PREBIOTIC EFFICACY OF HERBAL EXTRACTS ON GROWTH OF PROBIOTIC ORGANISM *LACTOBACILLUS ACIDOPHILUS*

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ABSTRACT

The present investigation was carried out to explore the prebiotic efficacy of the herbal extracts used in the traditional Siddha medicinal system of Tamil Nadu in the growth of probiotic organism *Lactobacillus acidophilus*. The aqueous extract of the five herbs were introduced in the growth media of the *L. acidophilus* and the growth curves were obtained. The growth of the organism was compared with the control sample which did not contain any herbal extract. The growth of the probiotic organism *L. acidophilus* significantly increased with the supplementation of these herbal extracts.

Key words: *Lactobacillus acidophilus,* Pre-biotic, Pro-Biotic, herbals, biological effect, strain growth

Introduction

Prebiotics are more useful as functional food than probiotics, since they can be added to more foods than probiotics, because of their ability to survive the digestive process in the upper gastrointestinal tract (Blaser, 1984). Prebiotic usage is a way of maintaining mucosal growth, mucosal function and water and electrolyte balance, providing the host with energy and nutrients and increasing resistance against invading pathogens. The human digestive tract is from birth, highly dependent on a supply of prebiotics for its growth and optimal function. Recent studies suggest that prebiotics that have been designed to produce quite selective changes in the composition of the micro biota (Vos *et al.*, 2007) may have benefits in irritable bowel syndrome (Silk *et al.*, 2009).

Lactobacilli, which are natural inhabitants in healthy human intestinal tract, have a long history of use in foods and fermented products. It is desirable that these bacteria have suitable general aspect, origin, identity, safety and bile resistance (Growth properties *in vitro* and during processing) and functional beneficial features (Holzapfel and Schillinger, 2002). It is shown that *Lactobacillus* probiotic strain can posses inhibitory activity towards the growth of pathogenic bacteria resistance to acid and bile, adherence to the intestinal epithelial cell and positive effects on the health of the host (Jacobsen *et al.*, 1999).

Beneficial gut flora can also be stimulated by non-digestible foods, which are known as prebiotics. They can be used as complementary and alternative medicine. Despite the impressive list of therapeutic attributes, probiotics are not commonly part of the medical practitioner's armamentarium of prescription drugs, but instead are available un-prescribed from retail outlets usually supermarkets, grocery shop and health food stores (Gibson and Roberfroid, 1999).

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It is only from the 1990s that the medical and business world has begun to focus on the importance of the gastrointestinal tract and flora for its health. There is a growing focus on the importance of medicinal plants and traditional health systems in solving the healthcare problems of the world. Most developing countries have viewed traditional medical practice as an integral part of their culture. Herbal medicine, also called "phytomedicine", is the use of therapeutic plants, plant parts or plant derived substances to aid in fighting against infections, diseases or enhancing overall health (Jonas, 1997). Treatment with herbal medicine prevents infection prophylactically eradicating the potential pathogens from stomach and bowel, and preserves the normal anaerobic flora (Simennoff *et al.,* 1996).

Ocimum sanctum Linn belongs to the family Laminaceae. It is used in the traditional medicine system as expectorant, diaphoretic, anti periodic stimulants and stomachic. Useful in cold, cough, fever, disorders of urogenital system and hepatic affections of children.

Cassia auriculata belongs to the family Caesalpiniaceae. Flowers of *Cassia auriculata* are traditionally used to treat fever, leprosy, eye injuries, whooping cough, chest disease, diabetes, skin diseases and jaundice. Pharmacological activity of this plant was evaluated on anti-oxidant and antimicrobial activity (Nageswara Rao *et . al.*, 2000). *Cassia auriculata* plant contains 18% of tannin; the flowers also possess Di-(2-ethyl) hexyl phthalate. Besides these, it contains alkaloids and resins. Presence of vitamins and minerals like calcium and phosphorous has also been reported and it is used in preventing diarrhea, dysentery and hemorrhages (Brahmachari and Aysti, 2000).

Achyranthus aspera is a perennial shrub, widely distributed throughout India and other tropical countries. *Achyranthus aspera* has been found to contain betain, achyranthine alkaloid, saponin containing oleanolic acid, glucose, galactose, rhamnose and xylose (Satyavathy *et al.,* 1987). The leaves contain glucoside, sterol, oleanolic acid, urosolic acid, ß-sitosterol, palmitic, stearic, oleic, linoleic, linonioc acids (Kirtikar and Basu, 1984). The ethonolic and aqueous extract of the entire plant is reported to possess strong hypoglycemic activity in rats (Dhar *et al.,* 1968).

Ionidium suffruticosum belongs to the family *Violaceae.* The herb, which is considered to be extremely beneficial to human, as a diuretic, tonic and demulcent (*Rathna purusha in* Sanskrit). The root is diuretic and is used in urinary affection and bowel complaints of children. This plant contain alkaloid, leaves extract has been used in traditional folk medicine to treat numerous disease. The leaves extract is a natural alternative to commonly used anti-inflammatory drugs like brufen and can be used with confidence for treating bone fracture.

Aerva lanata belongs to *Amaranthaceae* family. The roots are prepared to use as a diuretic and is reputed as a lithotriptic in urinary calculi. The plant occurs all over India in dry locality up to an elevation of 1000 m AMSL. The leaves are eaten as a pot-herb. The coolly spikes are used for stuffing pillows. The plant is antihelminthic and demulcent. It is also regarded as a valuable medicine for cough, sore throat, indigestion and wounds, and as specific for diabetes. A decoction of the plant is an efficacious diuretic and is useful in catarrh of bladder. In Bihar, the plant is used to cure diarrhoea, cholera and dysentery.

Materials & Methods

Plant material collection and identification

Herbs such as *Ocimum sanctum, Cassia auriculata, Achyranthus aspera, Ionidium suffruticosum, Aerva lanata* were collected from Tamil University Campus, Thanjavur, Tamil Nadu, India, during the month of January 2008 and shade dried. The collected herbs were identified and authenticated by M. Jegadeesan, Professor and Head, Department of Environmental and Herbal Sciences, Tamil University, Vakaiyur, Tamil Nadu. The specimens were deposited at the Tamil University Herbarium.

100 gm of all plant parts were cut into small pieces, followed by extraction through 1000 ml distilled water/alcoholic extract for 24h in water bath at 40°C and the process was repeated twice. The extract was then, concentrated and finally lyophilized to dry. The extract was re-dissolved in sterile water/alcohol for further experiments.

Collection of organism:

Lactobacillus acidophilus was collected from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh and strain collection account number is MTCC – 447. The organism was sub cultured in the growth medium under 37° C at aerobic conditions for 24 hours. The culture was maintained at 0 to 4°C under refrigerated conditions (Hansen and Mocquot, 1970).

Growth curve

Lactobacillus was propagated aseptically at 37°C in MRS broth (Difco) or on MRS broth supplemented with 1.5% agar. Erythromycin (5mg/ml) and/or chloramphenicol (5mg/ml) was added to MRS broth or agar when it was appropriate. For determination of the maximum specific growth rate of L. acidophilus strains, standardized inoculation was added to obtain an initial absorbance at 600nm (A_{600}). They were incubated at 37° C along with different concentrations (0.5, 1.0, 2.0. 3.0, 4.0, 5.0, 10, 20, 30, 40 and 50 mg/L) of herbal extracts for 18 hrs. The growth was monitored by determining the changes in $A_{600.}$ Each point represented the mean of three independent cultures.

Results and Discussion

The present analysis reveals that 50 mg/ L of dose of O. sanctum extract had significant increase on the growth of *Lactobacillus acidophilus* than the control sample (Fig. 1). The increase in the growth of *Lactobacillus acidophilus* might be due to the essential oil (Umadevi *et al*., 1998) in the extract of the leaves and flavonoids components that involved in the effective growth of these beneficial bacteria (Shah and Qadry, 1988). Reports evidence that essential oil improves intestinal microflora balance through the decrease of E.Coli and stimulation of *Lactobacillus sp.* prolifrations (Horbowicz, *et. al.* 2000) Further, the presence of polyphenolic compounds such as calic acid, caffic, chlorogenic (Norr and Wagner, 1992), also supports the growth promotive capacity of drugs. Probiotics, particularly *Lactobacillus acidophilus* strains, have been used with some success in helping to maintain a healthy balance of colonic micro flora (Elmer *et al.,* 1996, Pochart *et al.,* 1992). The growth increase observed in the *Ocimum sanctum* treatment may also improve its efficacy through eco flora replacement therapy with beneficial clinical effect in conditions of food allergy, gastroenteritis, intestinal inflammation and chemical exposure (Bjorksten *et al.,* 1999).

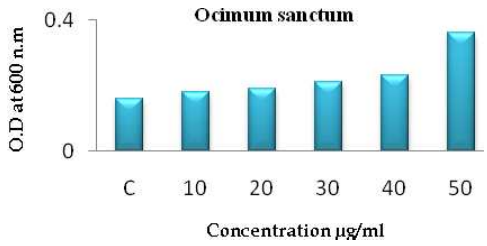

Fig.1 Growth characteristics of *L.acidophilus* at different concentrations of *O. sanctum* supplemented MRS medium

C.auriculata extract had shown significant increase in the growth of *Lactobacillus acidophilus* than control at the dose of 50 mg/L (Fig.2). Our present finding may be attributed to the prebiotic effect of flavanoids present in the flower extract of *C. auriculata*. Further the presence of Tannin may also be effective as prebiotic. The fermented probiotic product, propionic acid has been demonstrated to inhibit gluconeogenesis, stimulate glycolysis and inhibit biosynthesis of fatty acids (Wolever *et. al.,* 1995). It is reported further that LAB can colonize the intestinal epithelium may reduce glucose absorption from the small intestine (Tabuchi *et al.,* 2003). Pharmacological studies had proven hypoglycemic effect (Manickam *et.al.* 1997). Our finding with growth stimulatory efficacy of c. auriculata on probiotics also supports the above possible effect prebiotics.

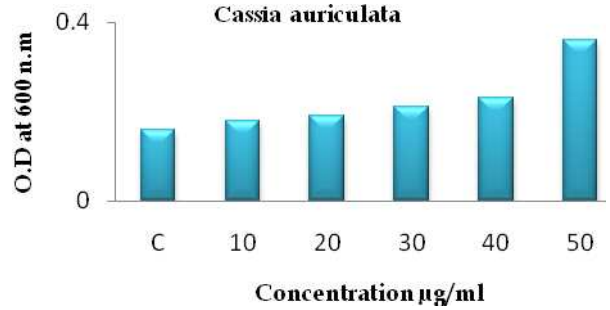

Fig.2 Growth characteristics of *L.acidophilus* at different concentrations of *C. auriculata* supplemented MRS medium

The observation from *in vitro* studies with *A. aspera* reveals that 50 mg/ L of dose of the plant extract *A. aspera* had shown increased growth of *Lactobacillus acidophilus* and it was twofold higher than that of control (Fig.3). Flavonoids have been shown to possess diuretic activity. Since the plant possesses flavonoids and triterpenoids, it has been claimed to treat renal dropsies. Betaine and achyranthine are the principal alkaloids identified from the whole plant (Kong *et al.,* 1976). The intestinal

microflora plays a considerable role in the endogenous metabolism of substances in the herbs and thus benefiting the human health. *L. acidophillus* has the capacity to hydrolyse phenolic compounds (Wang *et. al.* 2004) and thus the present study evidences the prebiotic capacity of *A. aspera* which is rich in flavonoids.

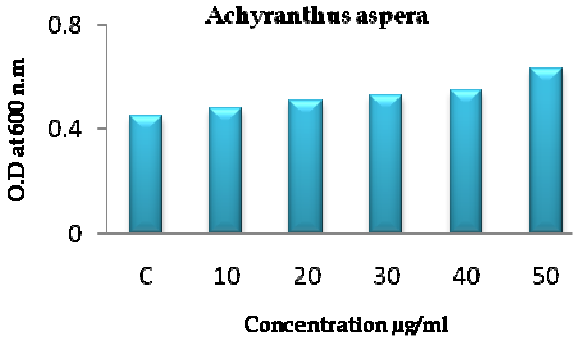

Fig.3 Growth characteristics of *L.acidophilus* at different concentrations of *A.aspera* supplemented MRS medium

Ionidium suffruticosum plant extracts at different concentrations are added into the MRS medium and the growth of the *L. acidophilus* was determined. The growth of the organism is maximum at the concentration of 50 mg/L of dose (Fig.4). The enhancement of *L. acidophilus* during Ionidium suffruticosum treatment may be evidenced to be protective against the suppressed intestinal microflora that occurs during stress condition(Tannock, 1983). The potential efficacy of plants as adaptogenic may also be contributed through the improvement of friendly bacteria in the intestine.

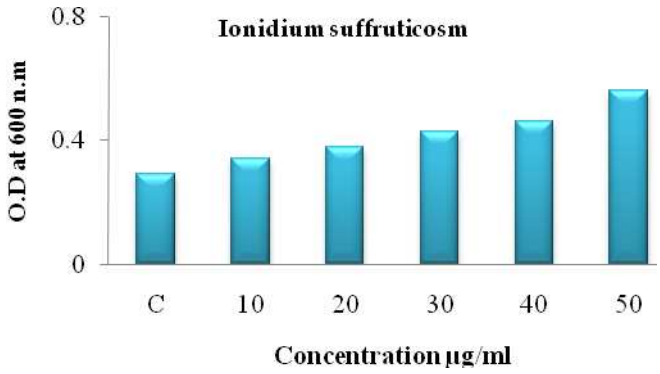

Fig. 4 Growth characteristics of *L.acidophilus* at different concentrations of *I. suffruticosm* supplemented MRS medium

The observation in our experimental studies with the *in vitro* growth curve of *Lactobacillus* showed significant influence on the multiplication of this lactic acid producing probiotic bacteria during the supplementation of water extract of *Aerva lanata* in the MRS growth media compared to control (Fig.5). *L. acidophilus* may reduce the symptoms of small bowel or bacterial over growth brought on by end stage kidney

disease (Simenhoff *et al*., 1996) and may reduce the severity of alcoholic liver disease (Nanji *et al*., 1994). Probiotic bacteria have been shown to preserve intestinal integrity and mediate the effects of a number of other diseases. *L.acidophilus* supplement had shown a marked improvement of intestinal motility in elderly patients.

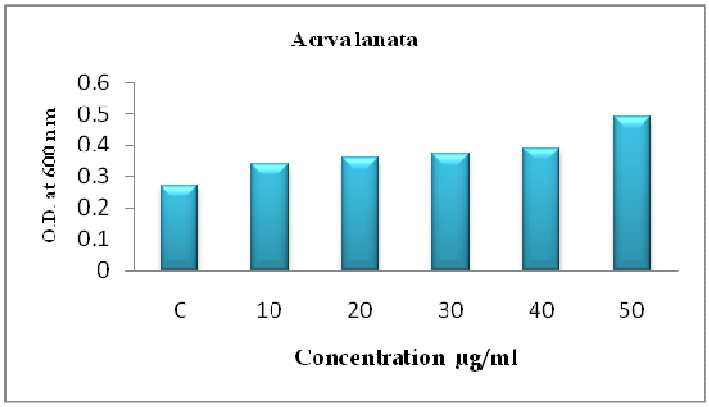

Fig. 5 Growth characteristics of *L.acidophilus* at different concentrations of A.*lanata* supplemented MRS medium

The richness of bioactive components in this plant extract could have growth stimulating effect on *Lactobacillus acidophilus* during the supplementation to MRS media. The possible influence on growth of this probiotic by *Aerva lanata* evidences the prebiotic capacity and health promotive efficacy.

Conclusion

The study revealed the growth promoting effect of herbal extracts on probiotic, *L. acidophilus* The finding reveals the prebiotic biological effect of these herbals by the selective stimulation on *L. acidophilus* which is reported to have positive endogenic intestinal activity. Our *in vitro* study confirmed the traditional utility of herbal drugs with beneficial effect from alleviation of diseases to therapeutic effect. The study reveals the prebiotic efficacy of herbal resources for customary/traditional utility in improving the beneficial bacteria that could be nutritive/pharmaceutical.

References

Bjorksten, B., Naaber. P., Sepp, E. and Miketsaar M. 1999. The intestinal microflora in allergic Estonian and Swedish 2- year old children. *Clin Experim allergy*; 29: 342-6.

Blaser, M.J. 1984. An endangered species in the stomach. *Sci. Am*. 292 (2)

Bramachari, H.B., and Aysti K.J. 2000. Hypoglycemic agents from endogenous plants. *J.Pharmacol*. 961; 13:381.

Dhar, L.M., Dhar, M.M., Dhawan, B.N., Ray, C. 1968. Screening of Indian plants for biological activity. Part I. Indian. J. Exp. Biol., 6: 232-247.

Elmer, G.W., Surawicz, C.M., and Mc Farland, L.V. 1996. Bio therapeutic agents, Review, a neglected modality of the treatment and prevention of selected intestinal and vaginal infection. *J. Am. Med Assoc*. 275 (11) :870-876.

Gibson, G.R. and Roberfroid, M.B. 1999. Colonic Microbiota, Nutrition and Health. Dordrecht: Kluwer Academic Press.

Hansen, P. A. & Mocquot, G. 1970. *Lactobacillus acidophilus* (Moro) comb. nov. *International Journal of Systematic Bacteriology* 20:325-327

Holzapfel, W.H., Geizen, R. and Schillinger, U. 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food grade enzymes. *International Journal of Food Microbiology*, 24:343-362.

Horbowicz, M. 2000. wstpowanie, biosynteza, walcei wooeci biocheni. *Nuak. Rol.* 2:3-17.

Jacobsen, C.N., Rosenfeldt Nielsen, V., Hayford, A.E., Moller, P.L., Michaelsen, K.F., Paerregaard, A., Sandstrom, B., Tvede, M., and Jakobsen, M. 1999. Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Appl. Environ. Microbiol.* 65: 4949-4956

Jonas, W.B. 1997. Alternative medicine. *Journal of Family Practice.* 45:34–37.

Kirtikar, K. R. and Basu, B. D. 1935. *Indian Medicinal Plants,* Second edition (Published by Lalit Mohan Basu, Allahabad, India) II:1492.

Kong YC, Hu Sy, Lau Fk, Che CT, Yeung HW, cheung S and wang Jcc, H. 1976. Potential anti-fertility plants from Chinese medicine. *Am J Chinese Med.* 4:105-128.

Manickam, M., Ramanathan M., Jahromi M.A., Chansouria J.P., Ray A.B. 1997. Antihyperglycemic activity of phenolics from Pterocarpus marsupium. *J Nat Prod*.60(6):609-10.

Nageswara Rao, G. 2000. Mahesh Kumar P., Dhandapani, V.S., Ramakrishna T., Toshimitsu nayashi. Constituents of *Cassia auriculata. Fitoterapia,* 71:82-83.

Nanji, A.A., Khettry, U., Sadrazadeh S.M.H.L. 1994. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). *Pro Soc Exp Biol Med,* 205,243-7.

Norr, H. and Wagner, H. 1992. New constituents from *O.sanctum*, *Planta Med,* 58:574.

Pochart, P., Marteau, P., Bouhnik, Y., Goderel, I., B o u r l i o ux , P. & Rambaud, J.C. 1992. Survival of Bifidobacteria ingested in a fermented milk during their passage in the human small intestine: an in vivo study using intestinal perfusion. *American Journal of Clinical Nutrition* 55:7&80.

Satyavathi, G.V., Gupta, A.K. and Tandon, N. 1987. In: *Medicina plants of India*, ICMR, New Delhi, II: 326.

Shah, C.S. and Qadry, J.S. 1988. Volatile oils, In *A textbook of Pharmacognosy* , BS Shah Prakashan, Ahmedabad, India:216.

Silk, D.B.A, Davis, A., Vulevic, J., Tzortzis, G., and Gibson, G.R. 2009. Clinical trial: the effects of a trans-galactooligosaccharide on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther.* 29:508–18.

Simennoff, M.L., Dunn, Sr., Zolner, G.P., Fitzpatrick, M.E., Emery, S.M., Sandine, W.E., and Ayres, J.W., 1996. Biomodulation of the toxic and nutritional effects of small bowel bacterial overgrowth in end-stage kidney disease using freeze-dried *Lactobacillus acidophilus*, *Miner Electrolyte Metab*:22

Su L., Shen L., Clayburgh D.R., Nalle, S.C., Sullivan, E.A., Meddings, J.B., Abraham, C., Turner, J.R. 2009. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. *Gastroenterology,* 136:551–563.

Tabuchi, M., Ozaki, M., Tamura, A., Yamada, N., Ishida, T., Hosoda, M., and Hosona, A., 2003. Antidiabetic effect of Lactobacillus GG in streptozotocin induced diabetic rats. *Biosci. Biotechnol. Biochem*., 67:1421–1424.

Tannock**,** G.W. 1983. The effect of dietary and environmental stress on the gastrointestinal micro- biota. In Human intestinal microflora in health and disease. New York**:** Academic Press: 517-539.

Umadevi, P., Bisht, K.S. and Vinitha, M.A. 1998. Comparative study of radtioprotection by Ocimum flavanoids and synthetic aminothiol protectors in the mouse, *Br J Radio*, 71: 782.

Vos, A.P., M'Rabert, L., Stahl, B., Boehm, G., Garssen, J. 2007. Immunomodulatory effects and potential working mechanisms of orally applied nondigestible carbohydrates. *Crit Rev Immunol* ;27:97–140.

Wang, X., Gongm X, Egashiyay and Sanda H. 2004. Purification and characteerisation of aferuloyl esterace from the intestinal bacterium *L. acidophilus. Appl. Environ. Microbiol.* 70:2367-72.

Wolever, T., Spadaflora, P., Cunnane, S., and Pencharz, P. 1995. Propionate inhibits incorporation of Colonic (c,3) acetate into plasma lipid in human. *Am. J. Clin. Nutr.* 61:1241-1247.