

General introduction and Review of literature

I. Ayurveda (Definition and history)

The Ayurveda, a holistic system of therapeutics and preventive medicines also known as the science of life has a trove of treasures of the basic principles which results into its survival since the time of inception around three thousand years back. Ayu is the main focus of consideration in ayurveda as it aims at the longevity of a disease-free life. Ayu is composed of four factors, namely *Sareera* (the soma/ body), *Indriya* (the sense organs), *Sattva* (the mind) and the *Atma* (the soul). The word, ‘Ayurveda’ is formed of two words *Ayu* and *Veda*. Ayu, as already described suggests the healthy life span and the Veda means the knowledge. Ayurveda, therefore means the knowledge of the life span.

The history of Ayurveda, the traditional Indian medical system, has been misunderstood and, outside of India, underappreciated for a variety of reasons. Of course, the many Ayurvedic scriptures, which span at least two millennia, are written in Sanskrit, a language that very few people are familiar with. The field of philosophy, literature, and comparative philology, whose study has dominated this branch of Indology in its relatively brief existence of little over two centuries, has more than enough material available for good Sanskrit academics to perform their research. Medical writings are rarely included in university curriculum and are never thought to be of popular appeal. Difficulties abound for the few scholars who are drawn to the subject, the most significant of which being the work involved in locating copies of the writings, which are customarily passed down through generations within families of hereditary physicians.

The primary sources of classical Ayurveda are six Sanskrit text bearing titles that refer to the names of their respective compiler-authors: the Compendium of Caraka (Carakasamhitā); the Compendium of Susruta (Suśrutasaṃhitā, composed shortly after the Carakasamhitā); the Compendium of Bhela or Bheda (Bhela- or Bhedasaṃhitā, dating to approximately 400–750 ce); the Compendium of Kaśyapa or Kāūyapa (Kāūyapasaṃhitā, written between fourth to sixth century ce); the Compendium of the “Heart of the Eightfold Science” (Astāṅgahriḍaya saṃhitā) of Vāgbhata; and the Summary of the Eightfold Science (Astāṅgasamgraha), which is also attributed to an author named Vāgbhata. Ayurvedic literature produced a great deal of original works and commentary during the medieval and early modern eras, reflecting both conceptual advances and exegetical attempts. The Rogaviniścaya or

Mādhavanidāna (ca. eighth century), the Śārṅgadharasamhitā (fourteenth century), and the Bhāvaprakāśa (at some point between 1550 and 1590) of Bhāvamiśra became known as "the shorter triad" (laghutaryī) from the nineteenth century onwards (Maas, 2018). It has been noted that Indian writers attempted to translate Sanskrit medical texts into regional languages during the later 19th century and to understand Ayurvedic sciences in the context of Western medical theories. The best example is Acharya Prafulla Chandra Roy's Hindu Chemistry, which explains the ancient understanding of Indian chemistry as well as the practice of Ayurvedic pharmaceuticals (Mukherjee *et. al.*, 2017).

II. Relevance of Ayurveda in present therapeutic scenario through literature review

The majority of the diseases that the ancient Ayurveda treated still affect people today. The modern Ayurvedic method is probably hindered by antiquated connections and reasoning, some of which may be out of date. The ancient approach was based on extensive clinical observations, intuition and insight, and interpretations that were mostly derived from the Tri-Dosha hypothesis and the Hindu Vedic philosophy. The times have evolved. Ayurveda needs to change to meet the needs of modern people without losing its essential qualities. Research publications have attempted to explain how Ayurvedic knowledge is relevant to contemporary needs and how to interpret it. The prospect of futuristic designer pharmaceuticals that are tailored to a patient's condition and derived from an Ayurvedic database has drawn the attention of academics. These treatments would correspond to a person's diagnosis based on dosha-prakriti and other etiologic factors as understood in Ayurveda (Chopra & Doiphode, 2002).

Plant-based formulations such as quinine, salicylates, digoxin, reserpine, colchicine, vincristine, and docetaxel played a significant role in the development of modern medicine and continued to serve as a source of inspiration for the discovery of novel therapeutic compounds. Those leads will probably run out and a fresh search will be necessary. Plant mass screening is probably going to be costly and ineffective. In light of this, a bioprospecting study is necessary to look for fresh insights from traditional plant-based medical systems like Ayurveda. The quest assumes additional significance when considering the socioeconomic impact and the community's innate acceptance and appeal of herbal remedies (Patwardhan, 2000; Das *et. al.*, 2023).

The difficult issues of medication standardization and validation (clinical and structure-activity relationship) confront modern Ayurveda. Numerous Ayurvedic medications that are already in widespread use can be quickly assessed in controlled clinical settings, despite the logistical and socioeconomic challenges in developing nations. Studies to determine pharmacology and mechanism of action for low clinical evaluation could be used as a sort of reverse clinical pharmacology paradigm. In the current drug development context, the latter would result in significant financial and time savings. However, it should not be overlooked in an overzealous quest to find more potent single molecules that the medicinal potency of these age-old herbal remedies likely rests in the plant habitat of the formulation and that a reductionist approach may compromise efficacy for safety. At least for chronic disorders and infectious diseases those face a threat of antibiotic resistance on a regular basis, that are hard to treat, Ayurvedic remedies could be put into trial. In the effort to make medicine more evidence-based, Ayurveda cannot be disregarded. Additionally, it might be possible to incorporate some Ayurvedic elements into contemporary medicine. In the latter case, the course and severity of the illness will determine whether Ayurvedic therapy is administered concurrently or sequentially (Ramli *et. al.*, 2023; Sharma, 2000).

Glaxo PLC has followed this interest by using its Natural Products Discovery Department's facilities and expertise to find new leads using such materials as templates. To find bioactive components, fermentation broths and extracts are screened. Joint plant medicine research programs have been established by numerous other academic institutions and multinational corporations, such as Bristol-Myers Squibb Pharmaceutical Research Institute, Bedrijf Geneesmiddelen Voorziening Suriname (BGVS), Searle, University of Ghana and BioResources, Virginia Polytechnic Institute, and Conservation International-Suriname. A number of these initiatives are funded by USAID, the National Science Foundation, and the National Institutes of Health (Mehta *et. al.*, 2022).

Over the past few decades, there has been a notable movement in the paradigm around Ayurveda and in the way, scholars view its applications. Ayurvedic therapy is based on the concepts of prakriti and tridoshas, which explain that each person has an individual constitution known as prakriti. Prakriti ascertains the unique way in which every person reacts to drugs, surroundings, and food elements. The recently developed science of "ayurgenomics" fills this

knowledge gap between genomics and Ayurveda and helps to explain inter-individual variations in how different people react to different treatments for different ailments. Studying inter-individual differences in patients with similar ethnic backgrounds is given particular emphasis (Jaiswal & Williams, 2017). Post-Covid 19 research on nature-based drug discovery is on a new high. More and more plants and herbal preparations are being screened for treating several different types of ailments including the most recent pandemic (Srivastava *et. al.*, 2023; Kamaraj *et. al.*, 2023). Several recent articles explore the potentials of Ayurveda based preparations in treating gastro-intestinal diseases like diarrhea and dysentery and multiple-antibiotics resistance (Sunil *et. al.*, 2019; Hassan Bulbul *et. al.*, 2022, Nedungadi *et. al.*, 2023).

III. Diarrhea treatment in Ayurveda

In classic Ayurvedic texts, diarrhea is referred to as "Atisara." The entire, highly detailed account of Atisara can be found in the Charaka Samhita. Both in the past and the present, atisara has been a prevalent issue. The words "ATI" (excessive) and "SARA" (passing of liquid stuff through anus) are combined to form the term "Atisara." This denotes an increased discharge of runny stools through the anus. In his commentary on the Sushruta Samhita, Dalhana mentioned that one of the hallmarks of Atisara is the increased quantity of watery stools passed.

Many single-drug treatments as well as compound formulations are mentioned for various forms of diarrhea in Ayurveda medicine. Numerous recent research has demonstrated the efficacy of various Ayurvedic medications in treating gastrointestinal disorders. Among these is Triphala churna, which is made up of an equal part of dried fruit powders from the three Ayurvedic plants: *Emblica officinalis* Linn., *Terminalia chebula* Retz., and *Terminalia bellirica* (Gaertn.) Roxb. Aqueous and methanolic extracts of Triphala have demonstrated noteworthy antibacterial activities against a variety of bacteria, with minimum inhibitory concentrations (MICs) ranging from 250 to 750 µg/ml. These bacteria include *Salmonella typhimurium*, *Escherichia coli*, *Enterococcus faecalis*, *Aeromonas hydrophila*, *Staphylococcus aureus*, *Bacillus cereus*, and *Shigella sonnei* and *Shigella flexneri*. The essential oils extracted from the aerial segments of *Achillea millefolium* Linn., an Ayurvedic plant, exhibit potent antibacterial activity against *B. cereus*, *C. freundii*, *E. faecalis*, *E. coli*, *S. typhimurium*, and *S. aureus*. The bacterial pathogens were found to be susceptible to the essential oils' strong antibacterial action, with minimum bactericidal concentration (MBC) values ranging from 2.5 to 5 µg/mL and 5 to

10 µg/ml, respectively. Similarly, root extracts of *Aconitum heterophyllum Wall.* have strong antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of 125 µg/ml (Kazemi, 2015).

PHFs or Poly Herbal Formulations are usually utilized in combination with mono-floral medicines to produce synergistic outcomes or additional therapeutic effects at low doses because the formulation contains many suitable phytochemicals. Additionally, by removing the requirement for patients to self-administer multiple herbal formulations at once, PHF can enhance patient compliance. PHF is a holistic therapy method that uses synergism to try and balance the body's doshas. Alternatively, the formulation's many plant components may work through various processes or bind to various biological targets in order to produce their desired effects. Pharmacokinetic and pharmacodynamic processes may control Ayurvedic synergism. Pharmacokinetic synergism involves one or more herbs that improve the absorption, distribution, metabolism, and elimination of the other herbs to maximize therapeutic outcomes while minimizing adverse drug reactions. Synergistic interactions pertaining to pharmacodynamics occur when phytochemicals that possess comparable therapeutic properties simultaneously target one or more receptor sites (Tiwana *et. al.*, 2023).

Arishtas (fermented decoctions) and asavas (fermented infusions) are examples of fermented Ayurvedic medications that can be used to boost their effectiveness, improve their absorption, and lessen their toxicity. Herbal medications are made using the asava-arishta process, a continuous hydro-alcoholic extraction technique, by fermenting herbal juices, decoctions, and sugars. Increased medicinal bioavailability resulting from a broader profile of active chemicals and decreased toxicity of certain detrimental plant components are two major advantages of utilizing the process of fermentation in preparing asavas and arishthas (Mishra *et. al.*, 2010).

IV. Present scenario of incidences of diarrhea

In India, diarrhea is the third most common cause of childhood death, accounting for 13% of all fatalities in children under the age of five each year. More than 5-8 million deaths are attributed to diarrhea each year, with children under the age of five in developing nations making up the majority of these deaths. Due to its link to malnourishment, early mortality, and financial strain on families, diarrhea is one of the biggest health risks to humans. The majority

of diarrheal episodes happen in the first two years of life, with the maximum incidence between the ages of six and eleven months.

Typically, the course of the non-specific approach to treating diarrhea aims to lessen the discomfort and inconvenience of frequent bowel motions. When acute diarrhea is not controlled by oral rehydration therapy (ORT), other treatments such as probiotics, antisecretory agents (bismuth compounds and octreotide), anti-infectives (ciprofloxacin or levofloxacin), spasmolytics (loperamide, atropine, hyoscine), or other antidiarrheal agents (α 2-adrenergic receptor antagonists, such as clonidine, and calmodulin inhibitors, such as zaldaride maleate) are used as a first line of treatment. Diarrhea is the most prevalent GI illness symptom, accounting for almost 1.7 billion occurrences of diarrhea in children worldwide each year. In fact, among children under five, diarrhea is the second most common cause of death and malnutrition. Thus, there is an urgent need for novel, safe, and effective therapeutics. Bacterial, viral, and parasite pathogens that cause diarrheal illnesses are frequently obtained and spread by contaminated food or drink (Rang *et. al.*, 2015). Diarrhea and gastrointestinal bacterial infections may be treated with plant species and preparations used in Ayurvedic medicine. But a lot of these treatments are still to undergo a comprehensive assessment for safety and efficacy. Significantly more research is needed to enhance our understanding of these treatments and enable their broader use in the management of diarrhea.

V. Problems with current therapeutic approaches in the treatment of infectious diarrhea

One of the biggest issues with world health is still infectious diarrhea. Patients experience at least three episodes of loose, watery stools each day, and a variety of etiologies, such as viruses (*norovirus*, *rotavirus*) and bacteria (*Campylobacter*, *Shigella*, *Salmonella*, *Vibrio*, *Escherichia coli*), as well as protozoa (*Entamoeba*, *Giardia*, *Cryptosporidium*) (The & Le, 2022). A common condition in infancy and early childhood is infectious diarrhea. An average of one to two episodes occurs annually in children under the age of three, with peak incidence between the months of six and eighteen (Koletzko & Osterrieder, 2009).

Most orally ingested microorganisms cling to the gut wall after overcoming immune defenses. They then penetrate the cell and change its metabolism. either directly or by producing toxins that damage the cell. Both pathogen and toxins can enter the bloodstream and induce cell death. They can also cause systemic symptoms like nausea, vomiting, chills, fever, and

hypotension. An empirical antibiotic treatment's potential clinical benefit should be assessed, as the pathogen in more than 90% of diarrhea episodes may remain unknown. Antibiotic therapy could possibly lead to the destruction of normal gut flora and development of antibiotic resistance (Baldi *et. al.*, 2009).

In endemic areas, enteropathogens have demonstrated rising resistance to first-line antibiotics over the previous 20 years, especially towards fluoroquinolones. Updating clinical practice recommendations is necessary in light of the rising dilemma of decreased susceptibility of chosen antibiotics for clinicians. High levels of resistance are manifested by older antibiotics like ampicillin, trimethoprim, sulfamethoxazole, and doxycycline. MICs of Azithromycin and fluoroquinolones were much higher than they had been ten years before. *Shigella* and *Salmonella* species have been shown to exhibit an increase in clinical resistance, frequently multidrug resistance, to both first-line antibiotics (such as azithromycin and fluoroquinolones) and alternative third-generation cephalosporins in recent years (Tribble, 2017).

Antimicrobials: A Global Alliance for Optimizing their Rational Use in Intra-Abdominal Infections, or AGORA, was established with the purpose of actively promoting the prudent and reasonable use of antimicrobial drugs in the treatment of intra-abdominal infections in contemporary medicine practice involving a task force of doctors, scientists and microbiologists of 79 countries. Despite the fact that the majority of practitioners are aware of the issue of antibiotic resistance, most undervalue it contributing to higher incidences of multiple antibiotic resistance and failure of therapy (Sartelli *et. al.*, 2016).

Antibiotics are useful in treating a number of infectious diseases, including diarrhea; however, if they are used inappropriately, frequently, or continuously, they may cause microbes to become resistant, which will reduce the effectiveness of treatment regimens. As a result, more than a few researchers are turning to medicinal plants in quest of a suitable solution (Hirudkar *et. al.*, 2020).

VI. Screening ayurveda based therapeutic preparations

Natural resources are too abundant with available solutions to problems human kind face on a regular basis. Diarrhea is an immense problem that takes away at an average of fifteen thousand lives, even before attaining the age of six years; every single day. Association of

infection caused by multiple antibiotic resistant pathogens would most likely aggravate the situation several folds. Giant pharmaceuticals are decreasing their spending on R & D related to antimicrobial development every year due to failure of finding any synthetic or semi-synthetic “superbug” killer single molecule or moiety through ultra-high throughput screenings and most modern means. Therefore, this is the high time when a guided exploration of nature, like an expedition of a lifetime should be planned to fight with the above-mentioned problem. Ancient knowledge of Ayurveda could definitely provide the guidance in such exploration.

The problems with most Ayurveda based preparations are presence of adulterants, unreliability of efficacy, under-reporting of adverse effects, extremely less emphasis given on how the formulation works, variability in composition and constant fight with skepticism of modern western science. However, the need for the exploration, to conduct a search within the arsenal of nature to find a cure would easily undervalue them all. As a result, the following aims and objectives were conceived to screen and manufacture Ayurveda based polyherbal preparation that work against multiple antibiotic resistant gastro-enteric pathogen and to reveal its mode of action to reach the primary end point of the current study.

VII. Aims and objectives

1. To evaluate suitable plant part(s)/ combination of plant parts efficacious against enteric pathogens on the basis of Ayurveda literature and existing practices of Ayurveda.
2. To standardize the preparation(s) in terms of dosage form, dose regimen and evaluating activity against multiple antibiotic-resistant pathogens.
3. To elucidate the mechanism of action of the potential preparation(s) using analytical chemistry, electron microscopy and fluorescence based bacterial cytological profiling.
4. To determine physico-chemical characteristics of the selected Ayurveda preparation using different analytical methods.
5. To collate necessary data of pharmacology and toxicity for initiating pre-clinical and/or clinical studies.