

ROLE OF PROBIOTICS IN HEALTHY SKIN WITH REFERENCE TO GUT SKIN AXIS

*Dr. Ritunanda Tripathi ** Dr. N.K. Prasad #Dr. Shuchidubey

* PG Scholar, ** MD (Ayu) Professor and HOD, #MD (Ayu) Reader Kriyasharirvibhag
PTKLS Govt (auto.) Ayurveda College Bhopal (M.P.)

ABSTRACT:

*In modern era, changed life style of human being for example busy life, unhealthy improper diet, use of chemical products and polluted environment has created several disharmonies in his biological system. All factors can cause changes in skin composition and plays major role in producing variety of skin diseases along with systemic involvement. Researchers have demonstrated the bidirectional connection between the gut and skin which plays a major role in the maintenance of homeostasis. Gut flora which produce neurotransmitters and modulate skin function. The skin and gut both operate as neuro-immuno-endocrine organs, and participate in essential communication with the nervous system, immune system, and endocrine system. Probiotics can disrupt the pathogenesis of skin diseases through immunomodulatory and anti-inflammatory actions. Probiotics can have great potential in preventing and treating the skin diseases including eczema, tropic dermatitis, acne, allergic inflammation, UV induced skin damage and as a cosmetic product. In our Ayurvedic classics several types of drug and diet have been described to maintain health which is being used today as a prebiotics and probiotics. **Aim:** To study the concept of probiotics and its role in the gut skin axis. **Methods:** Conceptual study based on modern literature, classical references and published research articles. **Conclusion:** Skin is one of the important presentable organs of the body and personality. So, disorders of skin affect not only on somatic level but also on psychological level too. In the current study, we describe how the gut microbiota communicates with the skin as one of the main regulators in the gut-skin axis.*

Keywords: Probiotics, Gut flora, Gut Skin Axis, Ayurveda

INTRODUCTION

The skin represents the largest organ in the human body and as such, its main function is to act as a barrier to extrinsic factors including physical, chemical and microbial threats. Healthy skin is a mirror of a healthy body and skin health has a powerful impact on emotional and mental wellbeing. The skin effectively performs its functions protection, temperature regulation, water retention, and more when in a state of homeostasis. As an organ undergoing constant renewal, effective epidermal

turnover, the process by which the skin regenerates itself, is essential to maintaining this state.

The small and large intestines provide residence for a vast community of bacteria and their metabolites and by products, which we call the gut microbiome. Similarly, thousands of microbial organisms and their by-products inhabit the skin, referred to as the skin microbiome. In both the gut and the skin, a harmonial balance in these microfloras is important in maintaining homeostasis.¹

The gut and skin are uniquely related in purpose and function with crucial immune and neuroendocrine roles.² The probiotics support intestinal health by inhibiting the over growth of toxic bacteria.

Lactobacillus and bifidobacteria also produce organic acid that reduce intestinal pH and retarded the growth of pathogenic bacteria. Probiotics influence immunity, lowering cholesterol anti allergic effect, Infections, Malabsorption etc. (Antioxidant property). Recent research has suggested that probiotics supplementation presents promising potential in the role of prevention and management of various skin disorders.

The word “probiotics” have not been mentioned in the ancient texts, but Indian medicine was certainly aware about the specific uses of dairy products, fermented diet and medicines for health benefits and was well-documented.

Aim:- To study the concept of probiotics and its role in the gut skin axis.

Material and Methods: Conceptual study based on modern literature, classical references and published research articles.

REVIEW OF LITERATURE:-

Gut Microbiome The “gut microbiome” refers to the diverse community of microbial organisms that normally inhabit the bowel and their by products. There are more than 100 trillion bacteria present in the human gastrointestinal tract, consisting of over one thousand different species colonizing the intestines.^{3,4} The role of the gut microbiome is thought to include proper development and functioning of the immune system, protection against infections, digestion of polysaccharides, and synthesis of vitamins. The intestinal microbiome protects against invasion by exogenous pathogens directly, by competitively binding to epithelial cells, and indirectly, by triggering immunoprotective responses. The symbiotic relationship between resident gut bacterial flora and the host is vital to the normal immune system development and homeostasis of the host and regulation of epithelial growth and differentiation.⁵ Short chain fatty acids (SCFAs), products of dietary fibers fermented by components of the gut microbiome, demonstrate a protective role against the development of inflammatory disorders including arthritis and allergy, in addition to colitis.⁶

Role of the Gut Microbiome in Skin Homeostasis

Epidermal differentiation or keratinization is a normal physiological process which turns the stratum corneum of the skin into a hard protective cover. When epidermal turnover functions appropriately, the resulting brick and mortar structure serves as an effective skin barrier with the ability to limit evaporation, preserve skin moisture, and protect from invasion by foreign organisms and substances.⁷ There is new evidence that the intestinal microbiome may impact cutaneous physiology, pathology, and immune response more directly, through the metastasis of gut microbiota and their metabolites to the skin. In cases of disturbed intestinal barriers, intestinal bacteria as well as intestinal microbiota metabolites have been reported to gain access to the bloodstream, accumulate in the skin, and disrupt skin homeostasis. DNA of intestinal bacteria has been successfully isolated from the plasma of psoriatic patients. These findings represent evidence of a more direct link between the gut microbiome and cutaneous homeostasis. The gut microbiome appears to have an influence on the skin microbiome. Short chain fatty acids (SCFAs) resulting from fiber fermentation in the gut can promote the growth of certain skin microbes, which influence immune defence and regulate skin inflammation.⁸

Gut Microbiota and Skin Allotaxis

The intestinal microbiome contributes to skin allotaxis, the restoration of homeostasis after a disturbance or stressor, through gut microbiota-mediated effects on both innate and adaptive immunity.⁹ Commensal gut flora can promote skin allotaxis by influencing T cell differentiation in response to various immune stimuli. These cells and their pro-inflammatory cytokines are thought to directly contribute to the pathogenesis of several chronic inflammatory dermatoses including psoriasis, Behcet’s disease, and contact hypersensitivity.¹⁰ Studies have demonstrated that gut bacteria can positively impact the response to disturbed skin barrier function. The gut microbiome has also been shown to support restoration of skin homeostasis after ultraviolet (UV) radiation exposure.

Dysbiosis and Skin Dyshomeostasis

Intestinal dysbiosis, a state of microbial imbalance, has the potential to negatively impact skin function. Intestinal dysbiosis results in increased epithelial permeability which then triggers the activation of effector T cells, disrupting their balance with immunosuppressive regulatory T cells. Pro-inflammatory cytokines further enhance epithelial permeability and set up a vicious cycle of chronic systemic inflammation. Gastrointestinal disorders can present with dermatological skin findings. IBD is linked to skin manifestations (pyoderma gangrenosum, erythema nodosum) and Celiac disease (dermatitis herpetiformis, vitiligo). Psoriasis is more commonly found in patients with Crohn's disease than healthy people.¹¹ Simultaneous gut and skin microbiome dysbiosis has been observed in several inflammatory skin diseases, such as Acne Vulgaris, psoriasis, and atopic dermatitis.

Acne Vulgaris

Although acne is caused by many factors, gut microbiome can play a key role in the progression and severity of the disease. The emotional stress that accompanies acne can impair the gut microbiome, most notably Lactobacillus and Bifidobacterium species. Psychological stress can cause intestinal microbes to produce neurotransmitters that can enter the bloodstream through the intestinal barrier, resulting in systemic inflammation. These neurotransmitters not only increase intestinal permeability, leading to both intestinal and systemic inflammation, but also directly access the circulation through the compromised intestinal barrier resulting in systemic effects.¹²

In Hypochlorhydria Low levels of acidity allows for the migration of colonic bacteria to distal parts of the small intestine, creating a state of intestinal dysbiosis and small intestinal bacterial overgrowth (SIBO). SIBO also results in the production of toxic metabolites, which can injure enterocytes, increase intestinal permeability, and ultimately lead to systemic inflammation.¹³

Atopic Dermatitis

Atopic dermatitis is the most common chronic pruritic inflammatory dermatosis. Skin barrier dysfunction and altered immune responses are primary players in the pathogenesis of Atopic Dermatitis. The low fiber and high fat content characteristic of the western diet fundamentally changes the gut microbiome, resulting in deficient production of immunomodulatory metabolites, particularly SCFAs.

Psoriasis

Psoriasis is an immune-mediated chronic relapsing-remitting inflammatory dermatosis. 7- 11% of inflammatory bowel disease (IBD) patients are diagnosed with psoriasis, making the association with GI inflammation particularly strong.¹⁴ It has also been theorized that the far-reaching effects of intestinal dysbiosis are the result of gut microbes and their metabolites breaching an impaired intestinal barrier and entering systemic circulation to directly target distant organs, including the skin and joints.

PROBIOTICS

The health exerting microbes are known as probiotics. According to WHO, probiotics are considered to be "live microorganisms which when administered in adequate amounts confer a health benefit on the host".¹⁵ They are postulated to exert a wide range of health benefits such as restoration and maintains of healthy gut flora, lowering cholesterol, IBD, malabsorption disease, management of lactose intolerance, urogenital and vaginal infection, protection against colon cancer, anti-allergic effect etc.¹⁶

Probiotics may alleviate abnormal alterations of the gut microbiome, referred to as "dysbiosis". Firstly they are believed to prevent pathogenic bacteria from colonizing the gastrointestinal tract, which would otherwise subsequently lead to disease. Secondly, they are thought to improve the barrier function of the colonic mucosa. Thirdly, probiotics may help modulate the immune system, which may help shift away from pro-inflammatory immune reactivity.¹⁷ Fourth, they may synthesize and secrete metabolites that may have nutritional benefits and anti-inflammatory effects.¹⁸

Probiotics are postulated to help in atopic dermatitis by improving the diversity of the intestinal flora and increase the barrier function of the skin and mucosa.¹⁹ Oral probiotics control skin inflammation by acting on both effector and regulatory T cells. Studies advocated for an acidophilus milk preparation, they would be referred to as probiotics.²⁰

PROBIOTICS IN AYURVEDA

Dadhi (Yogurt)

The origin of the science of modern probiotics lies in 1908 when Metchnikoff observed that the Bulgarian peasants lived longer because of the consumption of yogurt. Bacteria like *Lactobacillus*, *Bifidobacterium* and a few other single-celled organisms like yeasts (e.g. *Saccharomyces boulardii*) have been evaluated as probiotics.²¹

“Probiotics” as per modern definition refers to the specific live cultures of microorganisms while the Ayurvedic understanding of the products is more holistic. The medicinal benefits suggested may not be just due to the microflora but also to the prebiotic minerals and other components in the product. Several recent pharmacological studies showed the effect of Ayurvedic probiotics like curd on gut health.

According to *Maharshi Charka*, curd is appetizer, digestive, stimulant, aphrodisiac, strength promoting and nourishing.²² The watery portion of *Dadhi* is called *Mastu*. And it is light to digest, increases appetite, cleanses body channels, and pacifies thirst.

In cosmetic formulations, prebiotics can be applied to the skin microbiota directly and increase selectively the activity and growth of beneficial ‘normal’ skin microbiota. The use of honey and yogurt for skin beauty has been going on since ancient time. Buttermilk and goat’s milk powders traditionally used in Indian face mask preparations have soothing and emollient properties. They also contain vitamin A, B6, B12 and E. They would make beneficial alternatives to chemical bases and emollients.

Takra (Buttermilk)

Buttermilk is sweet, sour with astringency as subsidiary taste, hot in potency, easily digestible and may cause some dryness in body. It stimulates

digestive fire, alleviates minor poisons if consumed, decreases oedema, and helps to control diarrhea, anemia, hemorrhoids, and splenomegaly. It is helpful in anorexia, intermittent fevers, vomiting, excessive salivation, dysuria, and excessive body fat.²³ According to Acharya Bhavprakash a person who habitually takes *Takra* never suffers and being impregnated with *Takra*, disease does not attack him.

Asava and Arishta

Asava and Arishtas are self-generated herbal fermentations of traditional Ayurvedic system. Asava and Arishtas are used for the treatment of various problems in paediatrics, nervous system, blood and circulatory system, respiratory system, digestive and excretory system, urinary system, reproductive system, immune system, skin problems, worm infections, general illness and infectious diseases etc..²⁴ For treating skin diseases *Khadirarista*, *Loharista*, *Devdarvyarista*, *Sarivadyasava*, *Lohasava*, *Babularista*, *Chitrakasava*, *Gomutrasava*, *Lodhrasava*, *Nimbarista*, *Sarivadyarista*, *Panchtiktarista* are generally used.

Sandhanakalpana and fermented food materials described in *ayurveda* can be compared to probiotics. The microbes involved in this process mediate this process; enhanced therapeutic properties, which may be due to the microbial biotransformation of the initial ingredients of *Arishta* and *Asava* into more effective therapeutics as end products.²⁵ The antibacterial activity of *Takrarista* was reported.²⁶ Potential probiotic *L. plantarum* is isolated from fermented Ayurvedic medicine *Kutjarista*. (Kumar et al. BMC Microbiology 2011)

Kanji

Kanji is a unique Ayurvedic preparation which comes under *Shukta Kalpana* preparations. In Ayurvedic classics *Kanji* has been described as “*Kanjeeviyami*” i.e. ‘to who make alive’. The studies show the effect of *Kanji* on the digestion (*Jatharagni*). *Acharya Charaka* has described that a person dies when the function of *Agni* is stopped and the person stays happy and healthy when the *Agni* continues working in a proper way. In the Ayurvedic classics *Kanji* has been described as a digestive stimulant (*Dipaniya*) and it alleviates burning sensation and fever by external

(IJRMST) 2019, Vol. No. 8, Jul-Dec

e-ISSN: 2455-5134, p-ISSN: 2455-9059

application and reduces *Vata-Kapha* diseases and constipation by internal application.^{27, 28}

Kanji (an Indian functional food), a probiotics product, is a lactic fermented rice product. *Kanji* is a well known Ayurvedic lactic acid-based fermented product. It has been prescribed for a number of chronic diseases by Indian Ayurvedic practitioners. The study proves that the in vitro evaluation of the lactic acid bacteria isolated from *Kanji* has probiotics properties.²⁹

Dhanyamla

Acharya Susruta described the *Dhanyamla* (fermented gruel) in the *Madya* chapter and state that it is a good appetizer, light to digestion and act as a solvent. It improves blood circulation, immunity, skin complexion, relieves bodyache and muscle spasms.

Dhanyamla can be administered internally and externally. Oral application of the drug enhances appetite and digestive power. It is also used in enema therapy and purgation therapy to reduce obesity. Externally, *Dhanyamla* is used in massage therapy, fomentation (Pipe, Shower and Tub fomentation), *Shirodhara* and *Shiro Vasti*.^{30, 31}

Sukta

Fermented liquor known as *Sukta*. They act diuretics, pleasant, pungent in digestion, to bring on an attack of haemoptitis and prove curative in jaundice etc. *Acharya Bhavprakash* also described *Kanji*, *Tushodak*, *Sauveerak*, *Aarnal*, *Dhanyamla*, *Sandaki* etc. fermented material.

Madya

Ancient methodology of preparation of *Madya* (wine) was very scientific. The consideration of quality of *Madya*, its texture, contains duration etc. obviously indicates their appropriate and scientific approach to this end. According to *Acharya Charaka* *Madya* due to its subtle, heat, *teekshna* and *vishada* property clarifies the channels, removes *Malas* from *srotasas*, when *rasasamvahan* proper tissue formation start properly.³² It promotes good voice, health, *pratibha* (ingenuity) and complexions. *Acharya Susruta* view, a variety of *Madya* called *Arista* (fermented liquor) is highly efficacious owing to the concentrated action of a variety of drugs

entering in to its composition. It proves curative in large number of disease; tend to subtle the deranged humours of the body and a good appetizer.³³

DISCUSSION

Ayurveda is one of the most ancient medical traditions practiced in India, and has a sound philosophical and experiential basis. Knowledge of *Ayurvedic* herbs, herbal preparations and milk products, are now being effectively used in medicines, food supplements and personal care. *Ayurveda* explains a physiological process called "*Agni*", is responsible for digestion of food components and metabolism of tissues. Specific fermented milk products have been indicated to normalize "*Agni*" (metabolism).³⁴ Most of the diseases are generally resulted from the *Mandagni*. i.e. hypo functioning of *Agni* or slow digestive capacity.³⁵ *Ayurvedic* therapeutics always focuses on restoring normal status of *Agni*.

In Fermentation context, a strong symbiotic relationship between microorganisms exists that constitutes its microbiota. This natural microflora supports the immune system in various ways including the production of natural antimicrobial compounds (e.g. lactic acid) as well as activation of various signaling pathways and modulation of the inflammatory response.^{36, 37}

The relationship between human health and microbiota was first mentioned in 1907, by Elie Metchnikoff, when the enhanced longevity due to the intentionally present bacteria in yogurt was described.³⁸ Fermented food has been part of our diet. Some of the most ancient fermented foods used in history is wine, bread and milk products such as yoghurt. In addition, fermented food became famous after Werner Kollath first introduced the term "Probiotic". Most common species of probiotics belong in the families of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*. As these microorganisms are naturally found in the gut microbiota, most studies are focused on their effects in the context of the natural function in the gut and as preventive or therapeutic agents against disease development.

Medieval literature on dietetics, *Kshemakutuhalam* emphasizes the importance of including milk products in diet. Fermented milk products are known to normalize "Agni". Milk products like *Dadhi, Takra, and Navneet* are considered as *pathya* or *rasayana*, which can be correlated to modern probiotics and prebiotics that increase *Agni, Oja, complexions* etc.

CONCLUSION

The skin ecosystem is a complex environment covered with a diverse microbiota community. Topical applications of probiotics bacteria have a direct effect at the site of application by enhancing the skin natural defence barriers. Probiotics as well as resident bacteria can produce antimicrobial peptides that benefit cutaneous immune responses and eliminate pathogens. In this study we found that most of the fermented preparations (Sandhan Kalpana) and milk preparations like *Dadhi, Takra, Mastu* etc. described in ayurveda stimulates *Jatharagni* (strong action of digestive system) and show their influence on the (metabolism) body. In the field of Nutricosmetics 'eat and drink products in co-ordination with usual skin care routine for better overall results' the diet and medicines mentioned in ayurveda can prove to be a better option.

The future for beauty of body from probiotics that offer multifunctional benefits in the area of gut health and skin health with anti-inflammatory and immunomodulatory properties is bright. The dietary regimen and medicines mentioned in ayurveda classics may prove to be a good market in the field of probiotics in the future. This review may help healthcare industry and modern scientists understand various different trends of potential use to research on probiotics approaches to maintaining skin health and preventing skin diseases.

REFERENCES

- Barrett KE, Ghishan FK, Mercant JL. Physiology of the Gastrointestinal Tract. 4th ed. New York;
- The gut-skin axis in health and disease: A paradigm with therapeutic implications. O'Neill CA, Monteleone G, McLaughlin JT, Paus R Bioessays. 2016 Nov; 38(11):1167-1176. Elsevier
- Moschen AR, Wieser V, Tilg H. Dietary Factors: Major Regulators of the Gut's Microbiota. Gut Liver 2012; 6: 411-416 [PMID: 23170142 DOI: 10.5009/gnl.2012.6.4.411]
- D'Argenio V, Salvatore F. The role of the gut microbiome in the healthy adult status. Clin Chim Acta 2015; 451: 97-102 [PMID: 25584460 DOI: 10.1016/j.cca.2015.01.003]
- Neish AS, Jones RM. Redox signaling mediates symbiosis between the gut microbiota and the intestine. Gut Microbes 2014; 5: 250-25 [PMID: 24637602 DOI: 10.4161/gmic.2791]
- Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE₂. Kim YG, Udayanga KG, Totsuka N, Weinberg JB, Núñez G, Shibuya A Cell Host Microbe. 2014 Jan 15; 15(1):95-102. [PubMed] [Ref list]
- Mesenchymal Stem Cells from Adipose Tissue in Clinical Applications for Dermatological Indications and Skin Aging. Gaur M, Dobke M, Lunyak VV Int J Mol Sci. 2017 Jan 20; 18(1):. [PubMed] [Ref list]
- Am J Clin Nutr. 2002 Dec; 76(6):1249-55. Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers. Naruszewicz M¹, Johansson ML, Zapolska-Downar D, Bukowska H. Author information
- Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE₂. Kim YG, Udayanga KG, Totsuka N, Weinberg JB, Núñez G, Shibuya A Cell Host Microbe. 2014 Jan 15; 15(1):95-102. [PubMed] [Ref list]
- Skin manifestations of inflammatory bowel disease. Huang BL, Chandra S, Shih DQ Front Physiol. 2012; 3():13. [PubMed] [Ref list]
- Saarialho-Kere U. The gut-skin axis. J Pediatr Gastroenterol Nutr 2004; 39 Suppl 3: S734-S735 [PMID: 15167366]
- Vaughn A. R., Notay M., Clark A. K., Sivamani R. K. (2017). Skin-gut axis: the relationship between intestinal bacteria and skin health. World J Dermatol. 6 52-58. 10.5314/wjd.v6.i4.52 [CrossRef] [Google]

- Scholar [Ref list]
13. Review Acne vulgaris, probiotics and the gut-brain-skin axis: from anecdote to translational medicine. Bowe W, Patel NB, Logan AC *Benef Microbes*. 2014 Jun 1; 5(2):185-99. [PubMed] [Ref list]
 14. Skin manifestations of inflammatory bowel disease. Huang BL, Chandra S, Shih DQ *Front Physiol*. 2012; 3():13. [PubMed] [Ref list]
 15. Joshi Y.K., basics of clinical nutrition, jaypee brothers medical publishers ,reprint 2009)
 16. Agrawal Anjana, Udipi Shobha A ,Text book of Human Nutrition, jaypee brothers medical publishers, 2014 p.n. 532).
 17. Butel MJ. Probiotics, gut microbiota and health. *Med Mal Infect* 2014; 44: 1-8 [PMID: 24290962 DOI: 10.1016/j.medmal.2013.10.002]
 18. Stanton C, Ross RP, Fitzgerald GF, Van Sinderen D. Fermented functional foods based on probiotics and their biogenic metabolites. *Curr Opin Biotechnol* 2005; 16: 198-203 [PMID: 15831387 DOI: 10.1016/j.copbio.2005.02.008]
 19. Baquerizo Nole KL, Yim E, Keri JE. Probiotics and prebiotics in dermatology. *J Am Acad Dermatol* 2014; 71: 814-821 [PMID: 24906613 DOI: 10.1016/j.jaad.2014.04.050].
 20. Norman HJ: Lactic acid bacteria in the treatment of melancholia. *Br Med J* 1909, 1:1234-5. 11. Phillips JGP: The treatment of melancholia by the lactic acid bacillus. *J Mental Sci* 1910, 56:422-31.
 21. Narayan SS, Jalgaonkar S, Shahani S, Kulkarni VN. Probiotics: Current trends in the treatment of diarrhoea. *Hong Kong Med J* 2010;16:213-8.
 22. Gupta L.P., Biogenic Secrets of Food in Ayurveda, Chaukhambha Sanskrit pratisthan, first edition 1999, p.n.64
 23. Sharma PV. *Susruta Samhita*. Vol. 1. Varanasi: Chaukhambha Visvabharati; 1999. p. 173
 24. Asava and Arishta: An Ayurvedic Medicine – An overview, AK Mishra, A Gupta, V Gupta, R Sannd, P Bansal , *International Journal of Pharmaceutical & Biological Archives* 2010; 1(1): 24 –30
 25. Traditionally fermented biomedicines, arishtas and asavas from Ayurveda, S Sekar & S Mariappan, vol 7(4) October 2008
 26. In vitro antibacterial activity of Takrarist-An Ayurvedic formulation, Sandeep Bhardwas, Girish Achliya, Vijaya S Meghre, vol 4 (3) July 2005)
 27. Tripathi Bramhanand, Hindi commentator, *Charaka Samhita, Sutra Sthana*, Chaukhambha Orientalia, Varanasi, 27/192.
 28. Shastri Ambikadatta, *Sushruta Samhita, Sutrasthana*, 12th Edition, Chaukhambha Sanskrita Samsthana, Varanasi, 2001, 45/215, page 178.
 29. Screening of probiotic properties of lactic acid bacteria isolated from Kanjika, an ayurvedic lactic acid fermented product: An in-vitro evaluation Kanchi Bhasker Praveen Kumar Reddy, Ponnala Raghavendra, Basavanna Girish Kumar, Mahesh Chandra Misra, and Siddlingiaya Gurudutt Prapulla*
 30. Sharma PV, Editor. *Sushruta Samhita*. English Translation. Chikitsa Sthana. Varanasi. Chaukhambha Visvabharati 2010; 2:644. Chapter 38: 43-46.
 31. Singh RH. *Panchakarma Therapy*. Keraliya Pancha Karma. Varanasi. Chaukhambha Sanskrit Series Office. 2013; 1898
 32. Pt. Kashinath Shastri, *Charak Samhita of Agnivesha, Chikitsa Adhyay 8 ver. 166 vol 2*, Chaukhambha Sanskrit Sansthan Varanasi, reprint 2006 pg 300
 33. Kaviraj Ambikadutta Shashtri, *Sushruta Samhita, Sutrasthan Adhyay 46 ver. 194, 195, vol 1*, Chaukhambha Sanskrit Sansthan Varanasi, reprint 2005 pg 184.
 34. Dash B, Kashyap L. *Diagnosis and Treatment of Diseases in Ayurveda*. Vol. 2. New Delhi: Concept Publishing Company; 1994. p. LI.
 35. Shastri Hari Sadashiva Hindi translator, *Sarvangasundara Sanskrit Commentary by Arunadatta, Astanga Hridaya, Nidana Sthana*, Chaukhambha Subharati Prakashana, Varanasi, 2010, 4/19, page 513.

36. Cogen, A.L.; Nizetà, V.; Gallo, R.L. Skin microbiota: A source of disease or defence? *Br. J. Dermatol.* 2008, 158, 442–455. [Google Scholar] [CrossRef] [PubMed]
37. Grice, E.A. The skin microbiome: Potential for novel diagnostic and therapeutic approaches to cutaneous disease. *Semin. Cutan. Med.* Surg. 2014, 33, 98–103. [Google Scholar] [CrossRef]
38. Gordon, S. Ellie Metchnikoff: Father of natural immunity. *Eur. J. Immunol.* 2008, 38, 3257–3264. [Google Scholar] [CrossRef]