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# Immunomodulatory and antioxidative potential of milk fortified with *Asparagus racemosus* (Shatavari)

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**Abstract**
*Asparagus racemosus* is an important medicinal plant used in the traditional Indian system of medicine. Milk is often used as a carrier for delivering plant bioactives for imparting the therapeutic attributes of the medicinal plants. In the present study, immunomodulatory and antioxidative potential of milk fortified with *Asparagus racemosus* (*Shatavari*) was investigated using a freeze dried aqueous extract of *Asparagus racemosus* using mice as the animal model. Oral administration of test the material with (at 1%) or without milk to mice for 4 weeks resulted in a significant increase ($p<0.05$) in percent phagocytosis, proliferation of lymphocytes, reduced glutathione content and decreased lipid peroxidation. The immune enhancing and antioxidative property may be related to the antioxidant vitamins, saponins, glycosides, polyphenol and flavonoids present in the extract. These results clearly indicate that milk fortified with *Asparagus racemosus* may be effective against free radical mediated diseases and has immunomodulatory potential.

**Keywords:** Herb, *Asparagus racemosus,* Antioxidative, Immunomodulatory, Mice model

## 1. Introduction
Expanding knowledge of the role of physiologically active food components, from both plant (phytochemicals) and animal (zoochemicals) sources, has notably changed the role of diet in health. This has led to the development of a new generation of foods termed functional foods [1]. Functional foods are generally described as foods and beverages that provide health benefits beyond their inherent nutritional value. Increased consumer interest in improving overall health and reducing risk for specific diseases has fueled the demand for foods and beverages that provide health benefits beyond their traditional nutritional value (i.e., so-called functional foods) [2, 3]. Milk and dairy products, containing bioactive peptides, probiotic bacteria, antioxidants, conjugated linoleic acids and other biologically active components, have been associated with health benefits for many years [4]. In the traditional Indian system of medicine, *Ayurveda*, milk has been used as a dietary carrier for imparting the therapeutic attributes of the medicinal plants.
*Asparagus racemosus* Willd (family Liliaceae) is an important medicinal plant commonly known as *Shatavari* (Hindi). Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine. *A. racemosus* is a known *Ayurvedic rasayana* which increases longevity, imparts immunity, improves mental function and adds vigor and vitality to the body [5]. Its root paste or root juice has been used in the treatment of various ailments and as health tonic [6, 7]. The plant finds use in about 64 *Ayurvedic* formulations which include traditional formulations such as "*Shatavari kalpa*", "*Phalaghrita*", "*Vishnu taila*" [8] besides branded formulations being marketed by pharmaceutical companies [9]. Reports indicate that the pharmacological activities of *A. racemosus* root extract include antiulcer, antidiarrhoel, antidiabetic, adaptogenic, immunomodulatory activities, antioxytocin, anti-dyspepsia, cardioprotective, lactogogue and positive effect on neurological disorders [6, 9]. Previously it was reported that *A. racemosus* root extract contains steroidal saponins [10], alkaloids [11], polysaccharides, polyphenols, flavonoids and vitamins [12].
Fructo-oligosaccharides and other polysaccharides present in *A. racemosus* have been reported to be responsible for the immunomodulatory activity exhibited by it [13].

Besides these, Asparagus is a wellknown source of the amino acid Asparagine, a non-essential amino acid in humans. Asparagine is a beta-amido derivative of aspartic acid and plays an important role in the biosynthesis of glycoproteins and other proteins. A metabolic precursor to aspartate, Asparagine is a nontoxic carrier of residual ammonia to be eliminated from the body [14]. Asparagine acts as diuretic. It is known that chronic fat feeding could induce insulin sensitivity by down regulating insulin receptors. It has been demonstrated that supplementation with Aspartate (Asp) and asparagine (Asn) increases the response of glycogen concentration to modulate the glucose uptake by muscle. Supplementation with Asp and Asn could modify the action of insulin on muscle, thereby increasing the insulin sensitivity [15]. It was found that rats fed the asparagine-free diet synthesized less body protein and also oxidized asparagine at a less rapid rate, thereby suggesting that the weanling rats seem to have a short-term requirement for asparagine even though asparagine has not been considered as an essential amino acid [16].

Recently we have investigated the *in vivo* immunomodulatory and antioxidative effect of *Pueraria tuberosa* with milk as the carrier [17] as well as carried out its HPLC analysis [18]. The results suggested that milk works as a good carrier that effectively transfers the phytochemicals without affecting its functionality and the milk constituents interact with the bioactive components of *P. tuberosa* thus enhancing its health benefits. *A. racemosus* being a wellknown Ayurvedic Rasayana drug, the present study was thus taken up to investigate the feasibility of using it into milk system for harnessing its immunomodulatory and antioxidative potential. The Asparagus-fortified milk was also analyzed for the presence of the added Asparagus extract as well as for its major phytoconstituent marker, asparagine, using HPTLC analysis.

## 2. Materials and Methods

### 2.1 Chemicals

Asparagine and other chemicals viz. Dulbecco's modified Eagle's medium/nutrient mixture F-12 Ham, RPMI-1614 medium, lipopolysaccharides (LPS) (from *Escherichia coli* serotype 055:B5L-2880), Fetal Calf Serum (FCS), Concanavalin A (Con A), MTT (3 - [4, 5 - dimethyl thiazol - 2yl] - 2, 5 - diphenyltetrazolium bromide), TMB, Streptomycin sulphate and Penicillin-G were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB), Sodium pyruvate, Ferric chloride, Butylated hydroxytoluene (BHT), Sodium bicarbonate, Glutathione were purchased from HIMEDIA, Mumbai, India. Hydroxyethyl piperazine-N-2 ethane sulphonic acid (HEPES) buffer, Tris-base was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Solvents used for HPTLC analysis were purchased from Merck Chemicals, Mumbai, India.

### 2.2 Plant material and preparation of extract

The roots of *A. racemosus* were bought from the local market and authenticated. They were deposited in the departmental herbal drug museum of the Pharmacognosy Division, National Botanical Research Institute, Lucknow, India for future reference. Air-dried (40-50 °C), powdered roots (50 g) were extracted with hot water (5 × 400 mL) by continuous heating in a water bath at 100 °C for 5 h each time. The extracts were pooled together, filtered and concentrated at reduced temperature (60-65 °C) by rotary evaporation (Büchi, USA), and lyophilized (Freezone 4.5; Labconco, USA) under high vacuum (133 × 104 mbar) at −40 °C ± 2 °C to yield the freeze dried aqueous extract of *A. racemosus* (17 g).

### 2.3 Preparation of fortified milk

Fresh cow's milk was collected from Cattle Yard, National Dairy Research Institute, Karnal, India, and warmed to 40–45 °C. The warmed milk was filtered using muslin cloth to remove any foreign materials. The milk was then pasteurized at 63 °C for 30 min and finally cooled to 4°C. Subsequently, lyophilised aqueous extract of *A. racemosus* was weighed (enough for 1.0% content in milk). The extract was crushed with pestle and mortar in the presence of small quantity of milk (25 mL) and finally made upto the required volume. The aliquot thus obtained was used for further analysis.

### 2.4 Sample preparation for HPTLC analysis

Pasteurized milk containing 1% w/v of hot water extract of *A. racemosus* i.e. fortified milk and milk controls were kept in freezer at lower temperature (-10°C) for 24 h, frozen samples then freeze dried by FREEZONE® 4.5 lyophilizer. Dry lyophilized powder was defatted three times with hexane (1:5 w/v) and finally extracted with methanol:water (1:1) by warming on water bath. Isolated fractions were then dried under reduced pressure and temperature. The dried hot water extract (HWE) of *A. racemosus* as well as the dried aqueous methanolic extracts of fortified milk and control were reconstituted in methanol: water (1:1) and working solutions of 10 mg/mL concentrations were prepared. These were filtered through 0.45 µm membrane filters before being subjected to HPTLC analysis. Standard solution of asparagine (0.5 mg/mL) was prepared in methanol: water (1:1).

### 2.5 HPTLC Analysis

A Camag (Muttenz, Switzerland) HPTLC system, comprising of a Linomat 5 automatic applicator, a twin trough plate development chamber (20 x 10 x 4 cm), Camag TLC scanner 3 and win-CATs 4 software, was used for analysis. Precoated silica gel 60 F254 plates (20 x 10 cm, 0.2 mm thickness, Merck, Darmstadt, Germany) on aluminium sheets were used as adsorbent layers. Aqueous methanolic solutions of the standard and samples of known concentration were applied to the plates as bands 6 mm wide, 10 mm from the bottom of the plate. Plates were developed to a distance of 9 cm, with chloroform : methanol : ammonia :: 4 : 4 : 1 (v/v) as mobile phase, in a twin trough glass chamber previously saturated with mobile phase vapor for 15 min at 10 °C. After development the plates were dried, sprayed with ninhydrin spray reagent and heated at 105 °C for 10 min. The peak areas were recorded by scanning densitometry at 400 nm. The slit width used was 5 mm x 0.45 mm. Calibration curve for Asparagine was prepared by plotting peak areas *vs* concentrations (500 ng to 2500 ng) and the regression equation was computed.

### 2.6 Animals and experimental design

Male swiss albino mice weighing 25 to 30 g obtained from Small Animal House of the Institute (NDRI, Karnal, India), was kept in polypropylene cages in a well-ventilated room with a 12-h day/light cycle and were provided with water and a laboratory stock diet containing starch 50%; casein 15%; cellulose 10%; vegetable oil 10%; salt mixture 3.5%; vitamin mixture 1%; choline chloride 0.2%; methionine 0.3% and sucrose 10% *ad libitum*. The mice were divided into 3 groups of 6 animals each. Group 1 was fed stock diet with milk (C), group 2 was fed stock diet with *A. racemosus* extract (1%) (S) and group 3 was fed stock diet with *A. racemosus* supplemented milk (SM) for 4 weeks. This study was

approved by the Institute Animal Ethics Committee (IAEC), National Dairy Research Institute, Karnal, India. After completion of experimental regimen, the mice were fasted overnight and sacrificed under mild dose of anesthesia. The skin of mice was carefully removed, leaving the peritoneum intact. DMEM Ham's F-12 medium (5 ml) was injected into the peritoneal cavity and peritoneal exudates were collected. Blood was collected from the heart of all the animals and kept undisturbed at room temperature for 2 h. Serum was prepared by centrifugation of blood at 5000×g for 10 min at 4 °C and was frozen at -80 °C until the determination of immunoglobulin. After serum separation, RBC cells were washed 3 times by resuspending in isotonic saline (0.9% NaCl) followed by centrifugation at 1500×g for 10 min at 4 °C. The red blood cells were lysed in 4-10 volumes of de-ionized water (4 °C) by repeated gentle vortexing for 10 min. It was subsequently centrifuged at 10000×g for 15 min at 4 °C and the supernatant collected and stored at -80 °C. The peritoneum was opened, the liver, spleen and intestine were dissected out and the liver homogenate was finally prepared for analysis [19].

### *2.*7 *In vitro* phagocytosis

The phagocytic activity of macrophages was used to assess the nonspecific immune function. The activity of the harvested macrophages was determined with the help of a phagocytic assay technique using yeast as the test organism and the microscopic method [20]. Results were expressed as percent phagocytosis, number of macrophages with yeast cell internalized/100 macrophages.

### 2.8 Lymphocyte proliferation index

Lymphocyte proliferative responses in mice spleen were determined by MTT assay. Lymphocytes were separated from spleen and suspended in 1 ml of 10% FCS and β-mercaptoethanol containing medium [21]. The cell suspension was dispensed into each well of 96 well flat bottomed tissue culture plates. Ten µL of mitogens (LPS and Con A) were added to the wells containing cell suspension to evaluate the impact of herb extract on lymphocyte proliferation. Lymphocytes were cultured in the above medium at 37 °C for 48 h in a 95% relative humidity air atmosphere with 5% $CO_2$ in a $CO_2$ incubator. Proliferation of lymphocytes was measured by colorimetric reading of MTT reduction [22].

### 2.9 Antibody responses (IgG and IgA)

The total IgG levels in serum and IgA levels in intestinal fluid were determined using ELISA kit supplied by Koma Biotech, USA. The ELISA kit employed the principle of quantitative sandwitch enzyme immunoassay technique.

### 2.10 Antioxidative responses

The extent of lipid peroxidation, an index of oxidative stress was measured as thiobarbituric acid reactive substances (TBARS) formed. Lipid hydroperoxides were measured in RBC lysate [23] and in liver homogenate [24]. Reduced glutathione content was determined based on development of yellow colour in addition of DTNB to compounds containing sulphydryl groups. Reduced glutathione were measured in liver and RBC lysate [25].

### 2.11 Statistical analysis

All the values are expressed as mean ± SEM (Standard Error Mean) of mice from each group and significant differences among groups have been determined by one-way analysis of variance (ANOVA) using SYSTAT 6.0.1 software. Statistical significance was set at $P<0.05$.

## 3. Results and discussion

### 3.1 Phagocytic activity

In the present study, milk supplemented with the extract of *A. racemosus* and the extract alone were evaluated for their immunomodulatory and antioxidative properties. The effects of the two test preparations on nonspecific immune function was estimated by measuring the phagocytic activity of peritoneal macrophages. Administration of milk supplemented with the herb extract (51.16±2.83%) and the extract (44.50±3.88%) alone could significantly enhance the phagocytic activity of peritoneal macrophages as compared to the control group (33.16± 2.45%) (Figure1). However, no significant difference in phagocytic activity was observed between the extract and milk-extract mixture. Immunomodulating property of *A. racemosus* has been shown to protect the rat and mice against experimentally induced abdominal sepsis [26, 27]. Since *A. racemosus* is reported to be devoid of antibacterial action, protection offered by it against sepsis by altering function of macrophages indicates its possible immunomodulatory property [26]. Both, the herb extract and the milk supplemented with the extract enhanced phagocytic activity of macrophages.

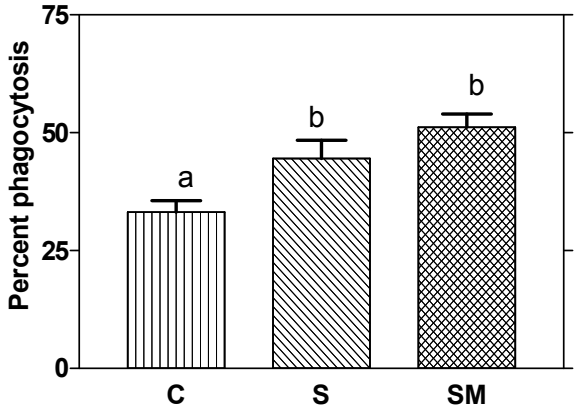



**Fig 1:** Effect of feeding *A. racemosus* and milk supplemented with *A. racemosus* on phagocytic activity of macrophages. Mean in each bar with different superscripts (a, b) were significantly different ($P<0.05$) from each other. (C: control, S: *A. racemosus* (*Shatavari)*, SM: *A. racemosus* (*Shatavari)* supplemented milk)

### 3.2. Lymphocytes proliferation index

The effects of herb extract and milk-herb extract preparation on cellular immune function was estimated by lymphocyte proliferation. The B lymphocytes, which produce antibody, and T lymphocytes direct and govern the character of antigen-specific immune reactivity. In the assay, spleen lymphocyte proliferation significantly increased in milk supplemented with the herb bioactives as compared to control group. However, no significant difference in lymphocyte proliferation was observed between the animals fed with herb extract diet alone and the control group (Figure 2). The result also showed that mitogen LPS has significant effect on lymphocyte proliferation as compared to Con A. In a similar study carried out earlier[28], *A. racemosus* roots showed a significant increase in proliferative responses in LPS or/and Con A stimulated lymphocytes and the increase in proliferation was concentration dependent.

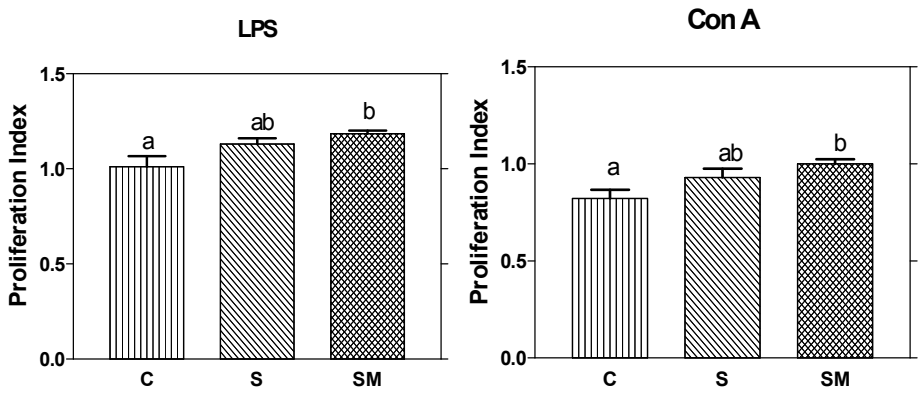



**Fig 2:** Effect of feeding *A. racemosus* and milk supplemented with *A. racemosus* on lymphocyte proliferation index. Mean in each bar with different superscripts (a, b) were significantly different ($P$<0.05) from each other. (C: control, S: *A. racemosus* (*Shatavari)*, SM: *A. racemosus* (*Shatavari)* supplemented milk)

### 3.3 Antibody (IgG & IgA) responses

IgG levels in serum represents the systemic immunity and IgA levels in intestinal fluid represent the mucosal immunity. In the present study, effect of feeding *A. racemosus* extract and milk added to the herb extract on the levels of IgG and IgA were investigated. Increase in the levels of IgG was observed in mice maintained on both the diets i.e. herb extract (171.27±9.6 µg/mL) alone and milk-herb extract mix (186.87±4.9 µg/mL) as compared to control (164.7±2.5 µg/mL). However, differences between the IgG levels in both the treatment groups were not statistically significant. Similarly, the IgA levels between the two treatment groups (herb extract - 4.31±0.24 µg/mL and milk-herb extract - 4.23±0.20 µg/mL) were also not statistically different from control (4.234±0.23 µg/mL). Thus the doses at which the herb extract (1%) was used in the diet were not effective in enhancing both the systemic and the mucosal immunity.

### 3.4 Anti-oxidative properties

Lipid hydroperoxides, an index of oxidative stress was measured in terms of TBARS in both liver homogenate and RBC lysate (Figure 3). Feeding *A. racemosus* extract and milk with herb extract significantly decreased the values of TBARS in liver homogenate as compared to control group indicating decreased lipid peroxidation. However, no significant difference was observed in TBARS values between mice groups fed with herb extract and milk added with herb extract. Similarly, the values of TBARS in RBC lysate were significantly lower in mice maintained on diets with herb extract and milk-herb extract mix as compared to control group. However, a significant decrease in lipid peroxidation was observed in the extract group as compared to milk with extract group.

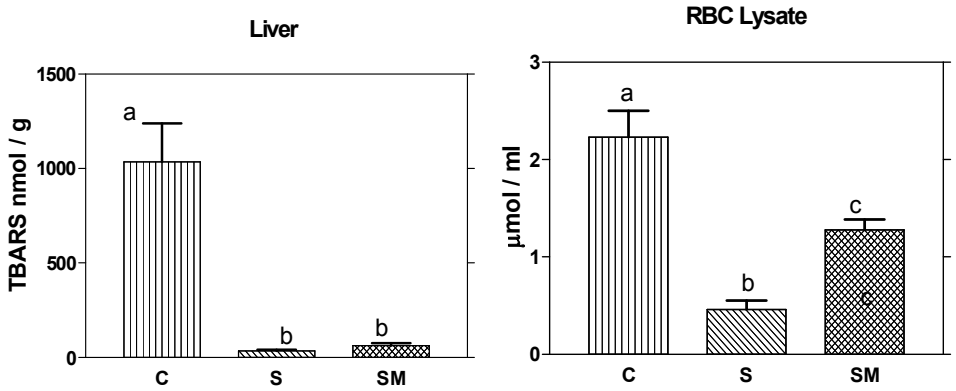



**Fig 3:** Effect of feeding *A. racemosus* and milk supplemented with *A. racemosus* on lipid hydroperoxide (TBARS) values of liver homogenate and RBC lysate. Mean in each bar with different superscripts (a, b, c) were significantly different ($P$<0.05) from each other. (C: control, S: *A. racemosus* (*Shatavari)*, SM: *A. racemosus* (*Shatavari)* supplemented milk)

Hepatoprotective activity of the *A. racemosus* root extracts were demonstrated *in vivo* by the inhibition of $CCl_4$-induced formation of lipid peroxides in the liver of rats by pretreatment with the extracts. $CCl_4$-induced hepatotoxicity in rats, as judged by the raised serum enzymes viz. glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase and total and direct bilirubin as well as oxidant enzyme viz. malondialdehyde were prevented, while antioxidant enzymes viz. superoxide dismutase (SOD), reduced glutathione and catalase were elevated by pretreatment with the extracts, demonstrating the potent hepatoprotective action of the roots of *A. racemosus* [29].

Glutathione, an antioxidant, helps protect cells from reactive oxygen species such as free radicals and peroxides. Feeding herb extract and milk supplemented with herb extract significantly increased reduced glutathione content of liver homogenate as compared to control group mice. However, no significant difference was observed in glutathione concentration between the mice groups fed with either extract alone or milk supplemented with the extract. Similarly, the glutathione content of RBC lysate was significantly higher in mice which were given milk supplemented with the extract as compared to either control group or the group maintained on diets with the extracts alone. However, no significant

difference in glutathione concentration was observed between extract and control groups (Figure 4).

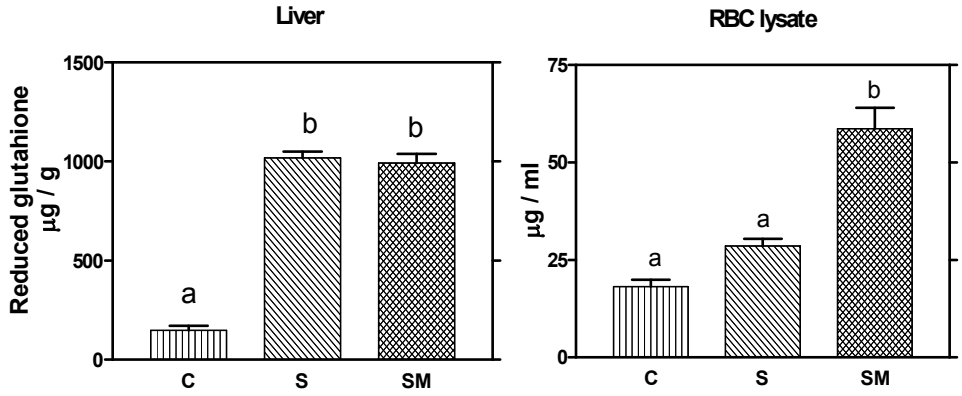
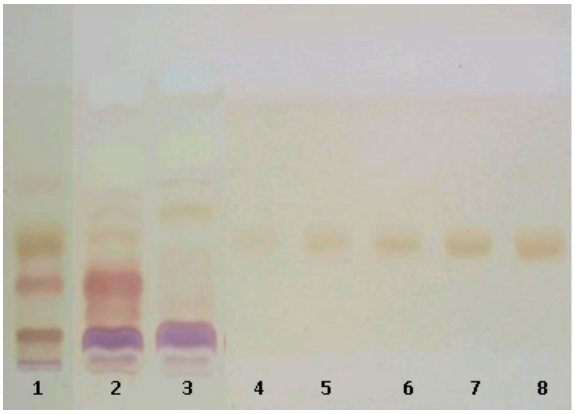



**Fig 4:** Effect of feeding *A. racemosus* and milk supplemented with *A. racemosus* on reduced glutathione content of liver homogenate and RBC lysate. Mean in each bar with different superscripts (a, b) were significantly different ($P$<0.05) from each other. (C: control, S: *A. racemosus* (*Shatavari)*, SM: *A. racemosus* (*Shatavari)* supplemented milk)

The antioxidant effect of an active fraction consisting of polysaccharides (termed as P3) fraction was more pronounced against LPO, as assessed by TBARS formation, while that of the crude extract was more effective in inhibiting protein oxidation. Both the crude extract and P3 fraction also partly protects against radiation-induced loss of protein thiols and inactivation of SOD. The inhibitory effects of these active principles, at the concentration of 10 µg/ml, are comparable to that of the established antioxidants GSH and ascorbic acid[30].The plant extract plays the role of an antioxidant by attenuating free radical induced oxidative damage. Mice treated with *A. racemosus* extract showed an enhancement in glutathione peroxidase (GSH-px) activity and reduced glutathione (GSH) content. Studies in the past have indicated that reduction in membranal lipid peroxidation (LPO) could eventually result in protective effect on the kainic acid induced excitotoxicity[31]. In another related study, *A. racemosus* root extracts were found to exhibit *in vivo,* antioxidant activity in rats, although the principles responsible for this antioxidant activity were not reported [32]. The antioxidant activity of *A. racemosus* could be mainly attributed to the presence of its phytochemical constituents, which act synergistically to alleviate the indices of oxidative stress. Like herb extract, milk supplemented with the extract was also found to enhance the antioxidative effect. The immunomodulatory and antioxidative effects of milk components viz milk proteins and some minor constituents in milk do find mention in the literature[33]. Therefore, in the present study when milk was supplemented with *A. racemosus* extract, it was found to have synergistic bioactivity.

**3.5 HPTLC fingerprinting and determination of sparagine**

In the present study, the *A. racemosus*-fortified milk was analyzed for the added Asparagus hot water extract (HWE) as well as for Asparagine, the amino acid present in *A. racemosus* using HPTLC analysis. The HWE as well as the dried aqueous methanolic extract of the fortified milk sample were reconstituted in methanol:water (1:1) and working solutions of 10 mg/ml concentrations were made for HPTLC analysis and analysis was carried out by using the conditions described earlier. The HWE was found to contain 0.4% Asparagine whereas the reconstituted fortified milk sample was found to contain approximately 0.15% Asparagine. No peak corresponding to that of Asparagine was observed in control milk samples. Figure 5A shows the HPTLC chromatograms of the Asparagus hot water extract (HWE), milk sample fortified with HWE, control sample and asparagine standard. Results of the HPTLC fingerprint analysis are presented in Table 1. Figure 5B shows the calibration curve obtained from asparagine standard (r = 0.99738). The identity of asparagine in milk samples was confirmed by comparison of its spectrum and $R_f$ with the authentic standard. Since the health benefits of the nutraceuticals or functional foods containing different botanicals is due to the presence of the phytoconstituents of the added botanicals, it is important to have a biological marker and also to be able to associate that biological marker with the quality of life. Asparagus is a well-known source of the amino acid Asparagine, a non-essential amino acid in humans. Asparagine is also known for its key role in the biosynthesis of glycoproteins. In addition, it is also essential for the synthesis of many other proteins. Human nervous system also needs this amino acid to be able to maintain equilibrium. Asparagine increases the resistance to fatigue, thus improving athletic stamina, and improves the smooth functioning of the liver. So, Asparagine benefits work best in the field of nervine health and liver protection [34].



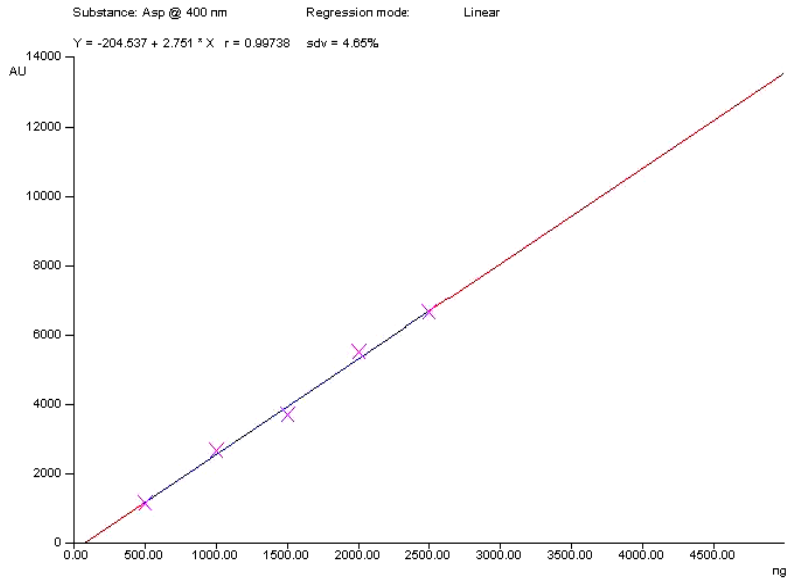



**Fig 5:** (A) HPTLC fingerprint profiles of Asparagus HWE (Track 1), fortified milk sample (Track 2), control sample (Track 3) along with Asparagine standard (Tracks 4-8); (B) Calibration graph for Asparagine standard solutions.

**Table 1:** TLC fingerprinting of Asparagus HWE, fortified milk sample and control sample after spraying with Ninhydrin reagent

| TLC band $R_f$ | Colour | *Asparagus racemosus* HWE | Fortified Milk Sample | Control |
|---|---|---|---|---|
| 0.08 | Magenta | √ | √ | √ |
| 0.20 | Reddish pink | √ | √ | x |
| 0.33 | Brownish yellow (Asparagine) | √ | √ | x |
| 0.40 | Reddish pink | x | √ | √ |
| 0.48 | Reddish pink | √ | √ | √ |
| 0.53 | Creamish white | x | √ | √ |
| 0.71 | White | x | √ | √ |

## 4. Conclusion

In conclusion, our studies suggest that aqueous extract of *A. racemosus* acts as a potential antioxidant by decreasing lipid peroxidation and increasing glutathione content. It also acted as an immunomodulator as was ascertained by increased activity of macrophages and proliferation of lymphocytes. When administered in combination with milk it had similar immunomodulatory and antioxidative properties. Thus, it can be inferred that milk fortified with *A. racemosus* may be effective against free radical mediated diseases and exhibit immunomodulatory potential. Also, the fortified milk samples were analyzed for the presence of the extract used for fortification as well as detection of asparagine through HPTLC analysis. These studies will be useful for developing milk nutraceuticals fortified with *A. racemosus* which has the potential to be included as an ingredient in health and functional foods leading to the construction of a health-promoting, disease-preventing diet with protective substances as well as assessing the quality of the fortified product.

## 5. Acknowledgements

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## 6. References

1. Abdel-Salam AM. Functional foods: hopefulness to good health. American Journal of Food Technology 2010; 5(2):86-99.
2. International Food Information Council (IFIC) Foundation. Consumer Attitudes toward Functional Foods/Foods for Health. 2007, Washington, DC.
3. International Food Information Council (IFIC) Foundation. Food & Health Survey. Consumer Attitudes toward Food, Nutrition & Health, Washington, DC, 2008.
4. Bhat ZF, Bhat H. Milk and dairy products as functional foods: a review. International Journal of Dairy Science 2011; 6(1):1-12.
5. Sharma PV. Cikittsastana. In Charaka Samhita. Chaukhambha Orientalis, Varanasi, 2001, 7-14.
6. Goyal RK, Singh J, Lal H. Asparagus racemosus - An Update. Indian Journal of Medical Sciences 2003; 57(9):408-414.
7. Pandey SK, Sahay A, Pandey RS, Tripathi YB. Effect of *Asparagus racemosus* (*Shatavari*) *rhizome* on mammary gland and genital organs of pregnant rat. Phytotherapy Research 2005; 19(8):721-724.
8. Unnikrishnan PM. Shatavari-Asparagus racemosus Willd. Amruth 1998, 18-20.
9. Bopana N, Saxena S. *Asparagus racemosus*-ethnopharmacological evaluation and conservation needs. Journal of Ethnopharmacology 2007; 110(1):1-15.
10. Hayes PY, Jahidin AH, Lehmann R, Penman K, Kitching W, De Voss JJ *et al*. Structural revision of *shatavarins* I and IV, the major components from the roots of *Asparagus racemosus*. Tetrahedron Letters 2006; 47(39):6965-6969.
11. Sekine T, Ikegami F, Fukasawa N, Kashiwagi Y, Aizawa T, Fujii Y *et al*. Structure and relative stereochemistry of a new polycyclic alkaloid, asparagamine a, showing anti-oxytocin activity, isolated from *Asparagus racemosus*. Journal of the Chemical Society, Perkin Transactions 11995; (4):391-393.
12. Visavadiya NP, Narasimhacharya RL. Hypolipidemic and antioxidant activities in *Asparagus racemosus* in hypercholesteremic rats. Indian Journal of Pharmacology 2005; 37(6):376-380.
13. Thakur M, Connellan P, Deseo MA, Morris C, Praznik W, Loeppert R *et al*. Characterization and

immunomodulatory screening of fructo-oligosaccharides of *Asparagus racemosus* Willd. International Journal of Biological Macromolecules 2012; 50(1):77-81.
14. National Cancer Institute Thesaurus (NCIT). http://ncicb.nci.nih.gov/xml/owl/EVS/Thesaurus.owl#Asparagine
15. Antonio H, Lancha Jr, Jacques R, Poortmans, Pereira LO. The effect of 5 days of aspartate and asparagine supplementation on glucose transport activity in rat muscle. Cell Biochemistry and Function 2009; 27(8):552-557.
16. Crosby LO, Cline TR. Effect of Asparagine on Growth and Protein Synthesis in Weanling Rats. Journal of Animal Science 1973; 37(3):713-717.
17. Sawale PD, Singh RRB, Kapila S, Arora S, Rastogi S, Rawat AKS. Immunomodulatory and antioxidative potential of herb (*Pueraria tuberosa*) in mice using milk as the carrier. International Journal of Dairy Technology 2013; 66(2):202-206.
18. Rastogi S, Katara A, Pandey MM, Arora S, Singh RRB, Rawat AKS *et al*. Physical stability and HPLC analysis of Indian Kudzu (*Pueraria tuberosa* Linn.) fortified milk. Evidence-Based Complementary and Alternative Medicine 2013.
19. Cohen G, Dembiec D, Mareus J. Measurement of catalase activity in tissue extracts. Analytical Biochemistry 1970; 34(1):30-38.
20. Hay FC, Westwood OMR. Practical Immunology. Blackwell science, London, 2002.
21. Kapila S, Jain S, Haque E, Sabikhi L, Dang AK. Immunoregulatory response of *Lactobacillus helveticus* fermented milk in mice. Milchwissenschaft 2008; 63(4):367-369.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65(1-2):55-63.
23. Asakawa J, Matsushita S. Coloring conditions of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids 1979; 15(3):137-140.
24. Uchiyama M, Mihara M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry 1977; 86(1):271-278.
25. Ellman GL. Tissue sulphydryl groups. Archives of Biochemistry and Biophysics 1959; 82:70-77.
26. Dahanukar S, Thatte U, Pai N, Mose PB, Karandikar SM. Protective effect of *Asparagus racemosus* against induced abdominal sepsis. Indian Drugs 1986 24(3):125-128.
27. Thatte UM, Dahanukar SA. Comparative study of immunomodulating activity of Indian medicinal plants, lithium carbonate and glucan. Methods and Findings in Experimental and Clinical Pharmacology 1988; 10(10):639-644.
28. Gautam M, Saha S, Bani S, Kaul A, Mishra S, Patil D *et al*. Immunomodulatory activity of *Asparagus racemosus* on systemic Th1/Th2 immunity: Implications for immunoadjuvant potential. Journal of Ethnopharmacology 2009; 121(2):241-247.
29. Acharya SR, Acharya NS, Bhangale JO, Shah SK, Pandya SS. Antioxidant and hepatoprotective action of *Asparagus racemosus* Willd. root extracts. Indian Journal of Experimental Biology 2012; 50(11):795-801.
30. Kamat JP, Boloor KK, Devasagayam TP, Venkatachalam SR. Antioxidant properties of *Asparagus racemosus* against damage induced by gamma-radiation in rat liver mitochondria. Journal of Ethnopharmacology 2000; 71(3):425-435.
31. Parihar MS, Hemnani T. Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of *Asparagus racemosus*. Journal of Neural Transmission 2004; 111(1):1-12.
32. Bhatnagar M, Sisodia SS, Bhatnagar R. Antiulcer and antioxidant activity of Asparagus racemosus WILLD and Withania somnifera DUNAL in rats. Annals of the New York Academy of Sciences 2005; 1056(1):261-278.
33. Cross ML, Gill HS. Immunomodulatory properties of milk. British Journal of Nutrition 2000; 84(1):S81-S89.
34. Amino acids guide. Asparagine. http://www.aminoacidsguide.com/Asn.html.