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REVIEW ARTICLE

Maintenance of a balanced human gut microbiota - an emerging healthcare destination for herbal research

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ABSTRACT

The human gastrointestinal tract harbors a complex and dynamic population of micro-organisms known as 'gut microbiota'. The gut micro-organisms exert great influence on us during the states of metabolic/immunological homeostasis as well as manifestation of or protection from diseases. Relative composition of gut microbiota is now known to be influenced by the type of diet, geographic locations and nutritional status of the individuals. Gut microbiota most closely interact with the gastrointestinal tract, liver, skin and central nervous system and hence, aid in physiological functions such as digestion and absorption of nutrients, neuro-transmission, fat metabolism, and immune responses. Accordingly, disturbance in the gut microflora architecture normally lead to several health disorders like obesity, diabetes, hypertension, rheumatoid arthritis, inflammatory bowel disease, nervous depression, anxiety, cognitive decline, Parkinson's Disease etc. The metagenomic studies of human gut flora have suggested that the bulk of the microbial community is made up of nine bacteriophyta namely: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, Cyanobacteria and Spirochaetes. Several studies have also indicated a positive association of gut microbes with bidirectional regulation of intestinal and central nervous system (CNS) through neurotransmitter, endocrine, immune and metabolic pathways. An imbalanced gut microbiota has been shown to affect the progression of CNS diseases, including cerebral ischemia, Alzheimer's disease, disseminated sclerosis and hepatic encephalopathy. In addition, gut microbiota imbalance can also increase intestinal permeability that can lead to liver toxicity and several other pathophysiological complications. Intestinal microbiota contributes to diverse mammalian processes including the metabolic function of the drugs, both traditional as well as modern.

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A large number of herbal medicines have been found to act in association with gut microbiota because majority of herbal drugs are orally administered and invariably come in direct contact with these microbes. Since most herbal preparations are made up of a large number of plant-derived bioactive metabolites, their precise mode of actions on gut flora, body metabolism and disease management/treatment is often not fully explained. Recent developments in the field of gut metagenomics vis-à-vis bioavailability of herbal ingredients and their pharmacodynamics in disease conditions are providing fresh insights to these associations. Today a general consensus is that most traditional herbals treat disease by three primary modes: getting metabolized into more pharmacologically active metabolites by the action of gut microbiota; regulating the gut microbiota balance in terms of relative population of different bacteria particularly the Firmicutes-to-Bacteroidetes ratio (F/B); and by regulating the synthesis of fermentation products of the gut microbes such as acetate, butyrate and propionate etc.

The gut micro-flora architecture and its association with herbal medicines is a hot topic of discussion today. In last five years >13000 papers were published on studies related to gut microbiota. This review is an attempt to summarize our current understanding of this subject with sole intention of sensitizing the researchers' community of medicinal and aromatic plant sciences in India to validate and valorize our existing AYUSH prescriptions in the context of gut microbiota influences for enhancing their global acceptance.

INTRODUCTION

Gastrointestinal Microbiota - An Overview

The term *gastrointestinal microbiota* or *gut flora* or *gut microbiota* collectively represents the total microbial population that inhabits the digestive tract of a human body. In other words, the term microbiota encompasses the collection of all microbial taxa such as bacteria, archeae, fungi and protists [14,41,50]. Depending on their particular location of occurrence in the body, the microflora can be specified as 'the gut microbiota' that refers to those present in the intestinal tract or the 'oral microbiota' that speaks about the microbes inhabiting the oral cavity. Other occupied habitats include genital organs, respiratory tract, skin and gastrointestinal system [65,78]. The gut microbiota is now considered an important partner of human cells, interacting with virtually all cells in our body. The human microbiota is roughly estimated to have 10^{-13} – 10^{-14} microbial cells based on the number of bacterial cells ($3.8x10^{-13}$) derived from colon that harbors the densest number of microbes.

Before moving further for a discussion on this subject, it is better to get familiarity with five related terms that will find extensive usage in the subsequent text in this review. They are: ***Metagenome -*** the term that refers to the entire genetic material present in a sample. The metagenome is composed of the genomes of several individual organisms, for instance, the genomes of human cells and of the gut microorganisms that are present in the faecal material. ***Probiotics –*** the term used for live microorganisms that, when consumed in adequate amounts, confer a health benefit to the host body; ***Prebiotics -*** a term that refers to the typically non-

digestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of certain advantageous gut flora by acting as substrate for them and exert a health benefit to the host; ***Synbiotics -*** refers to the dietary supplements combining probiotics and prebiotics for a synergistic action and; ***Pharmabiotics -*** refers to pharmaceutical formulations of engineered probiotics, prebiotics, or synbiotics with a regulatory approval for sale as a drug and are engineered for optimized shelf life in the digestive tract [49,55,98] .

The gut microbiota has co-evolved with the host over thousands of years to form an intricate and mutually beneficial relationship [100,131]. It's a highly interactive biodiversity niche where greatest numbers of bacteria reside compare to any other part of the human body. This coexistence is not only commensal but symbiotic or mutualistic in nature as well [56]. The microorganisms perform a variety of useful functions, such as fermentation of the unused energy substrates, balancing of immune functions using end products of metabolism like propionate and acetate, preventing growth of harmful/pathogenic microbial species, producing vitamins such as biotin and vitamin K for the host body, producing hormones required for storing fats, strengthening gut integrity by shaping the intestinal epithelium, and regulating overall host immunity etc [65,78]. Alteration and imbalances of the gut microbiota and its microbiome, (a condition popularly known as dysbiosis) often results in many disease manifestations associated with indigestion and obesity. In extreme situation prevalence or depletion of some species may even initiate an immunological, inflammatory, neurological or even a carcinogenic event [52,101,144].

The microbial composition of the gut microbiota varies in different parts of the digestive tract. In the stomach and small intestine, relatively few species of bacteria are present. The colon, on the other hand, harbors the highest microbial density (up to 10^{12} cells per gram of intestinal content). Though the high acidic environment of the stomach do not favor the survival of most microorganisms but *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *Peptostreptococcus*, and certain types of yeast can also inhabit the stomach. *Helicobacter pylori*, a gram-negative spiral bacterium that establishes on gastric mucosa, can frequently cause chronic gastritis and peptic ulcers [14]. Though total number of bacteria species present in the gut may range from 300 to 1200, the bulk of this microbial load (>90%) is constituted by around 40 species. Over 99% of the bacteria in the gut are anaerobes and, according to an estimate the gut microflora can account for hundred times more genes in total in comparison to entire human genome. Gut bacteria can also make up to 60% of the dry mass of feces. Bacterial phyla like Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria constitute the bulk of bacteria population present in the gut [131]. About 30% of all bacteria present in the gut belong to genus *Bacteroides.* Other dominate genera may include *Clostridium*, *Faecalibacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, and *Bifidobacterium*. Fungal genera that have been detected in the gut include *Candida*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhodotorula*, *Trametes*, *Pleospora*, *Sclerotinia*, *Bullera*, and *Galactomyces*. *Rhodotorula* is most frequently found in individuals with inflammatory bowel disease while *Candida* is most frequently found in individuals with hepatitis B cirrhosis and chronic hepatitis B [65,78]. The small intestine contains a trace amount of microorganisms due to the proximity and influence of the stomach. Gram-positive cocci and rod-shaped bacteria are the predominant microorganisms found in the small intestine. However, in the distal portion of the small intestine alkaline conditions support the growth of gram-negative bacteria also [50]. In addition the large intestine contains the largest bacterial ecosystem in the human body. About 99% of the large intestine and feces flora are made up of obligate anaerobes such as *Bacteroides* and *Bifidobacterium.* Since gut microflora also constitute a major portion of faeces dry mass, it is an ideal source of gut flora for conducting any tests

and experiments. For this, the nucleic acids from faecal specimens are extracted and bacterial 16S rRNA gene sequences are generated with bacterial primers [1,13,14,126].

FACTORS AFFECTING ACQUISITION AND COMPOSITION OF GUT FLORA

According to one estimate, a normal human adult consumes about 60-65 tons of food through its gastrointestinal tract in an average life time. This food intake also pushes down an abundance of microorganisms from the environment that imposes a huge potential threat on our gut integrity and health. The different number of microorganisms inhabiting the GI tract has been estimated to exceed 1000, which encompasses <“10 times more bacterial cells than the number of human cells and over 100 times the amount of DNA content (microbiome) over the human genome. These extra genes add various types of enzymatic proteins that are otherwise not coded by the host genome and play a critical role in regulating body physiology and metabolism [56]. Gut microflora is normally composed of three enterotypes: *Prevotella*, *Bacteroides*, and *Ruminococcus* and the relative concentration of each microbial community is greatly affected by the diet we consume. For example, *Prevotella* is prevalent when diet is rich in carbohydrates and simple sugars whereas *Bacteroides* is more associated with diet containing proteins, amino acids, and saturated fats. One enterotype can therefore dominate over others depending on the type of food prevalent in a given geographic area [50]. The vegetarian diet normally allows a preferential dominance of a microbiome that is entirely distinct from that of meat eaters. In diets that are rich in meat and animal products, there are high abundances of Alistipes, Bilophila and Bacteroides groups which are all bile tolerant [56,60,63]. In individuals consuming this type of diet, the group Firmicutes which is associated with the metabolism of dietary plant polysaccharides is in low concentrations. Diets rich in plant-based foods normally favor a greater diversity in the microbiome and have greater percentage of Prevotella group members that can process plant fibers. In addition, the diversity of microbiota composition of fecal samples is significantly higher in adults than in children. The maturation of microbiota into an adult-like configuration takes place during the first three years of life, by which time the intestinal epithelium and the intestinal mucosal barrier are developed to tolerate or even support the growth of microbial flora. When microbiome composition changes with age, the composition of bacterial proteins produced in the gut also changes [65,68,78]. In adult’s microbiomes, enzymes involved in fermentation, methanogenesis and the metabolism of arginine, glutamate, aspartate and lysine are found more. In infant microbiomes, cysteine metabolism and fermentation pathways enzymes dominate [85,90,100]. Malnourishment is another important factor that determines the composition of gut microbiota. Malnourished children have less mature and less diverse microbiota than their healthy counterparts [33]. They also typically have more potentially pathogenic gut flora and yeast in their mouths and throats. Changes in the microbiome associated with nutrient scarcity can in turn be a pathophysiological/ clinical indication/cause of the malnutrition [100,103,138].

As mentioned earlier, the gut microbiome composition varies with geographic origin of the populations. For example, the US population has a high representation of enzymes encoding the degradation of glutamine and enzymes involved in vitamin and lipoic acid biosynthesis; whereas Malawi and Amerindian populations have a high representation of enzymes encoding glutamate synthase and á-amylase in their microbiomes. Scientists have opined that one of the reasons for this heterogeneity may be because US population consumes a fat-rich diet in comparison to corn-food-based diets of Amerindian or Malawian populations. Similarly, the fecal bacteria of European children are dominated by *Firmicutes*, while the fecal bacteria of African rural children are dominated by members of *Bacteroidetes* that may also account for a reduced incidence of non-infectious colonic diseases in them [14,41].

The mode of acquisition and establishment of gut microflora in intestine has always been a subject of debate [56,59]. Though it is generally believed that a gut flora as similar to that of an adult is build up within one to two years of birth through a parent-to-child transmission mode but, transfer from food, water and other environmental sources can also be a big determinant. Multiple lines of evidence suggest the presence of bacteria in the intrauterine environment [62]. This supports the view that microbial colonization may occur in the fetus itself. Some studies have also confirmed the presence of *Lactobacillus* and *Bifidobacterium* species in placental tissues and the amniotic fluid [85]. Bacteria from the mother and the surrounding environment can also colonize the infant's gut during birth and thereafter. Though the exact sources of infesting bacteria can largely be speculative, but these may include birth canal, hospital workers, breast milk, food and general environment of the place of birth with which the infant comes first into contact after the delivery [100]. The initial bacterial populations are generally facultative anaerobes that decrease the oxygen concentration in the gut, which in turn allows obligate anaerobic bacteria like *Bacteroides*, *Actinobacteria*, and *Firmicutes* to establish and multiply [131]. Breast-fed babies have a dominance of Bifidobacteria due to the higher contents of the growth factors in breast milk that supports their multiplication. Breast milk also carries prebiotic compounds that further allow these useful bacteria to multiply faster. In contrast, the microbiota of formula-fed infants has larger populations of the members of *Enterobacteriaceae*, *Enterococci, Bifidobacteria, Bacteroides and Clostridia*. Interestingly, the method of delivery also acts as a critical determinant of the gut microflora composition. Caesarean section in general has been shown to be disruptive to mother-offspring transmission of bacteria, which impacts the overall health of the offspring and increase the risks of disease such as asthma, and type-1 diabetes. According to a recent study using high end sequencing techniques[62], placentas retrieved from more than 500 women shortly after giving birth has been found to lack bacteria that reaffirms the idea that babies gain their microbiome at the time of birth. The workers of this study also compared bacterial sequencing signals from what they expected as possible contamination sources such as the DNA sequencing kits or the birth process itself. They found that certain microbes are more prevalent when women give birth vaginally rather than by cesarean section, suggesting thereby that these bacteria get on the placenta as it's delivered rather than living there before birth. The gut microbiome research community has begun to accept these results with some apprehension and reservations because gathering definitive proof of a sterile womb by testing the gut of the fetus itself is technically and ethically difficult. Therefore, till the time a more precise technical solution is in hand, collecting the placenta or amniotic fluid after birth offers a tentative window to know how a baby's microbiome might shape. In a recent publication on maternal microbiota in pregnancy and early childhood and its influence on susceptibility towards some chronic diseases in children, McDonald and McCoy [85] have elaborated that how the microbial bond shared between a mother and her new born child influences the childhood microbiota that imparts an ever lasting impression on child's health and development *vis-a-vis* disease. The paradigm belief that fetal development takes place in a sterile intrauterine environment is often being challenged by observations on the presence of bacterial genomic DNA within placental and amniotic tissues and culturable microbes in the first feces passed after birth. However, definitive evidence of colonization during fetal development with a bona fide microbiota (i.e., a live, persistent, and functional community of microorganisms) are still lacking. Nevertheless, seeding of early-life microbiota with maternal microbes, in all probability, greatly influence the risk of several childhood/adulthood illnesses. Scientists have question whether dysbiosis-associated microbiota phenotypes can be vertically transmitted from mother to child? In a study carried out to verify this, Kasai et al [63] found that out of 935 mother–infant pairs from Canada, the children of obese mothers had a three-fold higher risk of becoming obese at 1 year, and it increased to five-fold if the delivery occurred

through cesarian section. Similarity of the taxonomic signatures of the faecal microbiota during the first months of life of such overweight infants supported the fact that seeding of an "obesity-associated" microbiota can be a case of transfer of this phenotype from mother to its offspring.

THE PRIMARY ROLES OF GUT MICROFLORA

Initially the gut micro-flora was considered to play three major roles in a human life: defending the body against pathogens; fortifying host defense by developing and maintaining the intestinal epithelium and inducing antibody production there and; metabolizing otherwise indigestible compounds in food [14,41,50]. The symbiotic relationship between the gut microbiota and the host is regulated and stabilized by a complex network of interactions that encompass metabolic, immune, and neuroendocrine cross-talks [20,23]. These interactions are mediated by microbial metabolites that impart pleiotropic effects and act as signaling molecules in the regulation of host neuro-immune-inflammatory axes linked to gut and other body organs [17]. The gut microbial community defends body against pathogens by colonizing the space, making use of all available nutrients, and by secreting compounds that kill or inhibit unwelcome guests. Disruption of the gut flora normally results in the establishment of competing microorganisms like *Clostridium difficile* that are otherwise kept in abeyance. As the gut flora gets established, the lining of the intestines – the intestinal epithelium and the intestinal mucosal barrier that it secretes, also develop and create an environment that not only resist the entry of pathogenic invaders but also support the growth of commensalistic microorganisms. Specifically the goblet cells that produce the mucosa, proliferate and, the thicken mucosa layer allows the beneficial microorganisms to anchor and feed. Additionally, the development of gut-associated lymphoid tissue (GALT), which forms the part of the intestinal epithelium that can detect and reacts to pathogens, also develops during the time the gut flora is established. The GALT is tolerant to gut flora species, but not to other microorganisms [32,41,42,46,50,60,65-67,78].

Without gut flora, it will be difficult to utilize some of the undigested carbohydrates present in human diet because some members of the gut flora community produce enzymes that human cells lack for breaking down certain types of polysaccharides such as certain starches, fiber, oligosaccharides, and sugars that the body failed to degrade and absorb like lactose. Gut bacteria ferment carbohydrates into short-chain fatty acids by saccharolytic fermentation and make products like acetic acid, propionic acid and butyric acid that are used by host cells as source of energy and nutrients. Acetic acid is used by muscle, propionic acid facilitates liver production of ATP, and butyric acid provides energy to gut cells [78]. Gut flora is also known to synthesize vitamins like biotin and folate, and facilitate absorption of dietary minerals, including magnesium, calcium, and iron. Evidences are also accumulating to support the view that treatment with some probiotic strains of bacteria like *Enterococcus faecium*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Propionibacterium freudenreichii*, *Bifidobacterium breve*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Bifidobacterium infantis*, and *Streptococcus thermophilus* is effective in irritable bowel syndrome and chronic idiopathic constipation [17,19,35-37,39].

The immunological and developmental impact of the maternal and early-life microbiota extends beyond the gut mucosal immune system [29,41]. Conclusive experimental evidences are being provided for a "microbiota-mediated gut–brain axis" controlling neuroimmune development and susceptibility to neuro-developmental disorders like autism and different psychiatric illnesses [42,45,46,51,58,65,67,69,77]. The gut-brain axis represents a biochemical signaling between the gastrointestinal tract and the central nervous system. The gut-brain axis encompasses the central nervous system, neuro-endocrine and neuro-immune systems. Holistically, it includes the hypothalamic–pituitary–adrenal axis (HPA), sympathetic and parasympathetic autonomic nervous system, the vagus nerve and the gut microbiota. Certain commercially available strains of probiotic bacteria like *Bifidobacterium*

longum, *B. breve*, *B. infantis*, *Lactobacillus helveticus*, *L. rhamnosus*, *L. plantarum*, and *L. casei*and have been found to have the highest potential to be useful for correcting certain central nervous system disorders, However, whether changes to gut flora are a result of disease, a cause of disease, or both in the gut-brain axis is a matter that is yet not fully resolved. In a mouse study [102] gut microbes were found to release certain chemicals that affect the function of nerve cells that are important for overcoming fear responses and depression. The researchers also examined whether gut bacteria played any role in the learning and forgetting responses. They treated mice with antibiotics to totally get rid of the bacterial microbiome. They then played a tone and right after gave the mouse a mild shock, doing this multiple times. All of the animals quickly learned to associate the noise with pain, freezing when they heard the sound. Mice with normal microbiomes eventually forgot the connection within three days, and the noise no longer affected most of them, whereas the antibiotic-treated mice continued to react. Histological examination of the dissected brains of the two sets of mice revealed that a region of the brain known as the medial prefrontal cortex was primarily associated with the differences between the treated and untreated mice [102]. The excitatory neurons in this region that were liked with learning and memory process failed to appropriately develop when microflora was missing. The workers also observed substantial changes in the amounts of certain chemicals produced by the gut microbes that may help keeping the brain part in proper shape to combat sense of fear and appearance of symptoms of neuropsychiatric diseases such as schizophrenia and autism. Several bacterial species present in the gut flora have also been found to influence the immune system by producing selective cytokines. The cytokines drive our immune system to produce inflammation in order to protect itself. They also tamp down the immune response to maintain homeostasis and allow healing after an injury. *Bacteroides fragilis* and some *Clostridia* species present in the gut elicit an anti-inflammatory response whereas some segmented filamentous bacteria favor the production of inflammatory cytokines. Gut flora can also regulate the production of antibodies by the immune system. The immune system can be tailored by gut bacteria's ability to produce metabolites that can affect cells in the immune system. For example short-chain fatty acids can be produced by some gut bacteria through fermentation to stimulate a rapid increase in the production of innate immune cells like neutrophils, basophils and eosinophils. These cells are part of the innate immune system that tries to limit the spread of infection [14,41,50].

Gut microbiota also plays an important role in the metabolism of bile acids in the host [2]. Bile acids are derived from cholesterol in the liver and subsequently secreted into the duodenum to facilitate the fat digestion, and lipid metabolisms. About 95% of bile acids are reabsorbed in distal part of the small intestine and 5% unabsorbed bile acids are bio-converted into secondary bile acids (deoxycholic acid and lithocholic acid) by bile salt hydrolases secreted by several *Clostridium* species present in the colon [127]. The secondary bile acids are reabsorbed in colon and transported back to liver for reuse [2]. Secondary bile acids also possess antimicrobial properties against bile acid-intolerant microbes. They disrupt the microbial cell membrane integrity, causing spillage of intracellular contents, thus regulating the composition of the gut microbiota and protecting the host from an array of infectious pathogens [95]. In a recent publication [53] scientists have reported that secondary bile acids can modify body's immune response against infectious microbes by up-or down-regulate the differentiation of specific immune cell types. It was also observed in this study that secondary bile acids can also play anti-inflammatory roles because they are always present in stool samples of patients with inflamed colons but are absent in germ free samples. This observation reaffirmed the view that specific gut-residing bacteria play an important role in the production of secondary bile acids.

MICROBIOTA, HUMAN HEALTH AND DISEASES

As pointed out earlier in this review, disturbance in relative occurrence of different types of gut bacteria when a person is on a broad spectrum antibiotics therapy often affect the host's health due to improper digestion and metabolism of food. Though still in its very nascent stage, the present understanding of this subject do suggest that gut microflora and their unique metabolites, besides protecting the host body against invading pathogen, also regulate various host physiological and biochemical functions and homeostasis of immunity and the nervous system [41]. Some of the most notable diseases namely *Clostridium difficile* infection, inflammatory bowel disease, multisystemic autoimmune celiac disorder, obesity, colorectal cancers, and neuropsychiatric disorders have been found to be closely linked with imbalance of gut flora [3,5,11,16,18,19,21,22,69]. Hence, making dysbiosis corrections through faecal microbiota transplantation (popularly known as "Yellow Soup Therepy"), probiotic and prebiotic applications are being increasingly searched as new possible mode of medications to ameliorate these health disorders [141,146]. Dysbiosis can be induced by several factors such as antibiotic consumption, dietary components, mental and physical stress etc. The dysbiosis condition is often marked by impaired composition and functioning of gut microbiota by selective-enumeration of certain specific microbial members that eventually disturb the secretion of microbial-derived metabolites and cause diverse range of diseases of local or systemic orders [26,27,43,44,57]. Some of the most influential microbiota-derived metabolites in disease manifestations include: Short-chain fatty acids (acetate, butyrate, propionate, hexanoate, valerate); Indole derivatives (indole, indoxyl sulfate, indole-3-propionic acid; Secondary bile acids (deoxycholic acid, lithocholic acid); Choline metabolites (choline trimethylamine N-oxide, betaine); Phenolic derivatives (4-OH phenylacetic acid, urolithins, enterolactone, 8-prenylnaringenin, 2-(3,4-dihydroxyphenyl acetic acid, 3-(4-hydroxyphenyl) propionic acid, 5-(3,4-dihydroxyphenyl) valeric acid; Vitamins [(thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), pantothenic acid (B5), biotin (B7), folate (B11–B9), cobalamin (B12), menaquinone (K2)] and; Polyamines (putrescine, spermidine, spermine).

Metabolic disturbances

Microbiota is now known to modulate the body metabolism and enrichment of polysaccharides, amino acids, xenobiotics and micronutrients in the host body [8,29,38]. Therefore, it plays a very important role in host's energy harvesting mechanisms [47,50,65,80,97]. This was validated in a study where it was shown that germ-free mice had 40% lower epididymal fat and required additional 10–30% food for maintaining the same body weight as mice with normal microbiota [6]. Microbial-synthesized short-chain fatty acids can contribute up to 70% of ATP production in colon, with butyrate as the preferred fuel for colonocytes [112]. Gut microbiota also synthesize micronutrients and vitamins that are of beneficial value for both microbial and host metabolisms. Vitamin-K-producing gut bacteria (*Bacteroides fragilis, Eubacterium lentum, Enterobacter agglomerans, Serratia marcescens and Enterococcus faecium*) anaerobically produce vitamin K2 (menaquinone) that inhibits vascular calcification, elevates HDL and lowers the cholesterol levels. It thus contributes to lower risk of cardiovascular disorders such as atherosclerosis and coronary heart disease [48,64]. Gut microbiota is also an important source of vitamins B for our body [37,117]. Vitamins B5 and B12, in particular, are exclusively synthesized by intestinal microbiota and act as coenzyme for a large number of host metabolic processes like production of acetylcholine and cortisol that are required for normal functioning of the nervous system. Deficiency of vitamins B5 and B12 often led to gastrointestinal discomfort, insomnia, neuropsychological and hematological disorders [4,51].

Immune and nervous disorders

As pointed out in the previous section, gut linings composed of mucus and epithelial layer regulate the barrier integrity and act as an interface between the microbial load of the food and body's

internal environment. A disoriented gut barrier action normally results in increased gut permeability to commensal microbes, microbial derived products, virulence factors etc resulting in an aberrant immune-inflammatory responses such as inflammation, allergy, and dysregulated T-cell response-mediated autoimmune disorders [9]. Association of gut microflora with host immunity development has been demonstrated at both local and systemic levels in an experiment wherein differentiation/suppression of myeloid and lymphoid progenitor cells was reversed in germ free mice model after colonization with normal microbiota. This observation clearly suggested that gut microbiota could facilitate maturation of haematopoiesis (innate immunity) and lymphocytopoiesis (adaptive immunity) processes [66]. In another study [42], it was found that host microbiota and their SCFAs metabolites can also regulate the maturation and functionality of microglia, the tissue macrophage of CNS. Colonization of germ free mice with complex gut microbiota showed partial reversal in the initial immature microglia phenotype in such mice. Mice model with normal microbiota but deficit SCFAs receptor had also mimicked the defective feature of microglia observed in germ free mice. Thus it becomes evident that microbial-derived SCFAs play an important role in microglia homeostasis which in turn is crucial for CNS maintenance [96].

In several recent studies, scientists have also explored the possible link between the gut microbiome and Autism Spectrum disorders (ASD) of brain with symptoms like mood disorder, depression, Alzheimer's and Parkinson's. ASDs are generally characterized by social and communication deficits and repetitive or stereotyped behaviors [32,67]. Strong possibility of ASDs link with gastrointestinal dysfunction and microbiota dysbiosis have also been indicated [133]. One of the reasons for such a correlation might be traced back to impaired tyrosine kinase – MET (mesenchymal epithelial transition) signaling in all ASD patients which is important in brain development, gastrointestinal health and immune response regulation [58], ASD patients also register increased population density of *Clostridium*, *Bacteroidetes, Lactobacillus, Desulfovibrio*, and reduced number of *Bifidobacteria* [1,126]. Shultz et al. [122]. have also found that mice injected with propionic acid (PPA) via intra-cerebroventricular route showed intensive neuro-abnormalities with ASD-like symptoms. PPA is a SCFA that is frequently synthesized by above mentioned enteric bacterial species and its level is generally very low in normal healthy individuals. Application of probiotic *Bifidobacterium infantis* has been found to improve the behavioral abnormalities and gastrointestinal dysfunction in mice suffering with ASD [36] and hence, can qualify to become a potential therapy for these mental discomforts in humans after proper clinical evaluations. Initial efforts in this direction have indicated that *B. infantis* can neutralize the neurotoxic effect of excess NO in ASD [142].

Inflammatory Bowel Diseases (IBD) & Antibiotics-Associated-Diarrhea (AAD)

Inflammatory bowel diseases relate to multifactorial, idiopathic, persistent and recurring gastrointestinal inflammatory disorders [71,91]. Crohen's disease and Ulcerative colitis are the two most common forms of IBD [134]. In Ceohen's disease inflammation can occur anywhere along the whole gastrointestinal tract but in Ulcerative colitis it is restricted to the large intestine only. Relapsing diarrhea, fever and abdominal pain are the most common symptoms in both the cases. An inappropriate body's immune response against microbiota in genetically predisposed individual is likely to be the primary cause of inflammation. Several studies have observed the developments of antibodies against commensal microbial antigens and autoantigens. Also, it has been observed that these antibodies response patterns correlate with disease onset and its inflammation severity depending on the loss of microbiota species that affect the gut barrier function and gut immunity [134]. Altered immune barrier function subsequently increases the exposure between gut microbiota and epithelial cells that further enhance the stimulation of local immunity and gut inflammation. Nagao-Kitamoto et al [91] observed elevated pro-inflammatory gene expression in germ-free mice colonized by gut microbiota isolated from IBD

patients. Similarily, Colitis-prone germ-free mice colonized by IBD-associated-microbiota developed severe colitis compared to those that were colonized by healthy human microbiota. Another school of thought suggests that impairment in IBD-gut microbiome affects gut mucus barrier function by elevating the number of mucus-eating (mucolytic) gut microbial species that in turn, stimulates severe inflammatory response [61,118]. Based on these findings, most of the current therapeutic approaches for treating IBD generally target the aberrant pro-inflammatory immune response at intestinal mucosa. Antibodies targeting proinflammatory cytokines (anti-TNF-alpha, anti-IL12, anti-IL23) and employing a4b7-integrin antagonist to inhibit trafficking of T-lymphocytes to gut tissue are common therapies against IBD [26,43]. However, IBD often tends to recur in long-term and, therefore, development of a safer and more effective treatment is a serious clinical need. Since IBD is generally associated with decreased anti-inflammatory cytokines IL-10, development of novel genetically modified probiotic microorganism such as *Lactococcus lactis* for hyper-expressing IL-10 can be a good potential target for future research. Despite these exciting novel opportunities for future management and treatment of IBD, the less understood etiology of the disease that may vary with individual patients, designing a curative treatment that is effective to all is still a great challenge for all pharmacologists.

Antibiotics-Associated-Diarrhea (AAD) is another most commonly observed repercussion of microbial imbalance [10,24,57,81,113]. Changing the numbers and species of gut flora can also reduce body's ability to metabolize bile acids that can itself cause diarrhea [19,65,65,91,127]. A reduction in levels of native bacterial species can also disrupt their ability to inhibit the growth of harmful bacteria like *C. difficile* and *Salmonella kedougou*. Restoration of Bacteroides and Firmicutes classes of bacteria in the affected gut by faecal microbiota transplantation is becoming a powerful solution for reducing *C. difficile* infections. Available data on in vitro analysis of >1000 commonly traded medicines on growth of 40 different types of gut microbacteria suggests that nearly 24% of the drugs inhibited the growth of at least one of the bacterial strains [19]. Additionally, metabolites produced by some gut flora can also influence the host signaling pathways. The overabundance of some kinds of bacteria may also contribute to inflammatory bowel disease that can lead to sepsis, obesity and certain types of colon cancers. *Helicobacter pylori* can cause stomach ulcers by crossing the epithelial lining of the stomach. Additionally, it appears that interactions of gut flora with the gut-brain axis, coupled with hypothalamic–pituitary–adrenal axis mediated physiological stress have a role in IBD syndrome due to release of bacterial metabolites that trigger signaling in the enteric nervous system. IBD has often been found to be linked with shrinking of diversity of gut flora. Proteobacteria, Actinobacteria and *Enterococcus* dominate the gut flora in such a situation. Irritable bowel syndrome (IBS), on the other hand, is a disease caused by stress and chronic activation of the hypothalamic–pituitary–adrenal axis. Studies have shown that composition of mucosal microbiota is often get disturbed in IBS resulting in diarrhea or constipation. These microbial imbalances may include low levels of faecal *Lactobacilli* and *Bifidobacteria* and high levels of facultative anaerobic bacteria such as *Escherichia coli* [65].

It is appropriate to recall here that antibiotics are being used in modern medicine for multiple aims, ranging from growth regulation to disease therapy [19,29]. Several mechanisms of action for antibiotics have been proposed including inhibition of pathogen; reduction of microbial load and nutrient utilization by gut microbiota; enhancement of nutrient and calorie absorption by epithelium and; reduction of microbial-derived anti-metabolites [19]. Since germ-free individuals often suffer from a deficient immune system, using antibiotic treatment to deplete all or specific microbes is widely employed today as an alternative approach to study how the change in gut microbiota can affect host microbe interactions and host immune system [11,57,113, 133].

Allergy, Asthma, and Diabetes mellitus

Allergy, asthma, and diabetes mellitus are also now being linked to imbalances in the gut flora [40,84]. For rising cases of asthma in the developed countries it is now generally believed that lower exposure of children to sufficient microbes may result in lower prevalence of gut bacterial taxa that boost immunity. Also, the western diet that lacks sufficient whole grains and fiber contents with overabundance of sugars may also result in lower supply of short-chain fatty acids (SCFAs) that affect immuno-modulation and cause asthma. The connection between the gut microbiota and diabetes mellitus type 1 has also been linked to anti-inflammatory SCFAs such as butyrate [65]. Butyrate has been shown to decrease insulin resistance and hence, gut communities low in butyrate-producing microbes may increase the chances of acquiring diabetes mellitus type 2. The composition of the intestinal microbiota also plays an important role in the development of pre-diabetic conditions marked by insulin resistance [114]. Microbiota of type 2 diabetes patients have relatively lower population of butyrate producing bacteria *Roseburia intestinalis* and *Faecalibacterium prausnitzii* in the back-drop of a proinflammatory environment, increased expression of microbial genes involved in oxidative stress, reduced expression of genes involved in vitamin synthesis, and higher intestinal permeability [116]. Such microbiota also includes a significantly lower prevalence of Firmicutes and an enrichment of Bacteroidetes and Proteobacteria [114]. In a few studies, the presence of a high number of opportunistic pathogens like *Clostridium*, *Bacteroides, Eggerthella and Escherichia* spp was also found associated with microbiota of diabetic patients [44]. These Gram-negative bacteria stimulate the immune system resulting in release of inflammatory cytokines that can contribute towards the development of insulin resistance and the deficiency of insulin signaling in the muscle, adipose tissue, liver, and hypothalamus [92]. The intestinal microbiota of diabetes patients also favors the progression of the disease by affecting several other related factors like body weight and intestinal hormones. Hence, administration of beneficial probiotic combinations can hinder disease advancement by altering the glucose metabolism, insulin resistance, appetite pattern, food intake, body weight, and several gastrointestinal pathways [110]. In a recent study, it has been found that administration of *Saccharomyces boulardii* is effectively associated with glycemic control, cardiovascular protection and improvement of inflammatory profile in animal model [18].

Gut microbiota composition can also influence the treatment of diabetes patients through its implication on obesity [110]. Western diet appears to drive and maintain changes in the gut flora that in turn decides how much energy is derived from food and how that energy is utilized. Western food diets that often lack fibers and other complex carbohydrates increase the amount of energy generated by the gut flora that in turn contribute to obesity and metabolic syndrome. There are growing evidences that also indicate that microbiota can influence eating behaviors which can lead to the host consuming more food and eventually acquire obesity and diabetes. Since gut-brain-liver axis plays a dominant role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose through glycogenesis or gluconeogenesis pathways, the gut microbiota composition can also regulate the glucose homeostasis in the liver and provide potential therapeutic option to treat obesity and diabetes [47,94,109,137,139].

Celiac Disorders

Celiac diseases are potential risk factor of autoimmune disorders caused by microbiota dysbiosis. Celiac disorder is a multi-factorial immune-mediated disturbance in small intestine characterized by permanent intolerance to dietary gluten (such as gliadin peptides) and prolamines in genetically predisposed individuals [89]. Gluten triggers the activation of adaptive immune response in mucosa, inducing TH1 and TH17 mediated production of pro-inflammatory cytokines and stimulation of innate immune response that lead to enterocyte killing [128]. This causes cytoskeleton rearrangement and increased gut permeability that finally results in transportation of luminal antigens

to the sub-mucosa. The celiac disorders are also influenced by environmental factors including gluten consumption, breast-feeding duration as well as gut infections [83]. Gut dysbiosis in celiac disease patients shows a remarkable decrease in Gram-positive bacteria. Such bacterial misbalance can be partly restored if patients are put on a gluten-free diet. Patients with celiac disorders show a marked decrease in the ratio of total Gram-positive to Gram-negative bacteria, particularly a significant reduction in number of "health-promoting" Bifidobacteria and elevation of numbers of Gram-negative Bacteroides–Prevotella groups [36,83,90]. It is now generally believed that preferential overgrowth of pro-inflammatory endotoxin-secreting Gram-negative microbiont actually contributes to the gluten intolerance.

Obesity

Gut microflora has now been integrated as an identified factor that along with genetic, behavioral, environmental and other metabolic factors plays an important role in the progression of obesity that affects almost 600 million people in the world today [139]. Obesity sets in when energy intake is high over decreased energy expenditure in the body. It is characterized by excessive fat accumulation that leads to higher body mass index (BMI) above or equal to 30 kg/m^2 [14,139,]. It can be classified into obesity level 1 (BMI= 30–34.9 kg/m^2), level 2 (BMI = 35–39.9 kg/m^2) and level 3 (BMI > 40 kg/m^2). Obesity accounts for >2.5 million deaths per year. Higher fat accumulation and increased BMI raises the risk of hypertension, diabetes type 2, dyslipidaemia, cardiovascular diseases, insomnia, musculoskeletal disorders like osteoarthritis and several types of cancers like that of breast, ovary, prostate, liver, gallbladder, kidney and colon [8,22]. Gut microbiota composition has also been found to be an important factor behind obesity [14,16,63,72,103]. In majority of cases a higher proportion of members of Firmicutes phylum and a lower amount of Bacteroidetes have been found to prevail in obese microbiota when compared to that of lean individuals [12,63,68,72]. However, this generalizations can change with geography and diet consumed by different individuals. For example, Bacteroides enterotype is more predominant in individuals with higher protein and animal fat diets whereas, the Prevotella enterotype is more in people with higher carbohydrates and fibres intake [27]. Peters et al. [103] found larger abundance of Streptococcaceae and Lactobacillaceae families and a decreased numbers of Christensenellaceae, Clostridiaceae and Dehalobacteriaceae members in the microbiota of an obese American population of adults. Cani et al. [21] observed that when mice are treated with a high-fat diet (HFD) for four weeks, a reduction in the abundance of the genera *Lactobacillus*, *Bifidobacterium and Bacteroides-Prevotella* occurred in compared to mice fed with a standard diet. In comparison, Cui et al. [35]. found a negative correlation between the bacterial genera *Hungatella, Oscillibacter, Odoribacter* and obesity and a positive correlation between the genera *Isosphaera* and *Methylotenera* with obese genotypes. *Faecalibacterium prausnitzii* that was normally found in high abundance in normal lean healthy adults is considered a butyric acid producers and consequently beneficial for health [80]. Metagenomics studies have also suggested that obesity-associated dysbiosis features may include a significant increase in butyrate-producing Firmicutes and a decrease in Bacteroidetes in the distal gut microbiome. This metabolic disturbance is often accompanied with a higher content of starch-degrading glycoside hydrolase and SCFAs (butyrate and acetate), fasting glucose, insulin and increased energy harvesting capacity. Since obesity is an outcome of gut microbiota dysbiosis, selective modulation of microbial communities through dietary alterations is a promising therapeutic option for managing this disease [13]. Prebiotics like oligofructose, inulin, fructooligosaccharides, galactooligosaccharides, arabinoxylan etc were frequently found to have a beneficial impact on reducing obesity [20,93,123,125]. Similarily, administration of *Lactobacillus rhamnosus* preparations has been found to decrease plasma cholesterol, triacylglycerides and white adipose tissue in animal models. Likewise, the Insulin-type fructans (a class of prebiotics) can preferentially support the growth of *Roseburia* and *Clostridium* species in mice

model. On contrary to this, feeding arabinoxylan was found effective in selective nourishment of *Bifidobacterium, Roseburia, and Bacteroides* species that favor anti-adipogenic activity in obese mice [39]. Burokas et al. [20] have demonstrated a link between the gut microbiota, prebiotics and antidepressant effects that suggest involvement of gut-brain axis (GBA) in this overall scenario. It may be recalled here that GBA outlines a bidirectional communication between cognitive and emotional centres of the brain with peripheral intestinal functions [23]. Besides carbohydrate substances, polyphenols and polyunsaturated fatty acids have also been used as obesity reducing prebiotics for gut microbiota modulation [13]. Polyphenols selectively favors the growth of beneficial genera *Eubacterium, Roseburia and Faecalibacterium* that are butyric acid producers that reverse the obesity symptoms [80,112]. Proposing a possible model for combined action of prebiotics and probiotics on gut microbiota composition and reduction in obesity, O'Connor et al. [97] have suggested that such prebiotics modulate the gut microbiota that in turn affect a cascade of metabolic processes and culminate in the improvement of lipid profiles, reduction of blood pressure, improvement of glucose homeostasis and reduction in inflammation. Co-administration of probiotics further synergizes these metabolic alterations after getting stimulated by prebiotics. Role of physical exercises in obesity management through weight reduction is also very well documented [138]. Recent studies have suggested that most of such beneficial effects of physical exercise are mediated through altered metabolic activity of the gut microbiota [25,30,31,33,38,87]. Exercise improves selective microbiota diversity that can improve mucosal immunity and synthesis of desired SCFAs (butyrates) under different nutrients intake regimes. Clarke et al. [30] found a higher population of bacterial species *Akkermansia muciniphila* and *F. prausnitzii* in the microbiota of athletes that are important for good intestinal health and a control over obesity. In general, exercise favors an increase of Bacteroides and a decrease of Firmicutes bacteria [70]. Though exact mechanism behind exercise and positive gut modulation for controlling obesity is still not fully understood, it is likely that these modulations result in increased short chain fatty acid production, alteration in intestine pH, reduction in gut transmit time and increased excretion of antimicrobial primary bile acids [31,87,99,108,111,114,123,125].

Cancers

Role of gut flora in induction and progression of certain types of cancers is relatively a new dimension of microbiome research that can very well turn out to be a game changer in cancer treatments [65,74,124]. Linkage between the composition of microbiota and cancers largely revolves around the ability of gut microbes to influence inflammations and immunological disturbances in the body that can create a favorable environment for tumor development [52]. Scientists are now getting increasingly excited to give a closer look to the interaction of gut flora with available cancer treatments for increasing their efficacy. Linkage between Infection of *Helicobacter pylori* with gastric cancer was first reported in early 1990s and in last two decades several other members of gut flora, particularly the ones that initiate an inflammatory response by disrupting the mucus lining, have been shown to be associated with cancer growth [65]. They can also promote the proliferation of cancer cells by making the cells resistant to prevailing antineoplastic therapies. Colorectal cancer (CRC), which is the fourth leading cause of cancer related mortality worldwide is also a multifaceted diseases associated with gut microflora [5]. Li et al. [74] using a mouse model, have shown that germ-free mice registered a significantly lower colonic tumor incidence as compared to that of conventionally raised mice that also had other distinctive phenotypes of CRC like rectal bleeding, anemia and infiltration of inflammatory cells due to dysfunctional intestinal epithelial barrier. 16S rRNA sequencing of faecal microbiota of CRC patients has indicated massive enrichment of *Bacteroides fragilis* [140]. Nevertheless, some gut bacteria can also help in fighting a cancer by activation of auto-immune system of the body. For example, cyclo-phosphamide – a common cancer drug - has been

found to damage the mucus layer of the intes-tine that allows certain gut bacteria to travel into the lymph nodes and spleen where they activate specific immune cells that help killing the tumor cells. In microbe-free mice, the drug does not show any anticancer efficiency. Mice fed with a particular bacterium, *Bacteroides fragilis* again responded better to the drug than those without it [124]. A surface molecule from the bacterium *B. fragilis* was also found to boost the number of T-helper (TH1) cells by pushing infection-detecting dendritic cells in the lymph nodes to produce IL-12, a pro-infammatory molecule. These observations were further substantiated when researchers transferred bacteria from the human participants into the intestines of mice with comparable cancers. Rodents who got 'beneficial' bacteria developed smaller tumors than did mice that received microbes from people who hadn't responded to treatment. Researchers in US in collaboration with pharmaceutical companies have launched mega projects on exploring the efficiency of faecal microbiome transplant from people who respond to a cancer immunotherapy treatment with a checkpoint inhibitor in to the intestine of non-responders. It is anticipated here that faecal transplants would reshape the gut micro-biome of the non-responders in a beneficial way. Though faecal transplants have shown very promising scenarios in case of non-cancer diseases like bowel infections caused by *Clostridium difficile,* their effectiveness in cancer management needs more careful assessments in terms of selecting the donors and proper screening of the faecal material before it is transplanted in the recipients. Some companies are also toying the idea of preparing pills containing a set of only few specific desired spore-forming bacteria (particularly the *Bifi-dobacterial* strains) purified from the donors' faeces [52]. Human trials of these approaches would, however, be challenging because of too many unknown associations and side effects. Changing the make-up of an individual's microbiome would always carry the risk of possible predis-position with other health issues.

Several ongoing studies in the field of gut microflora, therefore, clearly suggest that gut microbiome should be seen as an integral part of precision medicines of the future because it not only contributes to individual's variability in all dimensions of a disease situation but is also a modifiable factor that can help in developing novel categories of therapeutics. For example the gut microbiome can be used to develop personalized blood glucose responses in accordance with specific diets that different individuals consume in different parts of the globe.

CORRECTING THE IMBALANCES OF GUT MICROBIOME – A POTENTIAL CLINICAL DESTINATION FOR HERBAL MEDICINES

Gut microbiota can be a potential new territory for targeting herbal drugs and dietary supplements in human healthcare [73,121]. Besides offering new potential solutions to several metabolic and immune disorders, this approach can also be gainfully employed to change the fate of several other modern medicines, making them more efficient and versatile at lower dosages [60,69,100,143]. Microbiota can also modify a parent herbal drug molecule in such a way that it is either absorbed faster in the body or it is derivatized into a more efficacious molecule with better pharmacodynamics. One of the base-line attraction and advantages of this approach resides in the fact that most of herbal drugs are orally administered and hence inevitably come in contact with intestinal microflora. They thus enjoy better chances for making necessary corrections in microbial populations and also up- or down-regulate the activities of desired or undesired metabolites produced by the gut flora. Moreover, gut microflora secrete an array of enzymes that can have major implications in drug stability. Dietary herbs are also consisted of hundreds of chemical constituents, some of which can also act as substrates for these bacterial enzymes. Therefore degradation or modification of herbal drugs by the microbiota can ultimately lead to regulation, lose or magnification of their biological activities. This also makes the metabolism of herbal medicines in our body more complicated than single compound drug of modern medicine. Being single molecule entity, it is much easier to chase the pharmacokinetics of a modern

medicine than a herbal formulation consisting of a large number of natural chemicals. Their concentration also varies in a large range and component-component metabolic competition for absorption is common. Based on in vitro and in vivo bioactivity evaluation studies with selected herbal medicines, the influence of intestinal microbiota on drugs' gut metabolism/modification has been characterized in recent years and, some very positive insights on the enhancement of bioactivity of botanical drugs vis-à-vis their parent compounds are in offing. The subject is advancing rapidly and is summarized here with the help of few exciting successful case studies.

Case Study-1: Bioactivity Enhancement of Ginsenosides of Panax

Panax species (Ginseng) are one of the best selling medicinal herbs in the Oriental as well as Modern Systems of Medicine [15,106,135,136]. The roots of this plant are important constituents of herbal supplements and functional foods used as revitalizer, aphrodisiac, immunomodulatory, ant-stress and anti-ageing formulations [28,34]. The world market of ginseng preparations in the western world is estimated to be around 2040 million US dollars in 2013 [7]. South Korea, Vietnam, China and USA are the major global supplier of ginseng roots/extracts in the herbal trade. The impressive range of bioactivities exhibited by ginseng is attributed to the presence of triterpenoid dammaranes, i.e., ginsenosides in the plant's roots [129,141,147]. Ginsenosides are broadly classified in two categories: 20(S) protopanaxadiols (Rb1, Rb2, Rc, Rd, Rg3, Rh2) and 20(S) protopanaxatriols (Re, Rg1, Rg2, Rh1), based on the differences in their aglycone structures. Like other herbal medicines, ginseng is always taken orally. In their native form, the bioavailability of ginsenosides is low because of their incomplete absorption in the gut [105]. After oral administration, ginseng has been found to be extensively metabolized by gut bacteria [54,76,115]. The most exciting popular metabolic modification is deglycosylation reactions by intestinal bacteria. Ginsenosides Rb1, Rc, Rb2, Rb3, and Rd are converted to form Compound K that has much wider bioactivity profile than parent molecules [107,141]. Compound K (20-O-D-glucopyranosyl-20(S)-protopanaxadiol) does not occur naturally in ginseng. It is a biosynthesized by intestinal bacteria via the stepwise cleavage of sugar moieties at C-3 position. Various pharmacologic actions of ginseng such as antidiabetes, hepatoprotetion and antiinflammation, have been found to be mediated by this compound. In the triol group of ginsenosides on the other hand, Rg1 and Re are converted to compound Rh1 and F1. Tawab et al. [132] reported that compound K, F1 and Rh1 were the major metabolites that reached the blood circulation of human volunteers that were orally fed with ginseng capsule. However, this biotransformation and mucosal uptake timing varied among volunteers that might be because of the different composition of the gut microbiota in them.

Case Study-2: Curcumin and turmeric dietary supplementation affecting human gut microflora composition

The therapeutic potential of turmeric (*Curcuma longa*, Zingiberaceae) rhizome and its bioactive constituent curcumin as anti-inflammatory, antioxidative, and neurotrophic agent is very well established today [104]. However, clinical trials have produced heterogeneous results. Though such heterogeneity can arise due to difference in source material/ or type of preparations used for testing (i.e., raw turmeric, turmeric extracts, turmeric essential oil, curcuminoid fractions and curcumin), but poor bioavailability of curcuminoids can also be a major potential factor behind these mixed clinical results. It is appropriate to mention here that curcumin and curcuminoids are loosely used terms in scientific literature. Normally turmeric contains 2-6% curcuminoids and the curcuminoid fraction has 80% curcumin, 18% demethoxycurcumin, and 2% bisdemethoxycurcumin [104]. Moreover, turmeric and curcumin also affect gut microbial diversity and impart health benefits by modulating intestinal barrier function. Gut microbiota in return, can also biotransform curcuminoids to produce more bioactive metabolites. Recently, a randomized, double-blind, placebo-controlled clinical trial to evaluate the effects of turmeric,

curcumin, and placebo on the dynamics of gut microbiota community in healthy humans was carried out [104]. In this study thirty adult participants were divided in three groups of ten each. Three different treatments were administered in these three groups of participants. These treatments consisted of: (i) 1000 mg turmeric+ 1.25 mg BioPerine; (ii) 1000 mg curcumin (C3C) and (iii) placebo (content unstated). BioPerine is a standardized extract of black pepper fruit containing 95% piperine that has proven ability of enhancing the bioavailability of curcumin. C3C is a standardized curcuminoid extract containing 70-80% curcumin, 15-25% desmethoxycurcumin, 2.5-6.5% bisdemethoxycurcumin, and 1.25 mg BioPerine. All subjects were given three tablets with food (2x/d = 6000 mg/d turmeric or C3C) for eight weeks. All participants ate their normal omnivorous diets during the study. Morning faecal samples were collected after 4th and 8th week of study. Genomic DNA isolated from the faecal samples was used for amplifying the V3-V4 region of 16S rDNA for sequencing. Baseline microbiota profiles were compared to averaged profiles from four and eight week samples. The number of microbial species identified in different samples ranged from 172-325. The placebo group had an overall 15% reduction in species (175 at baseline vs. 149 averaged post-treatment); the turmeric group had a 7% increase in diversity (156 species at baseline to 167 averaged post-treatment and; the curcumin group had an average 69% increase (127 species at baseline to 215 averaged post-treatment). Gut microflora composition was found to vary independent of treatment effects in all the three groups. However, most changes in species abundance in the placebo group was between 2-10 fold whereas such changes were 10 to 100-fold or more in an average of 53% and 60% of turmeric and curcumin participants, respectively. Patterns of species abundance were similar in both active treatment groups, suggesting curcumin as the main driver of change. A uniform increases in *Clostridium*, *Bacteroides*, *Citrobacter*, *Cronobacter*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Parabacteroides*, and *Pseudomonas* species and less relative abundance of *Blautia* and *Ruminococcus* species was observed in these two groups. A prebiotic effect of turmeric may be ascribed to the polysaccharide catabolism and sugar metabolism, but why curcumin, that cannot provide direct energy to commensal bacteria, produced the same microbial signature was not clear in this study. Workers are now reasserting these results with experiments employing larger sample size, exclusion of piperine from the active treatments because piperine itself can promote the growth of some gut microbes, time lag monitoring of gut and serum curcumin levels, assessment of absorption dynamics of curcumin in small and/or large intestine etc.

Case study-3: Garlic extract – blood pressure control - gut microbiota

Garlic (*Allium sativum*, Amaryllidaceae) extract in herbal medicines is a well know treatment for lowering the central blood pressure, pulse wave velocity, pulse pressure, and arterial stiffness that are predictors of the onset of cardiovascular diseases. Hypertension is often linked to gut microbiota dysbiosis wherein a decrease in gut microbial richness and diversity occurred in terms of a significant increase in the *Firmicutes*-to-*Bacteroidetes* ratio that may also lead to intestinal inflammation. To investigate this association, Ried et al. [111] conducted a randomized, controlled double-blind GarGIC (garlic-gut-microbiota-inflammation-cardiovascular markers) trial at National Institute of Integrative Medicine (NIIM), Australia. Fifty two adults (aged 62 ± 10 years) with uncontrolled hypertension (systolic BP e”140 mmHg; and/or diastolic BP e”90 mmHg) were selected for this study with proper exclusion criteria. The participants were randomly asked to take two capsules daily of standardized garlic extract preparation or placebo (inert microcrystalline cellulose) for 12 weeks. Each garlic capsule contained 1.2 g of dry garlic extract powder and 1.2 mg *S*-allylcysteine. Systolic and diastolic BP, pulse wave velocity, pulse pressure and arterial stiffness was measured after 4, 8, and 12 weeks compared to baseline. Also, the fasting blood and stool samples were collected at baseline and after 12 weeks for checking the changes in serum levels

of the inflammation markers tumor necrosis factor-Q (TNF-Q) and interleukin-6 (IL-6) and, gut microbiota richness, diversity, and F/B ratios in them, respectively. Results of this study indicated a significant decrease in diastolic and systolic BP in the garlic group compared to the placebo group. Pulse pressure and mean arterial pressure were also significantly improved in the garlic group compared to control placebo. Garlic supplementation also improved the gut microbiota richness in terms of larger populations of *Lactobacillus* and *Clostridia* species. The *Firmicutes*-to-*Bacteroidetes* ratio (F/B) decreased slightly in the garlic group and increased slightly in the placebo group. Group differences in TNF-Q and IL-6 levels were not significant. The results of this trial are now being extrapolated on large scale with increased sample size over longer study times.

Case study-4: Microbiota-mediated health benefits of Gingko extracts

Ginkgo leaf extract is a popular herbal medicine and dietary supplement with several health benefits in cardiovascular disorders. The extract is particularly rich in a variety of bioactive secondary metabolites of flavonoid and terpenoid groups [130]. In order to examine the pharmacokinetics of Gingko extract under the influence of gut microflora composition, Chunlong et al. [29] chased the bilobalide, ginkgolide A, ginkgolide-B, ginkgolide-C, isorhamnetin, kaempferol, and quercetin pharmacokinetic profiles of orally administered Gingko leaf extract (600 mg/kg) in mice with or without ciprofloxacin pretreatment (150 mg/kg/day for 3 days). It was observed that in antibacterial-treated mice the maximum plasma concentration of isorhamnetin was significantly increased when compared with the control group. The concentration of two other flavonol glycosides kaempferol and quercetin also registered a slight improvement in the treated mice over the control group. The study clearly indicated that oral administration of antibacterials like ciprofloxacin can influence the bioavailability of isorhamnetin by suppressing gut microbial metabolic activities. Another report on this subject has also indicated that microbiota compositional changes in normal, diabetic and diabetic nephropathic rats can also modulate the in vitro biotransformation rates and residence times of the 14 bioactive components of Gingko leaf extract [130]. These workers also observed that microbiota of diabetic rats favored longer intestinal half-life of terpene lactones present in the Gingko extract than in normal rats. These findings also suggest that gut microbial enzymes can be engaged in the metabolic reactions of flavonoids in different ways and may eliminate glycosides, glucuronides, and sulfates of flavonoids to produce flavonoid aglycones that are further biotransformed by gut microbial enzymes to produce a variety of products for their own nourishment [88]. For example, quercetin glycosides may be metabolized by gut microbial glucosidases to yield quercetin, from which gut microbiota can generate products such as 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, protocatechuic acid, and hippuric acid for their own nutrition.

Case study-5: Traditional Chinese medicines-diabetes treatment-microbiota

Traditional Chinese medicine (TCM) has many prescriptions for treating diabetes. Recent researches are suggesting that these herbal medicines can improve glucose metabolism by remodeling the gut microbiota [120]. This has opened an entirely new perspective of developing a new generation of hypoglycemic drugs that will act through microbiota-mediated mechanisms like excessive endotoxins, altered short-chain fatty acids (SCFAs) and disordered bile acid metabolism that regulate body's inflammatory, lipotoxic, oxidative and intestinal barrier related responses [17,109]. As summarized in earlier sections, there are compositional and functional alterations in the gut microbiota of patients with diabetes mellitus like a lower number of butyrate-producing bacteria *Roseburia* and *Faecalibacterium* and *Bacteroides* abundance [46]. *Folium mori* (Mulberry) has been recorded for the treatment of diabetes in TCM for its hypoglycemic effect. Feeding a diet having 20% Mulberry leaf powder to diabetic rats was found effective in reducing non-esterified fatty acid signaling pathway by restoring the proportion of

Bacteroidetes in intestinal microbiota [120]. Another Chinese herb *Dendrobium candidum* was also found effective in alleviating oxidative stress in liver and reducing the blood glucose of diabetic patients [146]. Likewise, berberine - the bioactive alkaloid of *Coptis chinensis* has also been found to be a very good gut microbiota modulator in diabetic animals and humans owing to its promotory effect on proliferation of *Bifidobacterium* and *Lactobacillus* communities. It also inhibits the growth of *Escherichia coli* and relieves chronic systemic inflammation [75]. Besides, polysaccharides extracted from herbal medicines are also potential prebiotics. Polysaccharides from the seeds of *Plantago asiatica* L. could also reduce bodyweight, decrease blood glucose, and repair damaged kidney function in diabetic rats [94]. They also increased the density of bacteria like *Bacteroides vulgatus*, *Lactobacillus fermentum*, *Prevotella loescheii*, and *Bacteroides ovatus* and promote the production of SCFAs. Polysaccharides extracted from several other Chinese herbs like *Maydis stigma, Morus nigra* and *Momordica charantia* etc were also found effective in the management of diabetes via regulation of intestinal microecology. In general terms, most of these ant-diabetic traditional herbs regulate the gut microbiota architecture either by: reducing the Firmicutes/ Bacteroidetes (F/B) ratio; (ii) increasing the anti-inflammatory bacteria such as *Bifidobacterium*, *Lactobacillus*, *Akkermansia,* and *Faecalibacterium*; (iii) increasing the SCFAs producing bacteria such as *Roseburia* and *Eubacterium;* (iv) elevating the concentration of SCFAs in the intestine; or (v) decreasing the abundance of pathogenic bacteria such as *Escherichia coli* and *Enterococcus*. *Lactobacillus*, the widely used probiotics, has also been found to reduce metabolism-related inflammation in diabetic humans [3]. Several other herbs can help in relieving the complications associated with diabetes either by their antioxidative effect or by stimulating body's own antioxidative pathways. Oxidative stress can not only destroys islet 5ØýÞ cells and insulin signaling pathways, but also contribute to more serious complications such as cardiovascular disease and diabetic nephropathy [40]. A healthy intestinal microbiota has been found to help in maintaining redox homeostasis whereas a misbalanced microbial community can lead to the production of epithelial reactive oxygen species (ROS) that can cause massive systemic intestinal injuries [108]. Chinese herbs such as *Dendrobium candidum, Fructus mori* exert their antioxidative actions by lowering the concentration of enterogenous endotoxins that are a major source of inflammation and oxidative stress in pancreas and liver. They regulate gut microbiota and repair the intestinal mucosal barrier and hence, reduce endotoxin damage. Several herbs have also found to help in management of diabetes by reducing the lipotoxicity [111]. Lipotoxicity refers to cell dysfunction or death caused by excess lipid accumulation in non-adipose tissues and aggravates the progression of diabetes. Reduced Firmicutes/Bacteroidetes (F/B) ratio helps in diabetes treatment in obese people by promoting the production of the acetate and butyrate metabolites that can down-regulate the expression of yellow adipogenesis-related genes in white adipose tissue and mitochondrial activity in muscles which in turn, increase the energy expenditure of the body [47]. Overall scenario thus suggests that management of diabetes can be the most viable target for herbal intervention because several base-line studies have univocally indicated that a large number of traditional herbs can exert multiple beneficial therapeutic effects on diabetic patients by regulating their gut microbiota in a way that can activate anti-inflammatory, antioxidant and antilipotoxic effects. In addition, these metabolic changes can finally help in restoring islet function and improving insulin resistance.

FUTURE PERSPECTIVES – WHERE AND WHAT TO FOCUS ON?

Herbal medicines are the main stay of health care service in almost all parts of the globe today. Herbals worth 72 billion US$ are estimated to be traded annually and the trade is enlarging with a growth rate of 15% to touch 3-5 trillion mark in 2050. Traditional herbal medicines are fully institutionalized components of human wellbeing systems in China, India, Europe and several other south east countries [79]. China enjoys the bulk of

global herbal trade share (>40%), followed by India, Vietnam, Malaysia, Thailand etc. Germany, USA, France, UK are the largest consumers of herbal medicines. In retail pharmacy in the US, the sale of herbal medicines has grown by approximately 20% annually with more than 20% population preferring herbal solution for their primary health care and wellbeing needs. Herbal medicines are constantly gaining confidence of people in the management/ treatment of diseases like malaria, diabetes mellitus, hypertension, nervous disorders, arthritis and hepatoprotection [119]. Increasing usage and popularity of herbal medicines has pushed the scientists and pharmaceutical companies to undertake detail clinical evaluation of their bioefficacy and mode of action.

Information catalogued in this review amply justifies two things. First, both historic and modern science have assigned and validated several important functions to our gut flora in maintenance of our health and, secondly the traditional medicinal herbs can play a significant role in this interactive association. Disturbances in gut microbiota composition have been associated with occurrence, development, and treatment of many health disorders and, bioactive chemical components of several medicinal herbs or their standardized extracts have been shown to interact and optimize the gut microbiota and help in treating the diseases. Lot of staggered information is available in literature on this subject but it needs systematic channelization and confirmation in human subjects. Clearly, the task in hand is big and demands focus, prioritization, value chain alignment and hand shaking/mutual trust between traditional healers, AYUSH practitioners, modern physicians, chemists, microbiologists and pharmaceutical companies. Single molecule plant-derived drugs like, morphine, reserpine, vincristine, ephedrine, paclitaxel, podohyllotoxin, artemisininbe, scopolamine, digoxin etc were readily adopted by modern medicine due to their better understood pharmacokinetics and mode of action. Crude herbal medicines on the other hand, suffer from the complexity of their chemical composition that poses several practical difficulties in defining their precise mode of action in pharmacological terminology. Moreover, lower content of active compounds in the herbal formulation preparations further complicates the issue of tracing their pharmacodynamic signature in the body due to poor bioavailability. Cumulative data available on this topic points out three possible generalized modes of actions by which herbal bioactives can influence the gut flora: (i) herbal bioactives are metabolized by gut microbes to yield a more potent derivative (drug); (ii) herbal components alter/ regulate the composition of gut microbiota and creates a more congenial metabolic environment for disease management or treatment and; (iii) promoting the synthesis of microbial metabolites that help correcting the disease *per se*. Clearly the focus of future research will revolve around these three possibilities. In addition to enhance the pharmacological activity of herbal compounds, the gut microbiota can also reduce the toxicity of certain ingredients [82]. For example, aconitine is decarboxylated, methylated, hydroxylated, and esterifed by intestinal bacteria into mono-, di-, and lipid alkaloids that have significantly less toxicity but exert similar pharmacological action as the parental molecule. Conversion of ginsenoside Rb1 and Re of *Panax ginseng* to more bioactive molecule compound-K and Rh1 by gut flora is another example of this in vivo value addition [86].

Evaluation of efficacy and mode of action of herbal ingredients is most severely suffers due to their poorly known chemistry. Hence, this is the first area that will occupy the future scientific attention the most. Chemists will soon be analyzing the herbs with an eye of a clinical biologist. There are hundreds of chemical ingredients in a herbal preparation and very few minors can be the active entities responsible for pharmaceutical action on gut flora *per se*. Precise quantification of these biomarkers has always been a challenge, and chemists will have to redesign and refine their analytical tool box to tackle this issue. Herbal medicines will get their due recognition only when known optimized amounts of these active ingredients are available in the final preparation. It will not only add to drug efficacy but would also take care of batch to batch inconsistency which is the most serious limitations of herbals today. We

define quality of herbs today on the basis of a few major molecules which might not be directly responsible for their clinical function on gut flora. Perhaps some other unknown minor component(s) may also be acting as the real biomarker for a given response. Higher chemical resolution of frequently used herbs can also change their quantitative dosages that are very crucial factor in studying drug interaction with microbiota. It may also be recalled here that most of the herbal medicine preparations involves drying, extraction, concentration and refinement, and drying of plant materials that finally result in a necessity of administering a higher daily dosage compared to Western medicine. No patient actually prefers this undesirable feature of herbal medicines. Another concern that will keep the scientists engage in the research on herbal medicines-vis-à-vis microbiota association would be the low absorption and bioavailability of the former due to oral mode of drug administration. This problem generally arise either due to low levels of the bioactive ingredient in the final preparations or their poor solubility, lipid permeability and gastrointestinal stability. For example most bioactive plant phenolics are unable to cross the lipid membranes of the intestinal cells. This is one reason to explain that why most herbal medicines are not absorbed via the circulation. Making herbals more bioavailable through co-administration of pro- and pre-biotics will therefore be another active area to focus in coming years.

Till recently, most information about the human gut microbiota were gathered through culture-based methodologies that were very labor and time consuming. Advent of culture independent high throughput sequencing techniques targeting the bacterial 16S ribosomal rRNA is making the identification job much easier now because this gene is present in all bacteria and archaea and contains nine variable regions (V1–V9) that allows species to be easily distinguished. Recently, the focus of 16S rRNA sequencing is shifting towards analyzing shorter sub-regions of the gene in greater details. Further refinement of more powerful and reliable estimates of microbiota composition and diversity by whole-genome shotgun metagenomics would be another upcoming area of this field. Data from Human Microbiome Project will further refine this cataloging search of human gut flora. This database has already identified 2172 microbiota species of 12 different phyla, >90% of which belong to Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Cataloging of roughly 9.87 lakhs of microbial genes of gut flora has already been done and the list is enlarging. It can be hoped that understanding of functional manifestations of this gene set will help us in developing more appropriate and precise herbal therapeutics for treating several life style diseases of modern times through microbiota corrections.

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