT) product was studied on rabbits fed on high cholesterol diet. Thc second part of the work was to compare its potential with olive oil as positive control and therapeutic drug atorvastatin in rats. The TC, triglyceride (TG), LDL-C and Atherogenic Index (Al) in all the groups at day 0 were not significantly different from each other. TC and TO levels did not show any significant change in normal rabbits following administration of Sal product for 18 days. The LDL-C, LDL/HDL ratio and Al levels were significantly decreased after SBT administration, whereas the high density lipoprotein cholesterol (I-IDL-C) and FITR levels were found to be significantly higher than the pm-treatment values. Feeding of high cholesterol diet to the rabbits resulted in significant increase in IC, TG, LDL-C, HDL-C, Al and decline in HTR. Administration of SBT after cholesterol feeding restricted further rise of TC and caused a significant decline in TG, LDL-C, LDL/HDL ratio and Al levels. In all the three groups although HDL levels were also increased following, cholesterol administration, the rise in HDL and HTR over the basal values in STIT treated animals was significantly higher than the non-treated animals.

In second phase there was a rise of IC at day 20 as well as day 40 in control rats. All other treated groups showed decline of TC after administration. The decline was most prominent in SET group both at day 20 and day 40. Control group showed a rise of RI both at day 20 and day 40. Rise was also found on day 20 in olive oil group but there was a decline on day 40. Decline of TG was found in statin as well as SET treated group both at day 20 and day 40. Plasma EDI_ showed a decline in control and olive oil groups both at day 20 as well as day 40. 1101--C level was also lower on day 20 in statist group but there was a slight increase on day 40. &illy in SET treated group there was a rise in KM in both the days. In conclusion, the present study suggests that SET product has significant anti-atherogenic activity when administered to normal or hypereholesterolemic animals. The cardioprotective effects of SET may be due to the presence of Omega-3, Omega-6 and Omega-9 fatty acids, tocopherols, phytosterols and p- carotene which in combination may have synergistic effects on cardiovascular health.

15 Dermal wound healing efficacy of Hippophae rhamnoides L.

Asheesh Gupta, Nitin K Upadhyay, Gaurav K Kesitari Defence Institute of Physiology and Allied Science, tucknow Road, Timarpur, Delhi-110054

Hippophae rhamnoides L., commonly known as Seabuckthorn (SBT), growing naturally in cold-arid regions of Asia and Europe is used in preparation of health foods, cosmetics and medicines. The study was undertaken to determine the wound healing efficacy of SBT extract in acute and chronic diabetic and burn woundsin experimental 1 rats and further its mechanisms of action were explored. Patent filed (II dia) application number — 8371DEL12009. Safety and dermal toxicity was investigated to ensure any adverse effects associated with the application of SBT extract. Phytochemical analysis and HPLC fingerprinting revealed that SBT-extract is rich in polyphenols and Ilavonoids and confirmed the presence of quercetin-3-galactoside, quercetin-3-glucosi de, kaempfero I and isorhamnetin. SBT extract was developed in two dosage forms i.e. topical ointment and extract incorporated hydrogel wound dressing. It was observed that the SBT extract possessed anti-bacterial activity against tested wound pathogens (Pseudomonets aeruginosa and Staphylococcus aureus). The SBT extract augmented the healing process as indicated by significant increase in wound contraction, hydroxyprolinc and hexnsamine contents. Healing activity of SBT was found better than Silver sulfadiazine and Povidone-iodine based ointments (standard care).The treatment also up-regulates the expression of growth factors (VEGF, TOF-Ii), extracellular matrix protein (collagen type-III), cellular proteins and matrix metalloproteinases (MMPs-2 & 9); which help in tissue regeneration and remodeling phases of wound repair. These results were further supported by histological examinations. The treatment also caused significant increase in endogenous antioxidants (GSH, SOD, CM' and vitamin C) and reduced production of reactive oxygen species in wound granulation tissue. The acute dermal toxicity studies showed that the SBT extract was safe up to a maximum dose of 2.0 g/kg body weight of the rats. In repeated dose dermal toxicity study, no adverse effects were observed in any of the experimental rats given 1.0 gm/ kg body weight of SBT extract topically up to 28 days. These results suggest that the Sea buckthorn extract possesses significant wound healing activity and have no associated toxicity or side effects.

16 Development of SSR markers for assessing genetic diversity in Seabuckthorn

PC Sharma
University of Biotechnology
GGS Indraprastha University, Dwarka sector 16C, New Delhi

Seabuckthom is upcoming as an important crop due to the presence of some prime medicinal and nutritional compounds, and as an ecosys tem restorer. Genomecharacterization using molecular tools has recently become an important area of Seabuckthorn research. One of the important activities in this field is the development of molecular markers for use in genome mapping, gene tagging and genetic diversity analysis. Unfortunately, this important aspect of research has largely been remained unattended in Seabuckthorn. Therefore, we have undertaken a project to develop polymorphic simple sequence repeat (SSR), also known as microsatellites, markers in Seabuckthorn and to assess their use in detecting genetic diversity in seabuckthorn populations. As a by-product of an EST sequenci ng proj ec t, we detected 56 probable m ic rosatel I ite markers from 1584 Seabuckthorn unigcncs. From our pilot study, we identified nine microsatel lite markers polymorphic in 14

Hippoplwe accessions of' DI HA R, DR DO. The EST-SSR markers also showed cross-amplification in H. salicilblia and H. Tibetan°. This study is being extended to assess genetic diversity in geographically diverse seabuck thorn populations collected from Leh and to find association with some morphometric traits. In another experiment, we have constructed random and inicrosatellite enriched genomic libraries in H. rhamnoides. Our method of library enrichment has proved very promising for yielding high frequency of microsatellite positive clones. These newly developed markers will be subsequently assessed for their use in seabuckthorn genome characterization.

17 Prevention of Gamma radiation induced Conditioned Taste Aversion (('TA) in Sprague-Dawley rats by Hippophae leaves

Vanita Gupta, Jagdish Prasad, Surender Singh, Manish Gupta, Mani' Bala* Institute of Nuclear Medicine and Allied Sciences. Brig. S.K. Majtundar Marg, Delhi 110054

The aim of this study was to investigate the protective effect of radioprotective herbal drug (prepared from leaves of Hipp'ophae rhainnoides). on "Co-y-radiation induced conditioned taste aversion (CTA) in male Spraguc-Dawley rats. CTA is an easily measurable behavioral change that occurs after radiation exposure in experimental rats. The vehicle and drugs were administered intraperitoneally (i.p.) to rats, without or before whole body "Co-y-irradiation (2 Gy). The CTA was assessed in terms of saccharine preference ratio (SPR). Ondansetrone, a standard anti-emetic drug prescribed during radiotherapy, was used as the positive control. The in vivo biochemical alterations were evaluated using standard methods and techniques. Whole body exposure to "Co-y-irradiation (2 Gy) caused loss of body weight (b.w.) and induced significant CTA and the effect was time dependent. One time i.p. administration of our drug, before irradiation, countered the radiation induced CTA as well as loss in body weight. The effect of herbal drug to counter radiation induced CTA, increased in dose dependent manner from 8 mg/kg b.w. to 12 mg/kg b.w. At 12 mg/kg b.w drug concentration, 100.3 % SPR was observed after day 3 of irradiation, which was maintained up to day 5. In comparison to Ondansetrone (70.0 % SPR),

administration of radioprotective herbal drug (12 mg/kg b.w.) provided better protection (100.3 % Sit) against ionizing radiation induced CTA after day 3. "Co-y-irradiation (2 Gy) significantly decreased ferric reducing ability of plasma (FRAP), increased plasma corticosterone as well as seroton in in jejunum and blood as compared to irradiated (2 Gy) animals. Administration of 12 mg,/kg b.w. drug concentration, prior to irradiation increased the total antioxidant status, decreased the corticosterone levels and reduced serotonin in jejunum and plasma at 24 and 48 h after radiation exposure. Present investigation suggested that our drug prepared from Hippophae leaves could he useful in preventing radiation-induced behavioral changes.

18 Cytoprotective effect of Seabucthorn on hypoxic stress in ghat cells: Mod illation of gain ill a glutamylcysteine synthetase

Jyotsna P", Rama Dutta, Gitika P and Dipti Prasad Defence Institute of Physiology and Allied Sciences, Lueknow Road. Timarpur, Delhi- 110054.

Astrocytes protect neurons against oxidative stress. Depletion of GSH in astrocytes leads to decreased ablilty of the astroeytes to protect neurons in culture. Astrocytes protect neurons with precursors of GSH synthesis and arc reported to have high GSH activity compared to neurons. Decreased GSH levels leave neurons vulnerable to oxidative stress and reflect the central role of astrocytes in supporting neuronal function. The present study reports the cytoprotective activity of alcoholic leaf extract of seabuckthom (Hippophae rhanmoides) by modulation ofgamma glutamylcysteine synthetase against hypoxia induced oxidative stress in C6 glial cells. Exposure of cells to hypoxia (3% 0,) for 24h and 481- with subsequent reoxygenation for lh resulted in decrease in GSH and increase in GSSG levels as well as decrease in GSH: GSSG ratio. Exposure of cells to 48h hypoxia showed more severe cellular damage as compared to 24h exposure. Pretreatment of cells with 200ugiml alcoholic leaf extract of Seabuckthom for 1 h. Seabuckthom influenced the synthesis of GSM in C6 glial cells, it was found to increase USE! levels. This could be due to increase in GSH due to ability of Seabucktliorn to quench free radicals ,thus sparing OSH and leading to its accumulation or inducing GSH synthesis,the inIZNA levels of the catalytic and regulatory subunits of gamma glutatnyleysteine synthetase, rate limiting enzyme, in GSH synthesis were determined. Treatment of cells with Seabuckthorn caused marginal increase in mkNA level of regulatory subunit and a marked increase in the inl(NA levels of catalytic subunit in seabuckthom pretreated cells confirmed that Seabuckthorn promoted GSH synthesis. These findings indicate that alcoholic leaf extract of Senhuck thorn has the ability to influence GSH synthesis.

19 Application of Seabuckthorn products as traditional medicine in Trans-Himalayas of Nepal

Youba Raj Pokharel Department of Forests Tree Improvement and Silviculture Unit, Hattisar, Kathmandu, Nepal

Seabuckthom is an important native plant species of dry temperate areas of Nepal Himalayas. in Nepal it grows wildly and cultivation is still not in practice. Two species of Seabuckthorn (Hippophoe solicifolia and Hippophae (ibeana) are knind abundantly in Northern part of the country in more than 22 districts from East to West. Present paper deals with the history of uses of Seabuckthorn in traditional medicine system. Basically boiled raw juice of the berries of both species is being used by traditional doctors (Amchis) and village folks for curing various health problems particularly skin diseases, cardiovascular, digestive and many other health ailments for human beings and the domestic animals as welt The area under present study lies on Mustang, a rain shadow district behind the I timalayas of Western Nepal.

20 Protective role of Scabuckthorn pomace against cadmium induced oxidative stress in hepatic and renal tissues of the poultry

Ankita Sharma, C Varshneya, Vinay Kant, Madhuri Mehta and Shivani Chauhan Department of Veterinary Pharmacology & Toxicology. College of Veterinary and Animal Sciences, CSK HPKV, Palainnur. It P. 176062.

The present study was undertaken to study hepatoprotcctive and rcnoproteetive effects of Seabuckthom pomace (SBTP) on cadmium induced oxidative stress in poultry. The study was conducted on twenty four two weeks old broiler birds and birds were divided into 4 groups: Group I (control), Group II (cadmium @25ingil, of' wafer), Group Ill (cadmium+SBTP I 000ppm) and Group IV (eadmium+SHIP I 0000ppm), respectively. The findings of the study revealed significant (P<0.05) increase in malondialdehyde levels in both kidney and liver following cadmium administration and a significant (P<0.05) decrease in reduced glutathione (GSH) level of kidney. There was no significant change (P>0.05) in the levels of GSH in-liver. The supplementation of seabuckthorn poinne in the feed at both the levels significantly (P<0.05) decreased malondialdehyde levels in both liver and kidneys. The dietary level of Seabuckthorn pomace at I 0000ppm produced a significant (P<0.05) increase in GSH levels of kidney, whereas, no change (P>0.05) was observed in the GSH levels of liver as compared to cadmium treated group. 'Me findings of the study revealed that cadmium induced oxidative effect and its protective effect was more pronounced in kidneys.

21 Anti-microbial activity of flavonoid rich fraction of Sea buckthorn leaves

Ri Tirpude* and MS Yogendra Kumar, Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timatpur, Delhi- I It) 054

Seabuckthorn (ifippophoe rhanmoides) is currently being cultivated in various arts of the world for its nutritional and medicinal properties. All parts of the plants are onsidered to be a rich source of bioactive compounds with various medicinal properties, n the present study, flavonoid-rich fraction (FRF) from Seabuckthom leaves, prepared by icid hydrolysis ,process-ka: investigated for its anti-microbial property. Total llavonoid ;ontent esbmated as rutm equivalent, was found to be 332.67 mg of FRE The major lavonoid compounds of FRF including tnyreetin, quercetin, kaetnpferol and isorhamnetin, vere qualitatively analyzed by reverse phase - high performance liquid chromatography. ntibacterial activity of the FRF, tested against certain medically important bacterial species ;bowed growth inhibiting effect against Eseherichia coil, Salmonella typ hi, Staphylococcus ?wens, Shigella dysentery. Streptococcus pneumoniae and Listeria monoctlogetts. The audy indicated that FRF of SBT has potent-broad spectrum anti-bacterial property.

22 Adaptogenie evaluation of Seabuckthorn (Ilippophae rhanmohles) fruit extracts on rats using C-H-R animal model

Priyanka Sharma Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur. Delhi- 110054.

Seabuckthom (Hippophae rhaomoides) is a well-known high-altitude shrub (2500-4000 m), native to Europe and Asia. Seabuckthom has many nutritional and medicinal benefits and all the parts of the plant are a good source of bioactive substances. Seabuckthom fruits are rich source of vitamin C and E, folic acid, carotenoids and various saturated and unsaturated fatty acids. The beneficial effects of seabuckthorn are well reported against cardiovascular diseases, mucosal injuries, and skin disorders. The present study was conducted to evaluate adaptogenic potential of various extracts of seabuckthom fruit using Cold-Hypoxia-Restraint (C-H-R) animal model. C-H-R is

a unique model in which the animal can be exposed to three different stresses-cold (5°C), Hypoxia (428 mm HO and restraint conditions. Thus adaptogenicity of any compound can be well established using this model. Both dry and fresh fruit-pulp was collected and vacuum-dried aqueous and alcoholic extracts were prepared. The different extracts of fruits were evaluated for their dose dependent adaptogenic activity on rats using C-H-R animal model. Various doses of the extract were administered in rats orally 30 minutes prior to C-H-R exposure. Out of these extracts the potent adaptogenic activity were found in seabuckthorn fresh fruit serial alcoholic extract (100 mg/Kg body wt.) and seabuckthom dry fruit aqueous extract (75mg/ Kg body wt.) .The HPLC analysis of the fruit extract showed presence of ascorbic acid and rutin which are potent antioxidants. Hence these antioxidants may be responsible for the adaptogenic activity found in the extract.

23 Antioxidant effects of Seabuckthorn leaf based herbal formulation

Naveen S and Farhath Khantun Biochemistry and Nutrition Discipline Defence Food Research Laboratory, Siddharthnagar, Mysore-57001I.

Seabuekthm (Hippophae rhatnnoides L. Elaeagnaceae) is used as a medicinal plant in Tibetan and Mango!lean traditional medicines. These plants are naturally grown in Ladakh region of Jammu and Kashmir, locally known as Tsermang or tasru-wpncler plant. The leaf extract of Seabuckthom is known to possess a significant anti-intlammatoly activity and has the potential for the treatment of arthritis. Seabuckthorn based herbal formulation was made by blending different herbs and spices with Seabuckthorn leaves as the major ingredient. This herbal formulation was evaluated for its antioxidative properies in normal and actylamidc treated cells to induce oxidative stress. In vitro antioxidant properties of the Seabuckthorn leaf based herbal formulation was evaluated and found to have 4.1ingig of phenolics and 2.2 mg/g flavonoids with an potency of 65% at a concentration of I mg. These parameters were compared with commercial herbal formulations as well as standard antioxidants such as BHA and BHT also. In vivo antioxidant properies were evaluated based on the DNA damage caused due to free radicals generation by acryl amide generation by employing the comet assay (single cell gel electrophoresis, SaiE). The tail length in acrylamide treated cells was 125.67+13.4p. There was comparatively less severe DNA damage seen in acrylamide + Seabuckthorn leaf based herbal formulation treated leukocytes. The comet tail length was 68.42+785p visa-vis the acrylamide treated rats. The seabuckthom leaf based herbal formulation was found to be efficient in reducing DNA damage induced by strong carcinogenic and neurotoxic acrylamide. Seabuekthorn leaf based herbal formulation thus could serve as a new source of natural antioxidants or nutracenticals with potential applications to reducing the level of oxidative stress and related health benefits.

24 Development and evaluation of Seabuckthorn leaves based herbal food formulation and baked foods

KR Anilakumar, Farhath Khanum & AS Bawa Biochemistry and Nutrition Discipline, Defence Food Research Laboratory, Mysore-5700 II.

Seabuckthom (Hippophae rhamnoides L. Elaeagnaceae) (Sill) is a nitrogen fixing deciduous shrub, native to Europe and Asia. It was used as a medicinal plant in Tibetan and Mangoliean traditional medicines. The leaves and berries have been traditionally used for treating radiation damage. burns, viral infections, gastric ulcers, etc. the contents of Vitamin C,vitamin E, carotenoids, trace elements, polyphenols and flavonoids of Seabuckthom berries and leaves are reported to be higher than the commonly used ones. A total of 18 amino acids and at least 24 phytochethicals have been identified in Seabuckthorn berries. We have developed herbal formulation and baked food viz. biscuits,rusk.cake and bun using SBT leaves as a major ingredient. The baked foods were found to be stable beyond 8 months with its rich content of antioxidants. Futher, the present investigation was aimed to evaluate the therapeutic effects of the SET based herbal formulation (SEW). The studies were conducted on the effect of prefeeding of SE-IF on hexachlorocyclohexane (HCH)- induced free radical stress in rats. Six groups of 6 male rats each were maintained fbr 12 weeks as (I) Control; (2) HCH (300ing/kg body weight) injected (3) I% Seabuckthorn based herbal formulation (SHF) incorporated diet (4) 1% SHP incorporated diet + HO-I (5)2% SHF incorporated diet and (6) 2% SHF incorporated diet + I-ICI I. Results revealed that HCH induction resulted in a significant lipid peroxidation with reduction in activities of glutathione (GSEIL superoxide dismutase (SOD), and Catalase and gl ucose-6-p hosphate dehydrogenase. Tfie prefeeding of SHF resulted in decreased hepatic levels of lipid peroxides and increased CiSH,GSH-peroxidase, GSH reduetase, SOD,Catalase and GSH-S-transferase activities. The study suggests that HCH induction resulted in free radicals, causing toxicity, which could be reduced by the incorpoiation of' herbal formulation in diet.

25 Immunomodulatory and hemolytic activities of Seabuckthorn glycoside

Sudipta Chanda, Bindhya Jayashankar, Divya Singh, Shweta, MS Yogendra Kumar, KP Mishra, Kshipra Misra and Lilly Ganju Defence Institute of Physiology and Allied Sciences, Lucknow koad, Timarpur, Delhi -110054

Seabuckthorn (Hippophae rhaninoides L.) is a member of the Elaeagnaceae family and is usually found at an altitude of 2000- 4500m in cold climates. Being an excellent source ofunsaturated fatty acids, Vitamins C and E, carotcnoids, phytosterols and fiavono ids, it has become the most sought after medicinal shrub. In this study, we have evaluated the stimulatory and hemolytic activities of glycosides (GLS) derived from Seabuckthorn leaves. Glycosides are compounds containing a carbohydrate and a non-carbohydrate residue in the same molecule. Balbic mice were intraperitoneally immunized with different doses of glycoside (2.25, 0.225, 0.0225 mg/kg body wt) in combination with and without antigens (TT and DT). After seven days of the 1st booster, antigen specific serum antibodies were estimated. The hemolytic activity of GLS was determined by using human red blood cells. GLS significantly stimulated the antigen specific IgG response in serum when compared with antigen alone and control groups. Hemolytic percentages of GLS treated RBCs were 1.15% and 0.15% at the concentration of 50 and 25 mg; L respectively. The results suggest that GLS significantly stimulated the antigen specific response against TT and DT in mice and showed no hemolytic effect.

26 Antioxidant activity of phenolic rich fraction of Seahnckthorn (Ilippophae rhanmoides) leaves in vitro and in vivo.

Maheshwari DT*, MS Yogendra Kumar, Vijay K Singh, Saroj K Verrna and Som Nath Singh Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi- 110 054

The antioxidant activity of phenolic rich fraction (PRF) obtained from 70% ehanolic crude extract of Seabuckthorn leaves by fractionation using hexane; ethyl acetate and water were investigated in vitro and in vivo. Total phenolic content estimated as gallic acid equivalent were found to be higher in ethyl acetate fraction (319.33+7.02 ingig of PRF). The major phenolic compounds of PRF like gallic acid, myreetin, quercetin, kacmphferol and isorhamnetin were quantitatively analyzed using RP - HPLC. The antioxidant activity determined by the DPPH method revealed that PRF had the highest antioxidant activity, compared to other fractions. PRF also exhibited a significant antioxidant activity in CCL4 induced acute oxidative tissue injury animal model. Oral administration of .50 and 75 mg/kg body weight significantly protected from CCL4 induced elevation in serum AST and ALT, elevation in hepatic LPO, depletion of hepatic 6SI-1 and decrease in the activities of hepatic antioxidant enzymes SOD, CAT and GPX. The data of the present study suggests that PRF of Seabu•kthorn leaves has the potent antioxidant activity against: free radicals, prevent oxidative damage to major biomolecules and afford significant protection against CCL4 induced oxidative stress and liver damage.

27 Ultrasonic cavitation technology for Seabuckthorn processing- A Review

Vandana Kirar, Radhey Shyam and Kshipra Misra Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur. Delhi-110054

Conventional processing of Seabuckthorn is largely restricted to thermal treatment, which provides high shelf life products. However, the major disadvantages of thermal treatment are the undesirable changes in sensory attributes and nutritional qualities as well. In order to prevent heat induced loss of phytochemical constituents and with the growing awareness of consumers for health, increased interest is focused towards the non thermal technologies of Seabuekthorn processing. Application of ultrasonic cavitation in Seabuckthorn oil processing have resulted in improved yield of oil, minimal loss of phytochemical substances and better retaining of nutritive and sensory qualities. Ultrasonic cavitation process involves a number of physiological effects in the sample leading towards cell disintegeration and facilitating release of matrix components, hence, resulting in improved yield. The area of ultrasonic cavitation is still wide open and with increased awareness the cavitation technology is also gaining importance in different applications for herbal research. This review paper presents the use of ultrasonic cavitation a non thermal processing technique for Seabuckthorn plant.

28 Genetic variability in economic traits of Seabuckthorn (Hippaphae D.Don)

DS Bhakunr, Ranjit Singh* and Z Ahmed** *Defence Institute of Bio-Energy Research, Field Station, Pithoragarli- 262 501 **Director, Defence Institute of Bio-Energy Research, Haldwani- 263139 (Uuttrakhand)

Seabuckthom (Hippophae salicifiilla D. Don) is a dioecious plant of the family Elaeagnaceae growing naturally in the hills of Kumaon and Garhwal in Uttarakhand at an altitude ranging from 8000ft to [2500 ft (MSL). Extensive survey was carried out for collection of promising gennplasm and to study the genetic diversity among the natural population of seabuckthorn for their possible utilization in crop improvement programme. Considerable phenotypic variations were observed in plant height, fruit colour and shape, leaf size and colour. These variations were confirmed at molecular level also. Simultaneously, plants with disease and insect resistance were also identified. The genetic variability is a basic prerequisite for any of the crop improvement programme and could be utill,x1 to develop superior genotypes for desirable traits. Selections have been made for the desired traits in natural population and a field gene bank of selected genotypes is established at Defence Institute of B io-Energy Research, Field Station, Anti (Josh imath) at 9500ft amsl. Agronomic packages and practices are being standardized for genotypes by giving equal and additive chance. These selected genotypes will be subjected to selection, hybridization, polyploidy and genetic engineering for getting the ideal plant ideotypes of seabuckthorn with desirable traits. In conclusion, molecular assisted breeding approaches in the crop improvement of scabuckthom will be very helpful to obtain plants with desirable traits and thus harnessing its full potential for the welfare of mankind.

29 Isolation of sex-linked genes in gender specific DNA marker in Hippophae rhanntoides

Xamal Das and Shailendra Godl Department olBotany, university of Delhi, Delhi

Hippophae rhanmoides L. (2n=24), commonly known as Seabuckthorn is known to be a promising source of vitamin, vast medicinal importance, mineral substances-Sodium salt, Potassium, Calcium, sugars, organic acid, pectin and tannins, triterpenoids, phospholipids, cumarin, catechins, leucoanthocyans, flavonols. alkaloid serotonin, as well as unsaturated fatty acids and other compounds. The oil and juice, commercially produced from its fruits have been shown to possess antibacterial, anti-ulcer, anti inflammatory (antiphlogistic), wound healing and immunomodulatory properties. It also has positive effect on heart diseases. This is why it has caught the eyes of researchers around the globe. From commercial point of view, female plant is more important than the male one. As only —10% male pants are required in the field to produce enough fertile pollens. In Seabuekthom, plant gender can be identified only after flowering which takes a minimum of three years. The only approach to discriminate morphologically similar sexes of Seabuckthorn is by chromosome analysis, but this is not a viable solution at commercial level. We used the technique called RDA (Representational Difference Analysis) to facilitate the isolation of sex linked marker. Compared to Subtractive hybridization, RDA has two additional element-representation and kinetic enrichment. For RDA, the DNA was sheared in the range of 100-500bp. After end repairing of DNA, different blunt end adapters were ligated specifically to the male and female. Female adaptor specific primer was biotin labeled to facilitate subtraction in which male was treated as TESTER and female as DRIVER. With the help of streptavidin coated magnetic beads, three rounds of subtraction were made in an increasing tester to driver ratio 1:80, 1:400 and 1:1400 respectively. After each round, enriched product was amplified with male specific

primer for compensation of DNA. A-tailing was done to the enriched fragment to facilitate ligation to the pGEMT easy vector for cloning. Positive clones were picked up on the basis of blue/white selection of culture grown in LB plates containing IPTG and X gal. Around 460 positive clones were picked up and squeezed. Presently, Insilico analysis is going on with these sequences.

30 Cytoprotective and antioxidant activities of Supercritical CO2 extract of Seabuckthorn (Hippophae rhamnoides) leaves.

Ruma nutta`, M S Yogendra Kumar, Swapna Sonale Rao ' K Udaya Sankar' and Dipti Prasad

Defence Institute of Physiology and Allied Sciences tucknow Road, Titnarpur. Delhi-110 054 'Food Engineering Department, Central Food Technological Research Institute, Mysore 570020

Seabuckthorn, family Elaegnaceae, is a widely used medicinal plant. The plant is currently being cultivated in various parts of the world for its nutritional and medicinal properties. All parts of the plants are considered to be a rich source of bioactive compounds with antioxidant properties. In the present study, an environmentally benign novel separation technique using supercritical carbon dioxide has been used for the extraction of Seabuckthom leaves (SBT) at different extraction pressures, temperatures and by addition of ethanol as an entrainer for first time to obtain compounds with high antioxidant activity. Total phenol content, estimated as gallic acid equivalent was found to be in the range of 13.21 — 29.64 ma/g of extract. Cytoprotective and antioxidant properties of SBT were evaluated against tertiary-butyl hydroperoxide (tert-BOOH) induced oxidative stress in murine macrophages (Raw 264.7). Exposure of cells to tert-BOOH (100pM) for 111 resulted in significant increase in cytotoxicity, decrease in mitochondria] membrane potential and an appreciable increase in reactive oxygen species (ROS) production was noted, which in turn is responsible for fall in intracellular antioxidant levels and GSH/GSSG ratio. Pretreatment of cells with SBT extract (25).t.g/m1) significantly inhibited cytotoxicity, ROS production and maintained antioxidants levels similar to that of control cells. These results indicate that supercritical carbon dioxide extract of SBT has strong cytoprotective and antioxidant activities.

31 Optimization of protein extraction procedures from Hippophae rhontnoides for dissecting its cold tolerance

Ravi Gupta and Renu Deswal Plant Molecular Physiology, Biochemistry and

Proteomies Laboratory, Department of Botany, University of Delhi, Delhi

Freezing stress is a major abiotic stress affecting crop productivity. Cold tolerant plants have developed mechanisms to cope up with the freezing conditions. For understand i ng mechanism of cold tolerance in plants, Hippophae rhamnoides- a cold hardy shrub, was selected. It can survive freezing temperatures up to -40°C and hence can be used as a model system for deciphering freezing tolerance.For analyzing the physiological manifestation of cold tolerance, droop test analysis was done. As there is scarcity of good protein extraction procedures for Seabuckthom, first aim was optimization of protein extraction procedures for total and apoplastic proteins. High

phenolic content interferes with the protein extraction procedures, a cleanup step was used to remove contaminants. Cold inducible changes in proteome were analyzed using SDS-PAGE and 2-DE. Up-regulated proteins were identified using MAL.D1-TOF/MS-MS. To test the purity of isolated apoplastic proteins, glucose-6- phosphate dehydrogenase (GPDH, a cytoplasmic enzyme) assay was done. To analyze the effect of freezing stress at protect= level, proteins were isolated from control, cold (4°C) and freeze (-5°C) treated seedlings and resolved on SDS-PAGE and 2-DE An 80 kDa heat shock protein and a 41.9 kDa actin were down-regulated while a 20 kDa hypothetical protein was up-regulated by freezing stress at -15°C. Rubisco degradation was observed at -15 °C and 4 °C. Total of 879 spots were observed in 2-DE gel profile of total proteins out of which the expression of around 70 polypept ides was altered due to freezing stress that represent only 8% of total proteome. As it is well documented that stress related proteins are secreted in apoplasto:ne. Sub-cellular analysis was done with apoplastome isolated using vaccum infiltration method. Protein extraction procedures for laboratory grown as well as field collected material have been optimized for Seabuckthorn for its further analysis.

32 Antioxidant activity of tiavonoid rich fraction of Seabuckthorn (Hippuphae rhatnnoides) leaves; Quantitative analysis of its major components by RP-HPLC.

M S Yogendra Kumar*, Praveen Mishra and K Udaya Sankar'

Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi- 110 054 'Food Engineering Department, Central Food Technological Research Institute Mysore 570020

In recent years, there has been a wide interest in finding natural compounds that could replace synthetic antioxidants, because of its possible toxicity and due to a suspected action as promoters of carcinogenesis. Seabuckthom (Hippophae Rhamnoides) is currently being cultivated in various parts of the world for its nutritional and medicinal properties. All parts of the plants are considered to be a rich source of bioactive compounds with antioxidant properties. In the present study, flavonoid-rich fraction (FRF) from Seabuckthom leaves was prepared by acid hydrolysis process. Antioxidant activity of FRF was evaluated using 2, 2-Dipheny1-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Averaged mg of Trolox equivalent (TE)/g of extract were found to be 390.71 and 372.70 as determined by DPPH and FRAP assays respectively. The reducing power of FRF increased with increasing amount of FRF; the equation of reducing power (y) and amount of FRF (x) was y — 2.4684x + 0.0351 (r2 = 0.99), indicating that reducing ability correlated well with amount of FRF. Chemical composition of FRF in terms of total flavonoid content was determined by a colorimetric method. Total flavonoid content estimated as rutin equivalent was found to be 332.67 mat of FRF. The major constituents of FRF including myreetin, quercetin, kaemphfcrol and isorhamnetin, was analyzed by reverse phase - high performance liquid chromatography carried out on C I 8 column, using acetonitrile: methanol (75:25) and water: orthophosphoric acid (99.7:0.3) as mobile phase with gradient elution and by ultra-violet detection at 370mn. Myrcetin. quereenn, kaemphferol and isorhanmetin, were found to be in the range of 0.62 — 11.49 mg/ g of FRE.

33 Development of green extraction process for Seabucktorn bioactives

Lalit D Kaglisvar, Sushant C Paul", Anuradha S Rekha S Singhala and Vandana 13 Patravaleb 'Food Engineering and Technology Departmem, Institute of Chemical Technology, Niatunga, Mumbai 'Department of Pharmaceutical Science and Technology, Institute of Chemical Technology. Matunga, Mumbai

Increasing efforts are being made to develop extraction processes with minimal ecological impact leading to reduced production of waste or avoiding the use of hazardous or toxic organic chemicals. Supercritical fluid extraction represents an efficient and ecofriendly technique for isolation of active components from different plant sources. Seabuck-thorn (SBT) (Hippophae than-mai& L.) seed oil having high nutracetiticak cosnicceutical and therapeutic activity has been extracted from dried SST seed powder using supercritical carbon dioxide (SC-COO. The combined effect of three independent process variables (temperature, pressure and time) on SBT actives extraction was examined using Box-Behnken design. Extraction was carried out at pressure ranging from 150- 350 bar, temperature from 35-55°C, and time of extraction from 30-90 min. The extract obtained was estimated for tocopherol (by HPLC) and carotene (by Speetrophotometry) content and compared with petroleum ether extract obtained by Soxhlet extraction. The antioxidant activity was estimated in terms of DPPH radical scavenging activity. The optimized conditions resulted in extraction efficiency of 82.87 ± 1.94 and 55.38 3.21% for tocopherols and carotenes, respectively, while free radical scavenging activity was 57.44 ± 2.84 InglinL. Further use of 2-propanol as an entrainer at 30% v/w of dried SBT seed powder at optimized conditions increased the extraction efficiency to 91.14 ± 0.36 and 69.61 ± 1.61% for tocopherols and carotenes, and lowered the free. radical scavenging activity to 38.97 ± 1.06 mg/mL.

34 Transcriptome analysis and development of microsatellite markers in seabuckthorn

IPrakash C. Sharma, /Rajesh Gliangal. 'Ankh Jain, ISaurabh Chaudhary.2Saurabh Raghuvanshi

University School of Biotechnology, GGS Indraprastha University. Dwarka. Delhi 110075, India Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021, India profpcsharrna raftnail.com

High adaptability to extreme environmental conditions of temperature, drought and salinity has generated keen interest among plant researchers in =buckthorn. Therefore, our laboratory initiated a project to develop an EST (Expressed Sequence Tag) database for =buckthorn (Hippophae rhamnoides L.) to identify stress responsive genes. As a prerequisite,, we developed a modified CTAB based method for the isolation of high quality RNA from seabuckthom. Subsequently, sequencing of about 4500 cDNA clones facilitated submission of 3412 high quality sequences in the EST database of NCBI. To overcome redundancy. ESTs were clustered using CAP3, yielding 1665 unigcnes. Functional annotation was performed by BLAST analysis against various public databases. Unigenes showing no similarity with existing entries

were considered as =buckthorn specific. Gene Ontology terms were assigned to respective unigencs using BLAST2G0 suite. About 50 unigenes showed high similarity to known proteins involved in various abiotic stress pathways. High throughput next generation sequencing is being currently used to generate comprehensive seabuckthom transeriptome Fifteen genes involved in able,* stress tolerance were selected for real time expression analysis by screening Arabidopsis mieroarray experimental data using Gencvestigator software. Genevestigator. We are also using serial analysis of gene expression (SAGE) for differential gene expression profiling. Our seabuckthom transeriptothe research will contribute significantly towards understanding abiotic stress management in plants, particularly seabuckthorn. Towards development of SSR (microsatellite) markers, EST-based SSRs were initially evaluated for assesing =buckthorn genetic diversity. Screening of 1584 unigene sequences using a mierosatellite search tool, MISA, identified 56 microsatellite positive sequences. PCR primers were designed for the amplification of 30 microsatellite loci. Two to five alleles were revealed by nine and eleven primer pairs in H. rhamnoides and H. salicifolia genotypes, respectively. None of the primer pairs detected polymorphism in H. tibetana genotypes. In another approach, random and microsatellite enriched genornic libraries have been constructed in H. rhamnoides for further isolation of mierosatellitc markers. For enriched libraries, seabuckthorn DNA was exposed in digestion ligation reaction with specific adaptors, followed by microsatellite repeat sequence capture with biotin labeled oligos and streptavidin beads. Nlicrosatellite positive sequences were subsequently cloned and sequenced. Our method of library enrichment has proved very promising yielding a high frequency of' microsatellite positive clones. These newly developed markers will be subsequently assessed for their use in seabuckthom genome characterization and diversity analysis, for which plant samples have been collected from diverse ecological conditions from Leh and Lahual-Spiti representing variations for altitude, soil texture, water availability and sunlight exposure. Morphometric data have also been recorded on these collections. Keywords: Transcriptome, ESTs, molecular markers. SSRs, genetic diversity

35 Study on the morphological variations in seabuckthorn (Hippophae rhainnoides ssp. turkestanica) populations growing in Lahaul-Spiti, dry temperate Himalayas

Virendra Singh, Manohar Lai, Reena Devi

Department of Biology and Environmental Sciences, College of Basic Sciences CSK Himachal Pradesh Agricultural University. Palampur. !IP. India
virendritqing112003(kahoo.com

In India, Iiippophae rhamnoides subsp turkestanica grows naturally in the high altitude regions of the Jammu & Kashmir; Himachal Pradesh and Uttarakhand provinces. The Lahaul-Spilt district (31044'57" and 3205957" N Latitudes and 76046'29" and 78041'34" E Longitudes: 11835 km2 geographical area) in Himachal Pradesh forms the part of cold desert and extends from 2400-7000 m arm!. In most woody plants, morphological and physiological characteristics at extremely variable across environmental gradients, particularly across attitudinal gradients. Hippuphac rhamnoides subsp nakeshunea, a deciduous species, occupies a wide range of habitats and altitude (2400-4500 in ains1) in the Lahaul-Spiti districts in the Himachal

Pradesh. Fifty five (55) female accessions and thirty seven (37) male accessions were studied in naturally growing population of seabuclahom in Lahaul-Spiti district. Hippophar rhamnoides subsp turkestanica grows in the Lahaul-Spiti district as shrub and tree form and mean height of female plants 115=0.16 at and male plant height 2.2310.20 in. In female accessions leaf length ranged front 151-8.24 cm (Mean 5.1810.17 cm) and width from 0.42-0.75 cm (Mean 0.56:t0.01 cm) and pt.-dice' length from 0.23-3.07 nun (Mean 2.1310.72 mm) and in male accessions leaf length ranged front 3.14-7.36 cm (Mean 5.06+0.18 cm) and width from 0.41-1.14 cm (Mean 0.6110.03 cm), and pcdiecl length from 1.17-3.31 inm (Mean 2.29+0.08 mm). The mature fruits are yellow. red and orange coloured along with persistent stylar end and acute, round and depressed shape of fruit tip. Fruit arrangement on secondary branches is profuse and zigzag. Fruit length varies from 0.67-9.6 nun (Mean 6.4610.18 mm) and width from 0.50-7.56 mm (Mean 5.7410.15 mm) and peduncle length from 1.15-3.33 mm (Mean 2.0710.07 nun). Total soluble solids ranged between 9.0-14.4o Brix. (Mean 12.0210.21° Brix.) and weight of 100 fruits varies from 8.0-29.5 g (Mean I 3. I 4±0.56 g). Seeds are deep red, brown and black coloured along with one longitudinal furrow. Seed length varies from 2.75-5.83 mm (Mean 4.0210.09 mm) and width from 0.96-33 mm (Mean 2.42t0.06 mm) and weight of 100 seeds varies from 0.69-1.86 g (Mean 0.96±0.03 g). The correlation between altitude and quantitetive parameter of female accessions (i.e. plant height, canopy diameter, leaf length and width, pedicel length, fruit length and width, peduncle length and seed length and width) wcrc tested and only some parameters showed significant correlation. A significant negative correlation has been found between the altitude and plant height (r=-0.329, p<0.02. n=55) and altitude and leaf length (r--0.560, r-0.01, n=55). Leaf length shows significant positive correlation with leaf width (1.-0A74. p<0.01, n-55) and pedicel length (r=0.448, r0.01, n=55). Fruit length show significant positive correlation altitude (r=0.327. p<-0.02, n-55); fruit width (r=0.602. p<0.01, n-55) and pedicel length(r=0.470, p<0.01,, n=55); and significant negative correlation with plant height (r--0.429, we.0.01, n=55) and canopy diameter (r-z-0 414, pc:0 01, trsiC) Sesvi length shows significant positive correlation with fruit length (r--0.317, p<0.05, n=-55) and seed width (r4.570. p<0.01. n=35). The cluster analysis of accessions has been done based on quantitative morphological characters of female accessions studied in Lahaul-Spiti. The cluster analysis of 55 female accessions resulted into seven (7) morphotypes of the Hippophae rhainnoides subsp. turkestanica. lt has been observed that plant height and leaf length decreased with increasing the altitude and nettled length of the leaf depends on the length of the leaf. Fruit length affected by altitude, plant height and canopy spread. The length of peduncle shows directly proportional relation to the length of fruit. Seed length cltssais on the fruit length. Dioecism and wind pollination make this species art obligate out-crosser. The two features coupled with occasional sexual polymorphism serve as the basis of genetic variation. This variation nuinifQas at the molphologicat cytological, ecological and molecular levels. It is concluded that wide variations exist in different populations of seabuclahom growing in this region. Therefore, conservation of the varied morphotyp=; am required by in-situ and ex-situ methods.

36 Influence of origin, harvesting time, and growth location on contents of inositols and methylinositols in sea buckthorn berries

Baoru Yang, lie Zheng, Heikki Kallio

Department of Biochemistry and Food Chemistry, University of Turku, Fl-200 14Turku, Finland baoru.vangautuft

Occurrence of inositols and methylinositols in sea buckthorn (Hippophoe rhamnoides) berries has been reported recently. These compounds play an important role in human physiology and may be important contributors to the widely shown health effects of sea buckthorn berries. Two studies were carried out to investigate the influence of subspecies/origin, harvesting date, and growth location on the abundance of inositols and methylinositols in sea buckthorn berries. In study I, the contents of inositols and meihylinositols in berries of throe subspecies of rhamnoides were compared. The influence of harvesting date and the impact of climatic conditions at growth sites on the content of these compounds were investigated in wild Chinese berries. The wild Chinese berries rhanmokks ssp. sinensis) contained higher levels of L-quebrachitol (IL-2-0-methyl-chiro-inositol) and tnetItyl-myo-inositol (average 615 and 58 mg/l00 nil- juice, respectively) than the Finnish (H. rhamnoides ssp. rhumnoides, 276 and Digit 00 mL juice, respectively) and the Russian (H. rhanmoides ssp. niongolica, 228 and 16 mg/100 nil_ juice. respectively) berries (Pc 0.001). The content of myo-inositol was higher in the Chinese and the Russian berries than in the Finnish berries (26 and 20 mg/100 ml. juice vs. 8 mg? 100 nth juice, P< 0.001). In the Chinese berries, the contents of methyl-myo-inositol and L-quebrachitol increased, whereas that of niyo-inositol decreased front late September to late November. The content of L-quebrachitol in the Chinese berries correlated negatively with the air temperature and the number of frost-free days. In the second study, wild berries of H. rhaamoides ssp. sinensis were collected and analyzed from nine natural growth sites in China in three consecutive years to investigate the influence of the latitude and altitude on the contents of inositols and methylinositols in the berries. The abundance of L-quebrachitol in the berries (mgt IOU la juice) followed the order: Inner Mongolia (1000)' Hebei (850) > I lelongjiang (720) > Qinghai (680) > Shanxi (590)> Sichuan (290). The berries from lielongjiang (140 mg/l00 ml. juice), Hebei (90 mg/100 mL juice). and Shanxi (80 mg/100 mL juice) were richer in methyl-myo.inositol than those from Qinghai (40 mg/l00 ml. juice). Inner Mongolia (40 mg' 100 rnL juice), and Sichuan (30 mg/ I 00 mL juice). itfyo-inositol content was the highest in berries from Hclongjiang around 50 mg/100 mL juice. whereas the levels in berries from other provinces were typically 20 mg/100 mt. juice. The content of L-quebrachitol in the wild Chinese berries correlated strongly and positively with the latitude and negatively with altitude. The two studies showed consistent results suggesting that the content of L.quebrachitol increases with elevation in latitude and decreasing temperature of growth location. The results indicate potential role of the compound in cold resistance of sea buckthorn.

37 Trace elements accumulation in sea-buckthorn fruits

-Gennady Xl. Skuridin,20Iga V. Chankina,2Konstalitin P. Koutscnogy, 'Valentina K. Kreymer, 'Nina V Baginskaya I. Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia 2. Institute of Chemical Kinetics and Combustion. Novosibirsk, Russia skuridin@bionansc.ru

Element complex of fruits of Siberian sea-buckthorn (Hippophae rhamnoides L. mongolica Rousi) growing in endemic conditions have been studied. Absolute tent and biological absorbance coefficient (SAC) of 22 elements: potassium (K), ium (Ca),

scandium (Sc), titanium (Ti), vanadium (V), chromium (Cr), anese (Mn), iron (Fe), cobalt (Co), nickel (Ni). cooper (Cu), zinc (Zn). arsenic (A), selenium (Sc), bromine (Br), rubidium (kb), strontium (Sr), yttrium (Y), Zirconium (Zr). niobium (Nb), molybdenum (Mo) and lead (Ph) have been determined by X-ray fitioresceni method with application of synchrotron irradiation (RFAS1). It was established that uptake level of Ti, Nb and Cr by sea-buckthorn fruits exceeds the same of mean Earth phytomass from 450 to 500%. Sea-buckthorn fruits absorbance coefficient of K. Fe, Ni, Mo, Br, Se, As and Zr is similar the same of mean Earth phytomass. Decreased fruits absorbance have been found for Mn (SAC is 28.6 times below than the same in Earth phytomass), Co (20.4 times below), V (16.4 times below), Ca (12.3 times below), Pb (10.8 times below), Sr (4.8 times below), Cu (3.4 times below), Rb (3.2 times below) and Zn (2.7 times below). It was shown that sea-buckthorn fruits do not concentrate both toxic elements Pb and

38 Trace elements accumulation in sea-bucktliorn root tubercles

'Cientiady M. Skuridin.

'Olga V. Charikina,2Konstantin P. Koutsenogy, 'Valentina K. Kreymer, 'Nina V. Baginskaya
I. institute of Cytology and Genetics SR RAS. Novosibirsk, Russia 2. Institute &Chemical Kinetics and Combustion, Novosibirsk, Russia skuridinai,,)bionet.nse.ru
Elements complex of root tubercles of Siberian sea-buckthorn (Hippophae rhamnoides L. ssp. mongolica Rousi) growing in endemic conditions have been studied. Absolute content and biological absorbance coefficient (BAC) of 22 elements: potassium (K), calcium (Ca), scandium (Sc), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), cooper (Cu), zinc (Zn), arsenic (As), selenium (Sc), bromine (Br), rubidium (Rb), strontium (Sr), yttrium (Y), zirconium (Zr), niobium (Nb), molybdenum (Mo) and lead (Ph) have been determined by X-ray fluorescent method with application of synchrotron irradiation (RFAS1). It was established that root tubercles uptake level (SAC) of several elements studied extremely exceeds the same of mean Earth phytomass: Zr (990 times more), Nb (640 times more), Ti (535 times more), Cr (135 times more), Fe (61.4 times more) and Mo (26.4 times more). Next elements were of less accumulation: Co (467%), Sr (423%), Ni (348%) and Br (325%). Sea-buckthorn root tubercles accumulation of Cu, Zn, Rb, V. Pb, As and Sc is similar the same of mean Earth phytomass. Decreased root tubercles absorbance have been found for Mn (SAC is 3.3 times below than in Earth phytomass). Ca (2.9 times below) and K (2.2 times below).

39 Trace elements accumulation in sea-buckthorn leaves

IGennacly M. Skuridin. 'Olga V. Chankina, 2Konstantin ft Koutsenogy. 'Valentina K. Kreyn)er, 'Nina V. Baginskaya

I. Institute of Cytology and Genetics SB RAS. Novosibirsk, Russia 2. Institute of Chemical Kinetics and Combustion, Novosibirsk, Russia slcuridin@bionet.nsc.ru
Elements complex of leaves of Siberian sea-buckthom (l-lippophae rhamnoides L. ssp. mongolica Rousi) growing in endemic conditions have been studied. Absolute content and biological absorbance coefficient (SAC) of 22 elements: potassium (K).

calcium (Ca), scandium (Sc). titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn). iron (Fe), cobalt (Co), nickel (Ni), cooper (Cu), zinc (Zn), arsenic (As), selenium (Sc), bromine (Br). rubidium (Rb). strontium (Sr), yttrium (V). zirconium (Zr), niobium (Nb), molybdenum (Mo) and lead (Pb) have been determined by X-ray fluorcsccnt method with application of synchrotron irradiation (REAM). It was established that uptake level (BAC) Ma range of elements significantly exceeds the same of mean Earth phytomass: Nb (1895%), Cr (910%), Ti (725%), Zr (635%), Sr (399%) and Fe (223%). Sea-buckthorn leaves accumulation of K, Ca. Ni. Br and As is similar the same of mean Earth phytomass. Decreased leaves absorbance have been found for Co (SAC is 8.3 times below than in Earth phytomass), Rh (6.7 times below), V (5.5 times below), Se (5.1 times below), Mo (4.8 times below), Mn (3.8 times below), Cu (3.8 times below), Pb (3.3 times below) and Zn (2.7 times below). It was shown that sea-buckthom leaves do not concentrate both toxic elements Pb and As.

Effect of solvent strength on total phenol content and DPPH scavenging activity of seabuckthorn extract

inharam Paul Attrey, 2Amrit Kumar Singh. jiyoti Katyal

I. Director. Amity Institute of Seabuckthom Research & Ex Director, Amity Institute of Pharmacy, 2. Research Fellow, AMity Institute of Seabuckthorn Research. 3.
II. Amity Institute of Pharmacy, Amity University Uttar Pradedi, Sector 125, Expressway, NOIDA - 201303, UT. (INDIA) dpattrey@arnity.edu, dpattrey@yahoo.co.in

Subject, i.e. research objective: Effect of solvent strength on total phenol content and DPPH scavenging activity of Scabucketom extract Experimental materials and methodology: Preparation of Extracts of 14ippophae rhamnoides: using hydro-alcoholic solvent of various strengths; Estimation of total phenol content; Evaluation of DPPH radical scavenging activity. Hypothesis used in the Research: Scabuckthom extracts have been found to show significant amount of phenolic compounds which may be responsible for its various activities including anti oxidant activity. Hydro-alcoholic extracts prepared by using solvent of various strengths may influence the total phenolic content which may be responsible for variation in different activities of Seabuckthom extract like anti oxidant activity. Main conclusions: The data provides variation in total phenolic content as well as in DPPH scavenging activity of Seabuckthom extracts prepared by using hydro-alcoholic solvent of various strengths.
Key words: Seabuckthom, total phenol content DPPH scavenging, hydro alcoholic extract, solvent strength

40 Detection of 5-hydroxytryptamine hydrochloride of Hippophae rhumnoides

L. from Qinghai-Tibetan Plateau 12Li mao cat rang, IStio You-nii 1. Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China: 2. Graduate School of the Chinese Academy of Sciences, Beijing, 100049, China ukaaiotmcn Samna), 5-hydrosytryptamine (5-HT), is a monoamiric neurotransmitter.

To our best knowledge, it plays wide biological roles in our body system. including cardiovascular system, respiratory system and the intestines system. Therefore it is critical to our metabolism. Sea buckthorn is a unique species that obtains abundant of

Serotonin. In this study, the content of 5-hydroxytryptamine hydrochloride of different parts of sea buckthorn from different areas of Qinghai-Tibet Plateau was determined by a novel, simple HPLC based pre-column derivatintion method. The method was found to bc simple, precise and rectilinear over a relatively wide range of concentrations (8.11 a 10-5 — 1.297a 10-3 mold.). This method can be applied to quality control tool of relative sea buckthorn industry.
Keywords: 5-hydroxyttyptamine hydrochloride; 14PLG; Hippophae rhantnoides L.; pre-column derwatization

41 Cleavage of f3-carotene to flavor compounds by the microorganism from seabuckthorn juice

Wang shulin, 2 Fan Mingtao
1. Qinghai university, Xining, Qinghai, China, 810016 2. College of Food Science and Engineering, Northwest A&F University Yangling, Shaanxi, 712100, China, wangs11970(0,163.com

This paper presents the first description of a bacteria exhibiting [3-carotene cleavage activity isolated from seabuckthorn juice. The kinetic of degradation of J3-carotene by the strain and enzyme has been studied, and the volatile compounds from degradation of p-carotene in cultures with the bacterial and mycelium-free culture media have been analyzed by the GC-MS. Nearly complete degradation of the substrate was observed with culture media of the strain after incubation for 24 h. About 40% of the initially added [3-carotene in mycelium-free culture had been degraded at 20min after the start of incubation. The norisoprenoids, such as 2,6,6-trimethyl-I-Cyclohexene- 1-carboxaldebyde,4-(2,6,6-trimethyl-l-cyclohexen- I -y1)-3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-l-y1)-3-Buten-2-ol, which may be from degradation of 13-carotene, can be determined in submerged cultures with the bacterial and mycelium-free culture after incubation. The phylogenctic tree of the strain was produced on the basis of morphology observation, experimental results of physiology, biochemistry and analysis of 16S rDNA sequence. The strain was identified as Staphylococcus sp.
Key words: 13-carotene bioconversion, enzymatic degradation, norisoprenoids, seabuckthorn juice, Staphylococcus sp

42 Determination of glucose, fructose and sucrose in seabuckthorn fruit honey by HPLC-ELSD

Jia Hongwei, Lin Tong, Yao Zhimin, ZhaoYu, Civan Dawei Qinghai Exit-Entry Inspection and Quarantine Bureau, Xining 810000 China jhw0820@l26.com

HPLC-ELSD was applied to the determination of glucose, fructose and sucrose in seabuckthorn fruit honey. The methods were studied and optimum conditions were found. After an appropriate ultrasonic pretreatment with water, the sugars were separated on a Zobrax-NH2 coloum (4.6 x250mm, Sum) by using acetonitrile: water (90:10, v/v) as the mobile phase, and flow rate was 1 ml/min, evaporator temperature was 70°C, evaporator gas flow was I .5L/min. The average recoveries for three analytes ranged from 96% to 103%, and the response value was linear between l0ttg-1000Rg and r was 0.992 to 0.997. With an average RSD of 1.41,1.54,1.83% (n=5) were obtained.

43 Selective and sensitive determination of fatty acids and amino acids in Hippophae rhamnoides L. fruit using pre-column derivatization HPLC method

1Li. mao cai rang, 2 Suo You-mil

1. Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 8 [0001, China 2 .Graduate School of the Chinese Academy of Sciences, Beijing, 100049, China

A novel, sensitive and selective HPLC based method was developed for determination of 20 amino acids (AA) and 18 fatty acids (FA) in Hi ppophae rharnnoides L. fruit (sea buckthorn fruit). The method is based on reaction of adding amino acids and 2{2-(dibenzocarbazole)-etboxy] ethyl chloroforrnate to borate butler of pH 8.5 to yield a highly fluorescent derivative, which could be measured at 390am (Excitation wavelength:300 urn), and fatty acids react with acridone-9-ethyl-p-toluene sulfonate in presence of k2CO3 to yield a highly fluorescent derivative, which could be measured at 505 nm (Excitation wavelength: 272nm). The separation of the derivatized fatty acids and derivatized amino acids has reached good baseline resolution by our established method. The detection limits was high, which reached to 1.0 nmol (calculated as the signal-to-noise ratio: 3) in both experiments. Excellent fir arity was observed with coefficients > 0.9990.
Keywords: Fatty acid; Amino acid; Rippophae rhatnnoides L.; HPLC; Pre-column derivatization

44 Seabuckthorn for protection against high altitude stress

Sawhney Indian Institute of
Technology Matadi, Mandi - 175001, Himachal Pradesh, India.
sawhney_49gyahoo.co.in, registrar@iitmandi.sc.in

Ascent to high altitude in man is associated with a variety of hypoxia induced disorders like acute mountain sickness, high altitude pulmonary edema, high altitude cerebral edema. Monts' disease, pulmonary hypertension besides causing a marked reduction in physical and mental performance. Although a variety of factors like extreme cold climatic conditions, low humidity, higher amounts of solar and ultraviolet radiations, and high wind velocity operate at high altitude-, the decline in barometric pressure leading to low availability of oxygen is the major contributory factor towards high altitude related ailments. At sea level, 11y/toxic stress leading to tissue hypoxia is a major life threatening complication during cerebral or cardiac ischemia. Since seabuckthom(SFIT) is able to grow undcr hypoxic conditions and natural wild growth of scabuckthom is well documented up to an altitude of 4800m in Western Himalayas, the plant may provide protection against hypoxie stress at high altitude and also at sea level. When (2-6 glioma cell were exposed to hypoxia for 1211 the hypoxic stress caused a marked increase in NO and ROS generation and decline in antioxidant levels, besides causing a reduction in mitochondria] transmemberane potential. Scabucktbom leaf extract provided a fair degree of protection to C-6 glioma cells suggesting that it has significant neuro protection activity under hypoxic environment. hypoxia induced DNA damage in the rat leukocytes could be prevented by treatment of animals with SBT leaf extract. The

hypoxic stress also caused activation of NP-k DNA binding which in turn resulted in an increase in secretion of inflammatory enplanes like TNF-c, IL-6. 1L-10 and MCP-I in the rat lungs. Both SBT seed oil and leaf extract were able to inhibit hypoxia induced NF-kit DNA binding and interleukin production thereby inhibiting hypoxia induced inflammatory processes. Similarly, hypoxia induced activation of hypoxia inducible factor-la (H1F-141), vascular endothelial growth factor (VEOP) and endothelial nitric oxide synthase (cNOS) could bc downregulated by pretreatment of animals with SIIT seed oil or leaf extract. Not only the VEOF gene expression but also the VEGF secretion in the lungs and plasma of animals subjected to hypoharic hypoxia could be curtailed by seabuckthom extracts. Maintenance of body temperature is most crucial for survival of any organism under stressful environment. When rats are exposed to cold-hypoxia-restrain stress, the animals can enter into a state of hypothermia if the rectal temperature falls below 230C. The S13T extracts were able to delay onset of hypothermia and recovery was faster in animals which received SBT preparations suggesting that hypoxia induced hypothermia can be prevented by SBT pretreatment. Similarly, when animals were exposed to lethal bypoxia of 9750m, pretreatment with SKI leaf extract or seed oil delayed onset of gasping and hypoxic survival time suggesting that SRI is able to enhance resistance to hnioxie stress. The hypoxia protection activity of seabuckthorn may be due to a variety of reasons like curtailments of oxidative damage, inhibition of stress hormone secretion and vasorclaxant activity which in turn may inhibit transvascular fluid leakage into the lungs and brain by maintaining membrane permeability. These studies suggest that seabuckthorn based preparations have high potential to be used for protection against high altitude hypoxia induced disorders.

45 Health effects of sea buckthorn berries:

investigations at the University of Turku, Finland

Heikki Kathy, Baoru Yang

Department of Biochemistry and Food Chemistry, University of Turku. Finland
herkki.kallioautu.11

Sea buckthorn (SB) has been a target of scientific investigations at the University of Turku since 1980s. In addition to taxonomic. chemical and sensory research of the berries, their health effects have deserved special attention. Nutritional effects of both entire berries and of their oil and ethanol soluble fractions have been investigated. Many of the hypotheses were based on F.astem, especially on Chinese traditions, claims and knowledge. Berries in the Scandinavian countries, both cultivated and wild ones, are commonly regarded as health promoting food ingredients also in Finland. The major genera are Vaccinium (bilberry, lingonberry), Rubtts (cloudberry, raspberry, arctic bramble), Ribes (currants), Empetmin (crowberry) and Ilippophad (sea buckthorn). Sea buckthorn berries rich in tlavonoids. oil soluble antioxidants and vitamin C were shown to lower concentration of the sensitive CRP in plasma. In addition, consumption of the juice indicated increase of the ratio of HDL cholesterol to LDL cholesterol and elongation of the lag phase of LDL cholesterol oxidation. Berries and especially their ethanol-soluble fraction suppressed the postprandial insulin peak. It was further shown, that the bioavailability of tlavonoids was increased by coincide supplementation of sea buckthorn oil. The results of consumption of SB thus indicate possible reduction of the risk of cardiovascular diseases in healthy

people. Sea buckthorn seed and pulp oils have been of special interest. The very recent studies showed the unexceptionally high protective, antioxidative effects of SB oils on the isolated DNA in vitro. The same was the case with DNA of rat liver homogenate in vitro. Whether the positive effects of sea buckthorn oils on dry eyes and atopic skin have the same mechanistic background, is not known. The oils investigated have all been isolated by aseptic CO2 extraction.

46 Isolation and synthesis bioaetive flavonols from Indian seabukthorn.

N. Pandurangan, Chinchu Rose, A. Bancrji, Amnia School of Biotechnology, Amrita Vishwa Vidyapcetham. Antritapuri,
Kollam fist. —690525, Kerala, India banasolc@yahoo.com & bancniasoke(rrediffmail.corn

In India seabuckthorn (5131) is represented by two main species, viz. Hippophae rhamnoides L. and H.salicifolia alone with smaller amounts of H. tibetana . Fruit and seeds are Icnown for its nutraceutical properties. The leaves of SBT are reported to be possess many bioactivities such as radioprotect ion, cancer prevention, wound healing and beneficial effects in cardiovascular conditions. Since most of the work was carried out using cnide extracts, it is difficult to relate the activities to their constituents. Also there is lot of variations in the constituents of SRI from different sources. Therefore it was felt that a carefid phytochemical analysis of extract of Indian seabuckthorn is warranted. The leaves were extracted with a battery of solvents! solvent mixtures to bring about broad separation depending on polarities. Since many of the activities were attributed to flavonoids, particular attention was paid to polar constituents. The extracts containing tannins and flavonoid glycosides were separated by the use of judicious mixture of solvents. Protocols using Gel Permeation chromatography were also developed. The constituents were separated. The gallo- and ellagitannias were isolated. Glucose and rhamnose were identified as sugars. Flavonoids were present as rhamnosides or glucosides. lsorhamnetin was the major constituents followed by quercetin and kaempferol with trace amount of myricetin. In contrast to H. rhainnoides, the leaves of H. salicifolia contained mainly quercetin followed by kaempferol and isorhamnetin. Since isolation of compounds from natural sources often present logistic problems (such as difficult accessibility, variation of constituents, seasonal occurrence), we developed a new short synthesis of polyhydroxy- as well as partial methyl ethers of flavonols . The isolation of active compounds and synthesis of flavonol library will be presented. Since antioxygenic activities are important in imparting bioactivities, the antioxidant activities of isolated compounds, extracts and synthetic compounds were determined using free radical scavenging activity (DPPH).

14 next

47 Cosmetic use of Hippophae rhanmoides winter twigs extracts to lighten skin pigmentation.

Alain MINHECK. AM Phyto-Conseil. Courbevoie, France. ameybeeVarclub-intemetir

Although Seabuckthorn fruit or seed oils, and fruit extracts are used m cosmetics formulations as emollients and antioxidants, the potential of branch extracts had not yet been examined. The present study showed that Hippophae rharnnoides winter twigs extracts have the highly valuable property to inhibit pigment formation in skin cells, whereas an extract of twigs collected in summer and bearing leaves had practically no effect. Young Seabuckthom branch extremities and shoots were harvested in winter (Februn or april) or summer (early September) in The Netherlands coastal dunes, particularly taking care not to collect too much woody material. After freeze drying, the twigs were ground to a powder which was extracted overnight either with methanol, 70% ethanol-water, or water. The dry extracts were then partitioned between water and butanol. and the fractions were characterized by HPLC and TLC. The different fractions were thereafter submitted to special tests on cultures of melanocytes (skin cells producing pigment) which allow to evaluate the amount of melanin pigment synthesized. These tests have shown that: stabuck-thorn winter twigs polar extracts inhibit the biosynthesis of melanin pigments by skin cells, a seahuckthom summer twig extract has practically no activity on mclanogenesis, the active compounds responsible for the whitening effect of Hippophac rhanmoides winter twig extracts are water soluble molecules, these active molecules seem to be indole derivatives. Therefore, Hippophae rhamnoides winter twigs extracts could be developed for brightening or whitening cosmetic formulations (Patent Application filed on February 28,2011). This new utilization of Seabuckthom is bound to further increase its economic importance by providing an industrial exploitation during the winter season.

48 Evaluation of effect of Seabuckthorn extract on cognitive impairment

IDharant Paul Attrey.2Annit Kumar Singh. 3.1yoti Katyal
I. Director, Amity Institute of Seabuckthom Research & Ex Director. Amity Institute of Pharmacy, 2. Research Fellow, Amity Institute of Scabuckthom Research. 3. Amity Institute of Pharmacy, Amity University Uttar Pradesh, Sector 125. Expressway. NO1DA —201303, U.P. (INDIA) dpattrey@amity.edu. dpattrey 'yahoo.co.in

Research objective: Evaluation of effect of Seabuckthom extract on cognitive impairment. Experimental materials and methodology: Extraction and characterization of Hippophae rhamnoides extract, toxicity/ Safely studies on lab animals. Efficacy studies of this extract on Scopolamine induced cognitive impairment. Hypothesis used in the Research: Seabuckthom, being a treasure house of a number of nutrients and phenolic compounds having excellent antioxidant and nutritional properties, will prevent/reduce cognitive impairment. Main conclusions: The data indicates that Seabuckthorn extract has very usefill nutrients like B Vitamins (including Vitamin 912), cobalt and folic acid. It was found to be safe and effective in cognitive impairment.
Key words: Seabuckthorn, Cognitive impairment, Phenolic compounds.

49 Possible mechanism of sea buckthorn fruit extract as a functional food in restraint-induced behavioral deficits and brain serotonin metabolism: Focus on 5-HT-IA receptors in depression

Farhat Batoo. "Asad Hussain Shah. 3Sycd Dilnawaz Ahmed, 3Zafar Saicd Saify, IDarakhshan Jabeen Haleem I. Neurochemistry and Biochemical Neuropharnmeology

Research Laboratory. Department of Biochemistry. University of tCarachi, Karachi-75270. Pakistan 2. Department of Plant Breeding and Molecular Genetics. Faculty of Agriculture. University of Azad Janunu and Kashmir, Rawalakot. Azad Kashmir Pakistan 3. International Centre for Chemical and Biological Sciences, FULL Research Institute of Chemistry University of Karachi. Pakistan. batoopit)uotedopk

Sea buckthorn (Hippophae rhainnoides L. spp. Turkestaniea) has been used for centuries in Europe and Asia as a folk medicine for its incredible nutritional and medicinal profiles. Natural products exhibiting antidepressant properties and accordingly, natural medicinal plants may he important sources of novel antidepressant drugs and the usage of plant extracts may be proven better in the management of stress and depression. Depression is an important global public-health issue and is associated with substantial disability. It is a chronic illness and has been estimated to affect unto 21% of the world's population. The present study was designed to investigate the antidepressant-like effects of aqueous fruit extract of SBT in animal models of depression. In first phase of study test rats were treated with oral administration of SRI-I-E (40 mgikg P.O.; 2 weeks) and controls received an equal volume of fresh water. In next phase for one)veck, two groups of animals were exposed to repeated restraint stress (one group from water treated and other group from SRI-FE treated). All groups of animals were separately submitted to forced swim test (PST), open field test (OFT) and elevated plus maze (EPM) tests for the bio-screening of fruit extract with antidepressant profile. Results revealed that the immobility time in the EST was significantly (p-s0.05) reduced and prolong struggling (numbers of jumps) was observed particularly in rats orally administered with SHT-FE (40 nig'kg P.O.) following one week stress when compared with their respective controls. Open filed ambulation, numbers of entries in GA and % time spent in OA were also significantly ($p<0.01$) increased and were more pronounced in SRI-FE treated rats following exposure to repeated restraint stress when compared with their controls. It is concluded that aqueous fruit extract of Sea buckthorn exhibited significant antidepressant-like effects in animal models of depression. This effect is supposed to be mediated via modulation of SLIT-IA receptors in rat brain and possibly explains the antidepressant-like effect of ST3T1FE. Thus it is suggested that SRT fruit extract play a beneficial therapeutic role and combat against a mental illness that is globally known as depression.

50 Effect of feeding seabuckthorn leaves for milk production in cross bred animals

V.K. Sharma, Virendcm Singh
Depn of Animal Nutrition, College of Veterinary & Animal sciences, LIPAgriculture University, Palampur-176062 (HP) India virendrasineh2003(ii(yahoo.com

The study was conducted to evaluate the effect of seabuckthorn leaves on the milk production by replacing the Crude Protein (('P) of the concentrate at 40 and 80 percent in two experimental groups. The control (inup was offered the dry grass s- oat green grass concentrate (1-1). The other groups were offered the same roughage diet except the concentrate Cl' replaced with the scabuckihom leaves at the rate of 40 and 80 per cent levels in the group T 2 and 'T3 respectively. The each group was consisting of 5 milk animals selected in a randomized block design. The replacement of the sealmekthom leaves was done slowly and slowly by increasing 10 per cent

leaves after every 3 days to meet the total replacement of 40 and 80 percent after 12th and 24th day of the experiment in the two treatment groups. The milk production trial was conducted for 60days after achieving the 80 per cent level of scabucktkom leaves in the last group. At the start of the experiment the milk yield. fat and protein was estimated keeping the animals on the routine ration of the University Dairy farm. 6th day onward the treatment ration were offered as mentioned above, At the end of the trial, for 10 days the milk yield. fat and protein per cent was estimated along with the 5days digestibility trial. Infore the Stan of the experiment the milk yield (titer/day) was recorded for 5 days for the selected animals offering them the feed being offend by the University Dairy and after that the 3 groups were made in a randomized design pattern, it was observed that there was no significant effect in the milk yield, milk protein and milk Mt per cent in all the animals. The daily record of the milk yield was kept. The milking was done twice a day. At the end of the 60 days experiment the milk was again tested for 5 days for milk protein, fat, total solids, lactose and ash per cent alone with FCN1 yield and milk yield. Milk production efficiency kg milk/ kg DM intake and kg FCM !kg DM intake was calculated from the data collected. At the end of the experiment the milk yield was 6.344.21, 6.9810.09 and 7.2310.30 in respect to TI. '12 and T3 treatment groups respectively and were highly significant (P-s.0.05) among the different treatment groups. An increase 9.46 and 14.03 per cent was observed in case of milk production in the 72 and 73 treatment groups as compared to T1 treatment group. The increase of 2.34 and 8.26 and 7.94 and 20.25 per cent was observed in case of milk protein and milk fat in 12 and 13 treatment groups as compared to TI treatment group. The milk production efficiency (kg milkfkg. DM1) and (kg FCNV kg D)41) values were also found to be significantly (Pss0.05) MOM in the T2, and 13 treatment glows as compared to TI treatment group. There was significant (P<0.05) increase in the milk production (lit/day) after the experiment and similar trend was seen in ease of milk protein and milk fat per cent. There was a significant (Pc0.05) increase in milk yield, protein and fat in both the treatment groups T2, and T3 as compared to TI treatment group where the seabuckthom leaves were added. This increase was 16.85 and 25.78 per cent in respect to milk yield and 13.25 and 23.31 per cent in respect to milk protein and 11.05 and 23.11 per cent was observed in the treatment groups 12, and T3 as compared to T1 treatment group.

51 Experimental study of proanthocyanidins extract from seabuckthorn seed on the effect of immune regulation in mice

'ton Yuansheng, IXu Rui. 'Wen Zhongping, Xi. Yidan. 2 Fan Bolin I. CONSCO Reabuck-thorn Co., Lid., Beijing,100032 2. Basel Provincial Center for Disease Control and Prevention ,Wulian,430079

This Study investigated the effects of immune regulation of proanthocyanidins extract from Seabuckthom seed in mice. The dosages in mice was 10, 20, 30 folds of human dosage(0.433, 0.867, 1.300g(kg). In addition and the negative control and solvent control were designed. Kunming mice (SPF degree) were continuous administrated orally, and then started the experimental observation. The experimental data were evaluated by 'I-test and P<0.05 was considered to be statistically significant. The results showed that compared with solvent control group and negative control gout), the proanthocyanidins extract from Scabuckthom seed significantly enhanced Con A-induced spleen lymphocyte proliferation of mice, and significantly increased DIOR-induced delayed allergy, it also significantly increased scrum hemolysin, and

significantly enhanced the ability of peritoneal macrophage phagocytizing chicken erythrocytes and enhanced the ability of carbon clearance in mice, In addition this extract significantly enhanced the function of antibody producing cells. On the other hands compare with the negative control and solvent control group, each dosage of the extract had no effect on spleen and thymus. In conclusion, Proanthoeyanidins extract from Seabuckthom seed can significantly improve thc immune system of mice, at the same time it will not affect the immune organs of mice, and has negative impact. Key words: Seabuckthom; Proanthocyanidin: Immune

52 Prophylactic efficacy of seabuckthorn oil and omcproazole in gastric erosions and ulcerations in dogs

'S.P Tyagi. A.C., 2Varshney, sAmit Kumar
Department of Surgery and Radiology, College of Veterinary and Animal Sciences CSK Himachal Pradesh Agricultural University. Palampur. H.P., India
sptyagivet@gmail.com

In the present study the comparative prophylactic efficacy of seabuckthom (Hippophae rhamnoides) seed oil and omeprazole in reducing the severity of dexamethasone-induced gastric erosions and ulcerations (GUE) was evaluated in dogs. 16 adult healthy dogs were randomly divided in to 4 equal groups. To induce CUE, Inj. 'Dexamethasone was administered @ I mg/kg intravenously once a day to all the animals for a period of 15 days. After 15 days, group I was kept as negative control and no treatment was given and spontaneous healing time of GUE lesions was recorded. Whereas, seabuckthom seed oil @ 5 raliklog in group II, omeprazole @ 0.7 mg/kg in group III and the omeprazole @ 1.5 mg/kg in group IV twice a day were administered orally starting from day I and continued till gastric lesions healing. The development of gastric lesions (erosions/ulcers) and their subsequent healing course was periodically evaluated by clinical. haematological and endoscopic examinations and compared in between the groups. The clinical symptoms of gastric erosions/ulceration and endoscopic ulcer index were significantly lesser in group IV followed by group II as compared to other groups till 12th day. The gastric lesions healing after discontinuance of dexamethasone on 15th day was fastest in group IV followed by group 11. III and I, however it was not much different in groups I and III. Key words: Seabuckthom, dog, gastric erosions and ulcerations

53 Effects of seabuckthorn polysaccharide on blood glucose of Normal Mice and Diabetic Mice

'Goo Fenipcia "Zeng Yang 'Du :ulna 'Ma .1 ixiong Meng 1. College of Life Sciences and Geography, Qinghai Normal University, Xining, China, 810008 2. Key Laboratory of Qinghai-Tibet Plateau Environment and Resource Education Department, Xining, China, 810008 3. Clinical Pharmacology, Affiliated Hospital of Qinghai University, Xining, China, 810007 zy-3®263.corn

Research objective: to explore die effects of Seabuckthom crude polysaccharide on the glucose tolerance in normal mice, as well as on the hyperglycemia in diabetic mice. Methodology: according to the mouse glucose tolerance model, with scabuckthom polysaccharide as the experimental drug, to respectively observe the

effects on normal mice's blood glucose with overload dose of sucrose, glucose. maltose, starch; to respectively observe the effects of different doses of seabuckthom polysaccharide on the model mice of alloxaminduced hyperglycemia and streptozotociminduced hyperglycemia. By analysis of the data collected from the former group (Glucose Tolerance Mice Model Group) and the latter group (alloxan (streptozotocin) model group), the starch group's blood glucose lowers most significantly (P<0.01), followed by the sucrose group (P <0.05); for the alloxan (streptozotocin) model group, after 2 weeks' and 4 weeks' drug administion, the hypoglycemic effect is more obvious for the high•dose group (P<0.0 I, P<0.05) and the efficacy rate of reduction is 31.72% (33.14%) and 45.70% (43.78 %); the low-dose group (P <0.05, P <0.05)'s efficacy rate of reduction is 28.02% (27.07%) and 3938% (36.43%). Main conclusion: Seabuekthom polysaccharide can improve glucose tolerance in normal mice. Scabuckthom polysaccharide has a good hypoglycemic effect on alloxan-induced and streptozotocin-induced hyperglycemia in mice, and the effect is proportional to the dose.
Kcy words: Scabuckthom crude polysaccharide, mice, glucose tolerance, the animal model of hyperglycemia

54 Immunomodulation by dietary seabuckthorn

Varshneya C
CSK I limachal Pradesh Agricultural University, Palampur . Himachal Pradesh (India)
evarsluteyalThgrnaileom eyarshneya@hotmailcom

The incorporation of medicinal herbs in vOTIOUS medicaments has been the hall mark of Indian traditional health care system. The herbs are being used since antiquity on the empirical basis. Therefore, there is immense need of pharmacological and scientific validation of the folklore claims of various herbal inedicamenB. Seabuckthom has a long history of its usage in various Indian and Chinese health care systems. Many of its therapeutic used such as anti-atherosclerotic, antiarrythmic. hepato -protective , skin protective anti ideerogenic actions have been vividly described. In this paper immunomodulatory actions of dietary seabuckthom am described.Thc immunodulation was assessed on the basis of Immoral, cellular and non specific immunity parameters. White albino rats were fed dietary levels of 200, 400 and 800 ppm of powdered berries of seribuckthom over a period of 2 months (56 days).There was significant increase in total serum protein and serum globulin and A:G ratio . A significant increase (P <0.05) was observed in the hacmagglutination titres at 800 ppm on day 42 of feeding. The macrophage function was significantly increased in nits fed 400 and 800 ppm in a dose dependent manner. The delayed hypersensitivity reaction to CAFE was found to be significantly higher(P <0.05) at 48 and 72 h in rats fed 800 ppm. The hismpathology of lymph node and spleen revealed a marked increase in B cell activity. In poultry birds dietary levels of 500 and 1000 ppm of seabuckthorn berries were fed in ration for a period of 62 days. Scabuckthom did not influence the growth of the poultry birds at the given dietary levels. Scabuckthom fed birds had higher A: G ratio in scabuckthom fcd birds at both the dietary levels. Significantly higher (P <0.05) HI titres against NCD virus and NBT positive cells indicating macrophage function) were also observed at both the dietary levels A sipiticantly higher delayed hypersensitivity reaction to DNFB was also observed in seabuckthom fed birds. A significant lymphocytosis was observed in birds fed seabuckthom at 1000 ppm dietary levels. The weight of spleen, bursa and

thymus were higher (P<0.05) in scabuckthom fed animals. flistopathology of the skin of scabuckthom fed birds showed sloughing of epidermis. formation of erosive and ulcerative lesions and infiltration of mononuclear cells apart from lymhofollicular reactions. The experimental studies have, thus, indicated stimulation of immune response following dietary intake.

55 Experimental study on effect of Tiangui Gengnian Soft Capsule on the mitochondrial functiong inflluence in aged female rats

Shentu Pingping. Wei Xiiahen, Wang Zhenlin, ct al TIAN KU! Medicine biology company Department of Xi'an fiaotong University,Xi'an (710061) weixiazhemir,hottnail.com

To investigate the effect of Tiangui Gengnian Soft Capsulc(TGC).which mainly consists of seatnickthorn fatty acid, on respiratory chain electron transport and oxidative phosphorylation of liver mitochondrial respiratory chain in aged female rats, in order to explore the mechanism of anti-aging. Methods Low(1.125g/kg),middlc(2.25glg)and high(4.5g/Icg) dose of TGC were administrated by gastrogavage to young and aged(20 months old) female rats for 90 days, and diethylstilbestrol(0.02mgekg) was used as a positive control. The activities of respiratory chain complexes. ATP synthatic. suecinate dehydrogenase (SDK Ca2+-ATPase and respiratory function(PCR.ADP/OPPR) were detected. Results The intervention of TGC could cause increase of respiratory chain complex I ,Ill, ATP synthase, Ca24-ATPase activities and ADPIO,OPR, which were significantly different to that in the aged group(P<0.05).There was no obvious effect of SDH activity level (P>0.05) . Conclusion The anti-aging effect of TGC is possibly realized by way of improving mitochondrial respiratory function and facilitating ATP synthesis, thus to adjust each system comprehensively. Key words: TianGui Gengnian Soft capsule; mitochondrion; energy metabolism; anti-aging

56 Investigation of antibacterial properties of seabuckthorn (1-lippophae rhanmoides L.) leaf extracts against common skin and wound microbial pathogens

Mandeep Sharma, Rajesh Chahota, llarshit Vcrmal, Akanksha Palial
Department of Veterinary Microbiology, DGCN College of Veterinary and Animal Sciences. CSK HPKV, Palampur - 176 062 rehahota(áyahoo.com

Different parts of seabuckthom (Hippophae rhamnoides L) plant arc reported to have antimicrobial properties. In this study, hof and cold niethartolic extracts of own H. rhamnoidcs leaves were tested against bacterial and fungal species commonly ass/36mM with skin and wound infections of domestic animals. Total 130 clinical bacterial and 15 fungal clinical isolates obtained from skin and wound infections including Staphylococcus aureus (70), Streptococcus pyogenes. (3). Klebsiella spp. (13), Pseudomonas acruginosa (10). Protons spp. (12). Bacillus spp. (23), Mycosporum gypseum (0), Trichophyton ntbrum (4) and Epidennophyton floceosurn (3) were tested by disc diffusion method. Different concentrations of leaf extracts i.e. 0.50%, 2.0%, 3.0%, 4.0% and 5.0% were listed against I z 108 efutinl bacteria inoculated on Muller-Hilton agar (NOW. Different bacterial and fungal species showed varied level of growth inhibition by scabuckthom leaf extracts but all isolates

of Klebsiella spp. Bacillus spp. were found resistant against both types of leaf extracts. The inhibitory ellbet of SAT leaf COMM at 5% concentration was observed to be maximum as compared to the standard drugs used as positive control. All the tested fungal isolates were found to be least sensitive to inhibitory effects of seabuckthom leaf extracts. When comparing the two types of extracts, the methanolic cold extract was observed to have better antimicrobial activities than the methanolic hot extract, may be due higher concentration of active ingredients. Minimum inhibitory concentration of leaf extract VMS also determined against various bacterial isolates by resazurin reduction method. The M1C against leaf extract sensitive bacterial isolates was found to be in range of 3% to 4%. These results showed the potential of seabuckthom leaves ingredients to be used as alternate antimicrobial agents.

57 Studies on effects of scabuckthorn (Hipphophae L.) leaf extract and seed oil on infected cutaneous wound healing process in rabbit experimental model

IRajesh Chahota, INfandcep Sharma, IlIarshit Verna, lAkanksha Paha!, 212.D. Paul, 3Arvind Shanna , 4N. P. Kuradey
I. Depanment of Veterinary Microbiolgy, DGCN College of Veterinary and Animal Sciences, CSK HPKV, Palampur - 176 062 2. Department of Veterinary Pathology, DGCN College of Veterinary and Animal Sciences. CSK HPKV, Palampur - 176 062 3. Department of Veterinary Surgery, DGCN College of Veterinary and Animal Sciences, CSK IIPK V, Palmnpur -176 062 4. Indian Veterinary Research Institute Regional Station, Palampur - 176 062 rertahop(alvahoo.com

Wound healing properties of leaves and seed oil of scabuckthom (Hipphophae L.) plant was investigated using rabbits as 171.4111%0 experimental modcl. Methanolic extract from scabuckthom green leaves and seed oil by cold press method was obtained from the local land races of the plant. Excision cutaneous wounds in 12 rabbits, divided into 4 groups including controls were surgically created on dorsal side. Wounds or all test animals were inoculated with pyogcnic Staphylococcus aurcus strain at 200 du per ml dose rate. In group-I. wounds were dressed with a sterilized vasalene base, while in group-II, 5% ma leaf extract was used for dressing. In group-Ill, dressing was done with seed oil and in group-IV wounds were dressed with 5% povidone iodine. The effects of topical applications were graded by clinical observations. haematological parameters and per cent wound contraction in all g ps. These observations were further coroborratcd by histopathological examination of biopsies taken from the skin. Results from these findings suggested that scabuckthorn leaf extract and seed oil may be used as natural antimicrobial agents and also they significantly enhance the rate of wound healing process in rabbits model.

58 Enhanced cAMP/PKA pathway by seabuckthorn fatty acids in aged rats

Hu Rui, Yuan 13inxin Wei Xivhcn, Zhao Lintel . Songlanlan
Department of Pharmacology; Xran Jiaotong University, ?Wan, China Tiunkui Biology Medical Company, Xi'an liaotong University. 710061, Xi'an, China vialliazhenceaottnailcom

Seabuckthom fatty acids were extracted by crushing and centrifuging from china seabuckthom fruit. We detected cyclic nucleotides concentration in serum of different

stages in aged rats (from 16 to 21 months), cyclic nucleotides concentration, PRA activity and PM activity in hepatic tissue in aged rats by seabuckthom fatty acids. Our data showed that the serum cAMP concentration decreased, accompany with the cGMP concentration increased and the imbalance of the cAMPicUMP ratio in aged process. This kind of change equally in the hepatic tissue, thc cANIP concentration decreased, PRA activity also decreased, but no change of the cAMP particularity PDF. activity. And the SBFAs raised serum cAMP level in different stages, and raised the cAMP concentration and PICA activity of hepatic tissue, but did not effect the eAMP particularity POE activity. Our study demonstrated that it is imbalance of the cAMP/cOMP ratio in aged process. SBFAs enhanced the a/NMI:A pathway, regulated cAMP/cGMP ratio in aged rats.

59 Modulation of Hypoxia-Induced Pulmonary Vascular Leakage in Rats by Seabuckthorn (Hippophae rhamnoides L.)

Jayamurthy Purushothaman,1, 2 Geetha Suryakumar,1 Dhananjay Shukla,1 Himani Jayamurthy,1, 2 Harinath Kasiganesan,3 Rajesh Kumar,1 and Ramesh Chand Sawhney1
1Defence Institute of Physiology and Allied Sciences, DRDO, Ministry of Defence, Timarpur, Delhi 110054, India
2National Institute for Interdisciplinary Science and Technology, CSIR, Industrial Estate, Pappanamcode, Trivandrum, Kerala, India
3 Gazes Cardiac Research Institute, Medical University of South Carolina, Charleston, SC, USA
Correspondence should be addressed to Geetha Suryakumar, geethasuryakumar@yahoo.com

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Cerebral and pulmonary syndromes may develop in unacclimatized individuals shortly after ascent to high altitude resulting in high altitude illness, which may occur due to extravasation of fluid from intra to extravascular space in the brain, lungs and peripheral tissues. The objective of the present study was to evaluate the potential of seabuckthorn (SBT) (Hippophae rhamnoides L.) leaf extract (LE) in curtailing hypoxia-induced transvascular permeability in the lungs bymeasuring lung water content, leakage of fluorescein dye into the lungs and further confirmation by quantitation of albumin and protein in the bronchoalveolar lavage
fluid (BALF). Exposure of rats to hypoxia caused a significant increase in the transvascular leakage in the lungs. The SBT LE treated animals showed a significant decrease in hypoxia-induced vascular permeability evidenced by decreased water content and fluorescein leakage in the lungs and decreased albumin and protein content in the BALF. The SBT extract was also able to significantly attenuate hypoxia-induced increase in the levels of proinflammatory cytokines and decrease hypoxia-induced oxidative stress by stabilizing the levels of reduced glutathione and antioxidant enzymes. Pretreatment of the extract also resulted in a significant decrease

in the circulatory catecholamines and significant increase in the vasorelaxation of the pulmonary arterial rings as compared with the controls. Further, the extract significantly attenuated hypoxia-induced increase in the VEGF levels in the plasma, BALF (ELISA) and lungs (immunohistochemistry). These observations suggest that SBT LE is able to provide significant protection against hypoxia-induced pulmonary vascular leakage.