Evaluation of the efficacy and safety of "Anti-Wrinkle cream" in the treatment of facial skin wrinkles: A prospective, open, phase III clinical trial

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ABSTRACT

Aging of the skin is the result of continuous "wear and tear" processes. Chronological skin aging is a universal and inevitable process, while in contrast, photoaging results from the UV rays of sunlight, and the damage becomes apparent in sun-exposed skin. The "Anti-Wrinkle cream" is a polyherbal formulation recommended for the management of skin wrinkling and this study was planned to evaluate the efficacy and safety of the "Anti-Wrinkle cream" in the management of facial skin wrinkles.

This study was a prospective, open, phase III clinical trial, conducted as per the ethical guidelines of Declaration of Helsinki. A total of 25 patients, of both sexes, aged from 35 to 65 years, with wrinkled facial skin, and who were willing to give informed consent were included in the study. Persons with known hypersensitivity to any of the ingredients of the formulation, any facial wound or abrasion, and who were not willing to give informed consent were excluded from the study. A baseline history was obtained in order to determine the patient's eligibility for enrolment in the trial. All the patients were advised to apply the "Anti-Wrinkle cream", twice-a-day for a period of 6 weeks. The subjective improvement evaluation was done by a predefined global grading system, which included the following gradations: "No improvement", "fair improvement", "remarkable improvement", "very good improvement" and "excellent improvement". All the patients were followed up for a period of 6 weeks, and at each weekly follow-up visit, the dermal safety and the improvement in skin wrinkling was evaluated. At the end of the 6th week, the overall performance of the "Anti-Wrinkle cream" was evaluated. The predefined primary end point was reduction in skin wrinkles, and the predefined secondary safety endpoints (for short- and long-term) were assessed by incidence of adverse events, and patient compliance to the therapy. All the adverse events reported or observed by the patients were recorded with information about the severity, date of onset, duration and action taken regarding the study drug. Statistical analysis was done according to intent-to-treat principles.

This study observed a significant improvement in the facial skin wrinkles, after a week's application in almost all persons and also, there were no clinically significant adverse reactions, with excellent overall compliance. This beneficial effect might have been due to the synergistic antioxidant, anti-inflammatory, and UVR protective properties of the ingredients, which also have excellent safety profile. Therefore, it may be concluded that "Anti-Wrinkle cream" is effective and safe for usage in the management of facial skin wrinkles.

ABBREVIATIONS

DNA	:	Deoxyribonucleic acid
LPS	:	Lipopolysaccharide
NO	:	Nitric oxide
PAF	:	Plasminogen activating factor
ROS	:	Reactive oxygen species
UVR	:	Ultraviolet rays

INTRODUCTION

Aging of the skin is the result of continuous "wear and tear" processes, which damage cellular DNA and proteins. Aging has been classified into 2 distinct types, viz. “chronological skin aging” and “photoaging” and both types have distinct clinical and histological features. Chronological skin aging is a universal and inevitable process, characterized primarily by physiologic alterations in skin function. In chronological skin aging, keratinocytes are terminally unable to form a functional stratum corneum, and the rate of formation of neutral lipids (which contribute to the barrier functions) gets slowed, resulting in dry, pale skin with fine wrinkles. In contrast, photoaging results from the UV rays of sunlight, and the damage becomes apparent in sun-exposed skin. Characteristics of photoaging are dry and sallow skin, displaying fine wrinkles as well as deep furrows, resulting from the disorganization of epidermal and dermal components associated with elastosis and heliodermatitis.[1]

The “Anti-Wrinkle cream” is a polyherbal formulation recommended for the management of skin wrinkling, and it contains the extracts of *Aloe vera, Papaver rhoeas, Vitis vinifera, Citrus limon, Solanum lycopersicum, Santalum album, Rubia cordifolia, Saussurea lappa, Lens culinaris, Symplocos racemosa, Amomum subulatum, Curcuma longa, Glycyrrhiza glabra, Valeriana wallichii, Vetiveria zizanoides* and oil of *Triticum sativum.* This study was planned to evaluate the efficacy and safety of the “Anti-Wrinkle cream” in the management of facial skin wrinkles.

Aim of the study

This study was planned to evaluate the clinical efficacy and safety (short- and long-term) of the “Anti-Wrinkle cream” in the management of facial skin wrinkles.

MATERIALS AND METHODS

Study design

This study was a prospective, open, phase III clinical trial, conducted at the Department of Dermatology of Apollo Hospitals, Chennai, India, as per the ethical guidelines of Declaration of Helsinki, from June to September 2004. The study protocol, case report forms, regulatory clearance documents, product related information and informed consent form (in Tamil and English) were submitted to the “Institutional Ethics Committee” and were approved by the same.

Inclusion criteria

A total of 25 patients, of both sexes, aged from 35 to 65 years, with wrinkled facial skin, and who were willing to give informed consent were included in the study.

Exclusion criteria

Persons with known hypersensitivity to any of the ingredients of the formulation, any facial wound or abrasion, and who were not willing to give informed consent were excluded from the study.

Study procedure

A baseline history was obtained in order to determine the patient’s eligibility for enrolment in the trial. The baseline assessment included personal data, a description of symptoms and details of past medical history (family history, history of possible exacerbating factor/s, etc.). All the patients were advised to apply the “Anti-Wrinkle cream”, twice-a-day for a period of 6 weeks. The subjective improvement evaluation was done by a predefined global grading

system, which included following gradations: “No improvement”, “fair improvement”, “remarkable improvement”, “very good improvement” and “excellent improvement”.

Follow-up and monitoring

All the patients were followed up for a period of 6 weeks, and at each weekly follow-up visit, the dermal safety, and the improvement in skin wrinkling was evaluated. At the end of the 6th week, the overall performance of the “Anti-Wrinkle cream” was evaluated.

Primary and secondary end points

The predefined primary end point was reduction in skin wrinkles, and the predefined secondary safety endpoints (for short- and long-term) were assessed by the incidence of adverse events and patient compliance to the therapy.

Adverse events

All the adverse events either reported or observed by the patients were recorded with information about the severity, date of onset, duration and action taken regarding the study drug. Relation of adverse events to the study medication were predefined as “Unrelated” (a reaction that does not follow a reasonable temporal sequence from the time of administration of the drug), “Possible” (follows a known response pattern to the suspected drug, but could have been produced by the patient’s clinical state or other modes of therapy administered to the patient), and “Probable” (follows a known response pattern to the suspected drug that could not be reasonably explained by the known characteristics of the patient’s clinical state).

Patients were allowed to voluntarily withdraw from the study if they experienced serious discomfort during the study or sustained serious clinical events requiring specific treatment. For patients withdrawing from the study, efforts were made to ascertain the reason for dropout. Non-compliance was not regarded as treatment failure and reasons for non-compliance were noted.

Statistical analysis

Statistical analysis was done according to intent-to-treat principles. The changes in various parameters from baseline values and the values after 6 weeks were evaluated by “Paired ‘*t*’ Test”. The minimum level of significance was fixed at 99% confidence limit and a 2-sided *p* value of <0.0001 was considered significant.

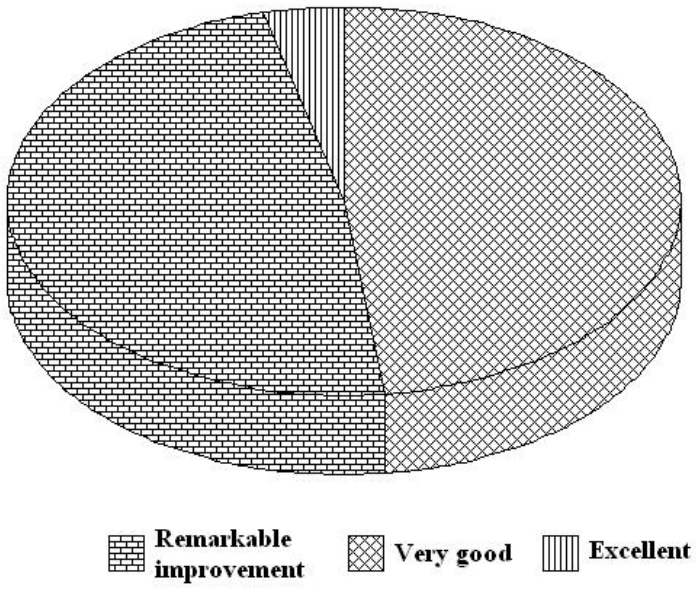

Figure 1: Response to drug treatment (Anti-Wrinkle cream)

RESULTS

A total of 25 (15 females and 10 males) patients were enrolled in the study. There was significant improvement in the facial skin wrinkles, after a week’s application, and the improvement trend continued, till the end of the study period. Out of 25 subjects, 12 (48%) had “remarkable” improvement, 12

(48%) had “very good” improvement, and 1 (4%) had “excellent” improvement in the facial skin wrinkles (Figure 1).

There were no clinically significant adverse reactions, either reported or observed, during the entire study period and the overall compliance to “Anti-Wrinkle cream” was excellent.

DISCUSSION

Complex biochemical processes precede aging, and it involves the ROS and free radicals. Results from various studies suggest that the antioxidative ability against ROS generation in the skin, possessed by antioxidant enzymes and low molecular weight antioxidants, is lowered age dependently.[2] A reduction in the number of epidermal Langerhans' cells is responsible for a decrease in delayed immune responsiveness in skin, as observed in the elderly, and reduced numbers of fibroblasts and mast cells are typical histologic findings in the aging human dermis. Collagen bundles also become fragmented, less elastic and more brittle. Telomere shortening at the end of chromosomes is probably the major mechanism of cellular senescence in skin.[3]

On the other hand that are the senescent keratinocytes become resistant to apoptosis and may survive for a long time (giving time for DNA and protein damage to accumulate with possible implication for carcinogenesis). The numbers of melanocytes decrease with age, and dysregulation of melanocyte density result in freckles, guttate hypo-melanosis, lentigines and nevi. The numbers of dendritic Langerhans cells also decrease with age and the cells get less dendrite, with reduction in the antigen-trapping capacity. Aging involves dermal changes, (such as damage to elastic and collagen fibers), giving thickened, tangled, and degraded non-functional fibers. Collagen intermolecular cross-links increase with age converting divalent cross-links into mature trivalent cross-links (of histidinohydroxylysinonorleucine)’ and the mechanisms involved are an enzyme-controlled process of maturation and a non-enzymatic glycosylation (the Maillard reaction), leading to cross-links in proteins (such as in collagen between arginine and lysine). Such kind of changes are also seen in case of patients with diabetes mellitus.[4]

Atmospheric pollutants are an important source of oxidative stress, and skin, which has a highly differentiated complex organizational structure, is particularly vulnerable to free radical damage because of its contact with oxygen and with other environmental stimuli.[5]

This study observed a significant improvement in the facial skin wrinkles, after a week’s application in almost all persons, and also, there were no clinically significant adverse reactions, with excellent overall compliance. This beneficial effect might have been due to the synergistic actions of the ingredients of “Anti-Wrinkle cream”, which have been documented by various researchers.

The principle constituents of *Aloe vera* are anthraquinones (aloe-emodin and aloin A/barbalin),[6] Cinnamoyl, p-coumaroyl, feruloyl, caffeoyl aloesin, aloemannan[7] acemannan, verectin[8] elgonica dimer A and bisbenzopyran.[7] *Aloe vera* has been long recognized as an effective remedy for wound-healing and other inflammatory skin disorders.[9] Esteban et al. identified a peroxidase, which may scavenge H_2O_2 in skin surface and thereby prevent free radical damage of the skin.[10] In another study, Byeon et al. demonstrated that *Aloe vera* contains multiple immunoprotective factors and oligosaccharides from *Aloe vera* prevent UVR-induced suppression of delayed type hypersensitivity (DTH), by reducing keratinocyte derived immunosuppressive cytokines.[11] In a study conducted by Heggers et al. treatment

with *Aloe vera* was found to expedite wound contraction, due to an increased collagen activity, consequently improving the collagen matrix and enhancing the breaking strength.[12]

The principle constituents of *Rubia cordifolia* are hydroxyanthraquinones, gallic acid, tannins[13] alizarin, rubimallin, beta- sitosterol and daucosterol.[14] Rubiadin, a dihydroxy anthraquinone, isolated from *Rubia cordifolia*, possesses potent antioxidant property, and prevents lipid peroxidation in a dose dependent manner.[15] Tripathi et al. reported that, *Rubia cordifolia* maintained the reduced glutathione content in the physiological system.[16]

The principle constituents of *Vitis vinifera* are flavonoids (gallic acid, catechin, epicatechin, resveratrol, gallocatechin, gallic acid, and ellagic acid)[17] and glycosylated stilbenes: (Z)-piceatannol-(3,5,3',4'-tetrahydroxystilbene)-3-O-beta-d-glucopyranoside and (E)- and (Z)-resveratrol-(3,5, 4'-trihydroxystilbene)-4'-O-beta-d-glucopyranoside, *which have* potent antioxidant potential.[18]

The principle ingredients of *Citrus limon* are volatile oils and the major component is limonene.[19] Naringin, a bioflavonoid predominant in *Citrus limon*, scavenges free radicals and reduces radiation-induced damage.[20] Calabrese et al. demonstrated the strong antioxidant activity of *Citrus limon* and reported a significant increase in the antioxidative potential of skin biosurface.[5]

The principle constituents of *Solanum lycopersicum* are lycopene, betacarotene, vitamin E,[21] lutein[22] and ferulic acid[23] all of which possess potent antioxidant potential. Balestrieri et al. observed that alpha-tocopherol in *Solanum lycopersicum* potentiates the effects of lycopene on the modulation of PAF and acyl-PAF biosynthesis in endothelial cells during oxidative stress.[24]

The principle ingredient of *Santalum album* is santalol[25], santalbic acid[26] and alpha-trans-bergamotenol.[27] Jagetia et al. demonstrated the NO scavenging potential of *Santalum album.*[28]

The principle constituents of *Saussurea lappa* are sesquiterpene lactones (costunolide and dehydrocostus lactone) and amino acid-sesquiterpene conjugates (saussureamines A and B), which inhibit NO production in LPS-activated peritoneal macrophages.[29] Jin et al. demonstrated the suppression of LPS-induced NO production by a dehydrocostus lactone of *Saussurea lappa*.[30] Cho et al. demonstrated that cynaropicrin, a sesquiterpene isolated from *Saussurea lappa* strongly inhibits TNF-α release from LPS-stimulated macrophage[31]

The principle constituents of *Symplocos racemosa* are salireposide and benzoylsalireposide.[32] The active constituents of *Amomum subulatum* are protocatechualdehyde and protocatechuic acid, which have potent antioxidant activity.[33] Dhuley et al. documented that, *Amomum subulatum* supplementation increases the antioxidant enzyme activities, and the lipid conjugated dienes and hydroperoxides, which are the primary products of lipid peroxidation.[34]

The principle ingredients of *Curcuma longa* are curcuminoids,[35] demethoxycurcumin, bisdemethoxycurcumin.[36] **A**lpha-curcumin, alpha-zingiberene, 1,8-cineole and zerumbone[37] Various studies have demonstrated the antioxidant potential of *Curcuma longa*.[38] The phenolic liposoluble 'co-antioxidants' of *Curcuma longa*, have a significant protective effect against age-related atherogenesis and immune dysfunction.[39] In a study by Ray et al., curcumin-treated cells showed a marked reduction in the expression of alpha-5-beta-1 and alpha-5-beta-3 integrin receptors. In addition, curcumin treatment inhibited pp125 focal adhesion kinase (FAK), tyrosine phosphorylation of a 120 kD protein, and collagenase

activity. Curcumin enhances the expression of antimetastatic proteins, tissue inhibitor metalloproteinase (TIMP)-2, nonmetastatic gene 23 (Nm23), and E-cadherin.[40]

The active ingredients of *Glycyrrhiza glabra* are glabridin, glabrene, licochalcone A, licoricidin, licoisoflavone B, isoflavonoids (3-arylcoumarin, pterocarpan, and isoflavan), gancaonols, vestitol, licoricone, 1-methoxyphaseollidin, gancaonol C, glycyrin, formononetin, isolicoflavonol, glyasperin D, 6,8-diprenylorobol, gancaonin I, dihydrolicoisoflavone A, and gancaonol B,[41] bioflavonoid licoagrodin, licoagrochalcones B, C, D, licoagroaurone, flavonoids (licochalcone C, kanzonol Y, glyinflanin B and glycyrdione A), isoflavone glycoside, licoagroside A, a maltol glycoside, licoagroside B[42], licoleafol, uralstilbene, 8-dimethylallyleriodictyol, sophoraflavanone B, gancaonin R, and 6-dimethylallyleriodictyol[43], flavonoids (glabranin, pinocembrin, licoflavone, wighteone)[44] glabrizoflavone[45] isoliquiritigenin[46] and aglycone beta-glycyrrhetinic acid.[47] *Glycyrrhiza glabra* has potent antioxidant activity.[48] In a study by Nose et al. the polysaccharide fractions of *Glycyrrhiza glabra* induced NO production by peritoneal macrophages.[49] In a study by Saeedi et al., *Glycyrrhiza glabra* has found to e an effective agent for the treatment of atopic dermatitis.[50]

The principle ingredients of *Valerina wallichii* are flavonoids (6-methylapigenin and hesperidin).[51] The chemical components of *Vetiveria zizanioides* are sesquiterpenes (valencene, 9-octadecenamide, 2,6,10,15,19,23-hexamethyl-2, 6,10,14,18,22-tetracosahexaene, 1,2-benzendicarboxylic acid, and diisooctyl ester).[52] Paranich et al. observed the tocopherol redox-system stimulation by *Triticum sativum,*[53] and Ferrando et al. reported the usefulness of *Triticum sativum* in diverse hyperkeratotic skin conditions (psoriasis, xerosis, pityriasis rubra pilaris, stuccokeratosis, seborrheic dermatitis, stasis dermatitis, and pityriasis lichenoides chronica).[54]

The excellent effects of "Anti-Wrinkle cream", might have been due to the synergistic antioxidant, anti-inflammatory, and UVR protective properties of the ingredients, which also have excellent safety profile.

CONCLUSION

Aging of the skin is the result of continuous "wear and tear" processes. Chronological skin aging is a universal and inevitable process, while in contrast, photoaging results from the UV rays of sunlight, and the damage becomes apparent in sun-exposed skin. The "Anti-Wrinkle cream" is a polyherbal formulation recommended for the management of skin wrinkling, and this study was planned to evaluate the efficacy and safety of the "Anti-Wrinkle cream" in the management of facial skin wrinkles.

This study observed a significant improvement in the facial skin wrinkles, after a week's application in almost all persons; and also, there were no clinically significant adverse reactions, with excellent overall compliance. This beneficial effect might have been due to the synergistic antioxidant, anti-inflammatory, and UVR protective properties of the ingredients, which also have excellent safety profile. Therefore, it may be concluded that "Anti-Wrinkle cream" is effective and safe for usage, in the management of facial skin wrinkles.

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References

1. Hashizume H. Skin aging and dry skin. *Dermatol*. 2004; 31(8): 603-609.
2. Yasui H, Sakurai H. Age-dependent generation of reactive oxygen species in the skin of live hairless rats exposed to UVA light. *Exp. Dermatol*. 2003; 12(5): 655-661.
3. Roupe G. Skin of the aging human being. *Lakartidningen*. 2001; 98(10): 1091-1095.
4. Wulf HC, Sandby-Moller J, Kobayasi T, Gniadecki R. Skin aging and natural photoprotection. *Micron*. 2004; 35(3): 185-191.
5. Calabrese V, Scapagnini G, Randazzo SD, Randazzo G, Catalano C, Geraci G, et al. Oxidative stress and antioxidants at skin biosurface: a novel antioxidant from lemon oil capable of inhibiting oxidative damage to the skin. *Drugs Exp. Clin. Res.* 1999; 25(6): 281-287.
6. ElSohly MA, Gul W, Murphy TP. Analysis of the anthraquinones aloe-emodin and aloin by gas chromatography/mass spectrometry. *Int .Immunopharmacol*. 2004; 4(14): 1739-1744.
7. Saleem R, Faizi S, Siddiqui BS, Ahmed M, Hussain SA, Qazi A, et al. Hypotensive effect of chemical constituents from Aloe barbadensis. *Planta Med*. 2001; 67(8): 757-760.
8. Yagi A, Takeo S. Anti-inflammatory constituents, aloesin and aloemannan in Aloe species and effects of tanshinon VI in *Salvia miltiorrhiza* on heart. *Yakugaku Zasshi.* 2003; 123(7): 517-532.
9. Kostalova D, Bezakova L, Oblozinsky M, Kardosova A. Isolation and characterization of active compounds from *Aloe vera* with a possible role in skin protection. *Ceska. Slov. Farm*. 2004; 53(5): 248-251.
10. Esteban A, Zapata JM, Casano L, Martin M, Sabater B. Peroxidase activity in *Aloe barbadensis* commercial gel: Probable role in skin protection. *Planta Med*. 2000; 66(8): 724-727.
11. Byeon SW, Pelley RP, Ullrich SE, Waller TA, Bucana CD, Strickland FM. *Aloe barbadensis* extracts reduce the production of interleukin-10 after exposure to ultraviolet radiation. *J. Invest. Dermatol*. 1998; 110(5): 811-817.
12. Heggers JP, Kucukcelebi A, Listengarten D, Stabenau J, Ko F, Broemeling LD et al. Beneficial effect of *Aloe* on wound healing in an excisional wound model. *J. Altern. Complement Med*. 1996; 2(2): 271-277.
13. Cai Y, Sun M, Xing J, Corke H. Antioxidant phenolic constituents in roots of *Rheum officinale* and *Rubia cordifolia*: Structure-radical scavenging activity relationships. *J. Agric. Food Chem*. 2004; 52(26): 7884-7890.
14. Qiao YF, Wang SX, Wu LJ, Li X, Zhu TR. Studies on antibacterial constituents from the roots of *Rubia cordifolia* L. *Yao Xue Xue Bao*. 1990; 25(11): 834-839.
15. Tripathi YB, Sharma M, Manickam M. Rubiadin, a new antioxidant from *Rubia cordifolia*. *Indian J. Biochem. Biophys*. 1997; 34(3): 302-306.
16. Tripathi YB, Sharma M. The interaction of *Rubia cordifolia* with iron redox status: A mechanistic aspect in free radical reactions. *Phytomed.* 1999; 6(1): 51-57.
17. Yilmaz Y, Toledo RT. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *J. Agric. Food Chem*. 2004; 52(2): 255-260.
18. Waffo Teguo P, Fauconneau B, Deffieux G, Huguet F, Vercauteren J, Merillon JM. Isolation, identification, and antioxidant activity of three stilbene glucosides newly extracted from *Vitis vinifera* cell cultures. *J. Nat. Prod*. 1998; 61(5): 655-657.
19. Zenner L, Callait MP, Granier C, Chauve C. *In vitro* effect of essential oils from *Cinnamomum aromaticum*, *Citrus limon* and *Allium sativum* on two intestinal flagellates of poultry, *Tetratrichomonas gallinarum* and *Histomonas meleagridis*. *Parasite*. 2003; 10(2): 153-157.
20. Jagetia GC, Venkatesha VA, Reddy TK. Naringin, a citrus flavonone, protects against radiation-induced chromosome damage in mouse bone marrow. *Mutagenesis*. 2003; 18(4): 337-343.

21. Seybold C, Frohlich K, Bitsch R, Otto K, Bohm V. Changes in contents of carotenoids and vitamin E during tomato processing. *J. Agric. Food Chem*. 2004; 52(23): 7005-7010.
22. Riso P, Brusamolino A, Scalfi L, Porrini M. Bioavailability of carotenoids from spinach and tomatoes. *Nutr. Metab. Cardiovasc. Dis*. 2004; 14(3): 150-156.
23. Bourne LC, Rice-Evans C. Bioavailability of ferulic acid. *Biochem. Biophys. Res. Commun*. 1998; 253(2): 222-227.
24. Balestrieri ML, De Prisco R, Nicolaus B, Pari P, Moriello VS, Strazzullo G, et al. Lycopene in association with alpha-tocopherol or tomato lipophilic extracts enhances acyl-platelet-activating factor biosynthesis in endothelial cells during oxidative stress. *Free Radic. Biol. Med*. 2004; 36(8): 1058-1067.
25. Hongratanaworakit T, Heuberger E, Buchbauer G. Evaluation of the effects of East Indian sandalwood oil and alpha-santalol on humans after transdermal absorption. *Planta Med*. 2004; 70(1): 3-7.
26. Lie Ken Jie MS, Pasha MK, Ahmad F. Ultrasound-assisted synthesis of santalbic acid and a study of triacylglycerol species in *Santalum album* (Linn.) seed oil. *Lipids* 1996; 31(10): 1083-1089.
27. Yu JG, Cong PZ, Lin JT, Zhang YJ, Hong SL, Tu G Z. Studies on the structure of alpha-trans-bergamotenol from Chinese sandalwood oil. *Yao. Xue. Xue. Bao*. 1993; 28(11): 840-844.
28. Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: A preliminary study. *J. Med. Food* 2004; 7(3): 343-348.
29. Matsuda H, Toguchida I, Ninomiya K, Kageura T, Morikawa T, Yoshikawa M. Effects of sesquiterpenes and amino acid-sesquiterpene conjugates from the roots of *Saussurea lappa* on inducible nitric oxide synthase and heat shock protein in lipopolysaccharide-activated macrophages. *Bioorg. Med. Chem*. 2003; 11(5): 709-715.
30. Jin M, Lee HJ, Ryu JH, Chung KS. Inhibition of LPS-induced NO production and NF-kappaB activation by a sesquiterpene from *Saussurea lappa*. *Arch. Pharm. Res*. 2000; 23(1): 54-58.
31. Cho JY, Baik KU, Jung JH, Park MH. *In vitro* anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from *Saussurea lappa*. *Eur. J. Pharmacol*. 2000; 398(3): 399-407.
32. Ahmad VU, Abbasi MA, Hussain H, Akhtar MN, Farooq U, Fatima N, et al. Phenolic glycosides from *Symplocos racemosa*: Natural inhibitors of phosphodiesterase I. *Phytochemistry* 2003; 63(2): 217-220.
33. Kikuzaki H, Kawai Y, Nakatani N. Diphenyl-2-picrylhydrazyl radical-scavenging active compounds from greater cardamom (*Amomum subulatum* Roxb.). *J. Nutr. Sci. Vitaminol.* (Tokyo). 2001; 47(2): 167-171.
34. Dhuley JN. Anti-oxidant effects of cinnamon (*Cinnamomum verum*) bark and greater cardamom (*Amomum subulatum*) seeds in rats fed high fat diet. *Indian J. Exp. Biol*. 1999; 37(3): 238-242.
35. Ukil A, Maity S, Karmakar S, Datta N, Vedasiromoni JR, Das PK. Curcumin, the major component of food flavour turmeric, reduces mucosal injury in trinitrobenzene sulphonic acid-induced colitis. *Br. J. Pharmacol*. 2003; 139(2): 209-218.
36. Rasmussen HB, Christensen SB, Kvist LP, Karazmi A. A simple and efficient separation of the curcumins, the antiprotozoal constituents of *Curcuma longa*. *Planta Med*. 2000; 66(4): 396-398.

37. Hu Y, Du Q, Tang Q. Determination of chemical constituents of the volatile oil from *Curcuma longa* by gas chromatography-mass spectrometry. *Se Pu*. 1998; 16(6): 528-529.
38. Baliga MS, Jagetia GC, Rao SK, Babu K. Evaluation of nitric oxide scavenging activity of certain spices *in vitro*: A preliminary study. *Nahrung*. 2003; 47(4): 261-264.
39. Miquel J. Nutrition and ageing. *Public Health Nutr*. 2001; 4(6A): 1385-1388.
40. Ray S, Chattopadhyay N, Mitra A, Siddiqi M, Chatterjee A. Curcumin exhibits antimetastatic properties by modulating integrin receptors, collagenase activity, and expression of Nm23 and E-cadherin. *J. Environ. Pathol. Toxicol. Oncol*. 2003; 22(1): 49-58.
41. Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T. Anti-Helicobacter pylori flavonoids from licorice extract. *Life Sci*. 2002;71(12):1449-1463.
42. Li W, Asada Y, Yoshikawa T. Flavonoid constituents from *Glycyrrhiza glabra* hairy root cultures. *Phytochem.* 2000; 55(5): 447-456.
43. Hayashi H, Zhang SL, Nakaizumi T, Shimura K, Yamaguchi M, Inoue K et al. Field survey of *Glycyrrhiza* plants in central Asia (2). Characterization of phenolics and their variation in the leaves of *Glycyrrhiza* plants collected in Kazakhstan. *Chem. Pharm. Bull.* (Tokyo). 2003; 51(10): 1147-1152.
44. Biondi DM, Rocco C, Ruberto G. New dihydrostilbene derivatives from the leaves of *Glycyrrhiza glabra* and evaluation of their antioxidant activity. *J. Nat. Prod*. 2003; 66(4): 477-480.
45. Iuldashev MP, Batirov EKh, Vdovin AD, Abdullaev ND. Glabrizoflavone: A novel isoflavone from *Glycyrrhiza glabra* L. *Bioorg. Khim*. 2000; 26(11): 873-876.
46. Kanazawa M, Satomi Y, Mizutani Y, Ukimura O, Kawauchi A, Sakai T, et al. Isoliquiritigenin inhibits the growth of prostate cancer. *Eur. Urol*. 2003; 43(5): 580-586.
47. Kroes BH, Beukelman CJ, van den Berg AJ, Wolbink GJ, van Dijk H, Labadie RP. Inhibition of human complement by beta-glycyrrhetinic acid. *Immunol.* 1997; 90(1): 115-120.
48. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK, et al. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochem.* 2003; 63(1): 97-104.
49. Nose M, Terawaki K, Oguri K, Ogihara Y, Yoshimatsu K, Shimomura K. Activation of macrophages by crude polysaccharide fractions obtained from shoots of *Glycyrrhiza glabra* and hairy roots of *Glycyrrhiza uralensis in vitro*. *Biol. Pharm. Bull*. 1998; 21(10): 1110-1112.
50. Saeedi M, Morteza-Semnani K, Ghoreishi MR. The treatment of atopic dermatitis with licorice gel. *J. Dermatolog. Treat*. 2003; 14(3): 153-157.
51. Marder M, Viola H, Wasowski C, Fernandez S, Medina JH, Paladini AC. 6-methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS. *Pharmacol. Biochem. Behav*. 2003; 75(3): 537-545.
52. Huang J, Li H, Yang J, Chen Y, Liu Y, Li, N et al. Chemical components of *Vetiveria zizanioides* volatiles. *Ying Yong Sheng Tai Xue Bao*. 2004; 15(1): 170-172.
53. Paranich VA, Cherevko O I, Frolova NA, Paranich AV. The effect of wheat germ oil on the antioxidant system of animals. *Lik. Sprava*. 2000; (2): 40-44.
54. Ferrando J. Clinical trial of a topical preparation containing urea, sunflower oil, evening primrose oil, wheat germ oil and sodium pyruvate, in several hyperkeratotic skin conditions. *Med. Cutan. Ibero. Lat. Am*. 1986; 14(2): 133-137.