

Gastro-enteric diseases like diarrhea caused by the infection of multiple antibiotic-resistant bacteria result in to severe morbidity and mortality among young children and elderly population. The major problems occur due to rapid loss of fluids causing dehydration and eventual depletion of electrolytes essential for maintaining life functions. Within the short window of appearance of symptoms and choice of therapy, diagnosing the gastro-enteric diseases with proper etiology is very difficult because the disease manifested by the common symptoms like passing of watery stool, nausea, vomiting, weakness and sometimes fever may or may not be associated with bacterial infection. In most of the cases, single antibiotic therapy or simple treatment with common anti-diarrheal drugs fail to cease the occurrence of the disease and symptoms if the ailing person was infected by a multiple antibiotics-resistant gastro-enteric pathogenic bacteria. Evidently, the association of antibiotic resistance is increasing with time due to several clinical, habitual and environmental reasons. The scarcity of a proper therapeutic agent for addressing both the incidence of diarrhea and the probable chances of associated infection is alarming and requires action.

Majority of the Ayurveda based medicines were supported by the observational findings of the practitioners over a myriad of diseases, disorders and symptoms for establishing the medicines therapeutic safety and efficacy. This current study was aimed at finding a suitable plant based natural therapeutic preparation which could counter both the problems of diarrhea and infections caused by multiple antibiotics resistant pathogenic bacteria localizing at stomach or intestines of a human being. The probable poly herbal therapeutic preparation would be evaluated for its safety and efficacy and an effort would be made to reveal the mode of action for further formulation development.

After thorough literature survey, interviewing the Ayurveda practitioners and chemists and evaluating marketed products of different categories like oral liquid preparations comprising

suspension or solutions, coarse dust products, encapsulated Ayurveda solid dosage forms and crude plant extracts, five different plant products were selected for preparation of a poly herbal hydro-ethanolic fermented formulation from among sixteen short listed plant materials based upon their potential to reduce diarrhea and also to exert anti-microbial actions. The poly herbal liquid preparation was prepared by following the basic instructions of Ayurvedic Pharmacopoeia of India and further evaluated for its safety on cytotoxicity study using human intestinal epithelial cell lines and rat intestinal cell lines. Oral toxicity studies were conducted to establish its safety prior to planning efficacy studies using rat model of diarrhea. The polyherbal preparation was found to be effectively arresting the symptoms of diarrhea by means of delaying the onset of symptoms and hastening the cessation of diarrhea as tested on castor oil induced animal disease model study and kaolin marked rate of gastric motility study also in rat model. The obtained results were compared statistically with medicines already in use for the treatment of diarrhea.

The poly herbal Ayurveda based natural preparation was further found to be effective in dose dependent inhibition of growth or/and killing the population of several different genus and species of bacteria isolated from different origins. The developed formulation was found to exert a bactericidal action upon four different pathogenic hospital isolates belonging to genus *Escherichia*, *Shigella* and *Salmonella*, collected from Gastric Tract Pathogen Repository where each species of bacteria was known and retested in lab to exhibit multiple antibiotics resistance phenomena towards several different classes of antibiotics. Minimum inhibitory concentration and minimum bactericidal concentration of the poly herbal formulation were determined by following several antimicrobial assay methods for validation. All three common scientific antimicrobial assay techniques comprising broth dilution method, Agar diffusion method and microdilution method followed by serial dilution and plating were used to establish the formulation's efficacy though *in*

*in vitro* assays against gastric pathogens and both Gram-positive and Gram-negative organisms. The formulation was evaluated thereafter on another animal model of burn wound infection and was found efficacious in topical usage to treat multiple antibiotics resistance also. One common mean of developing resistance among different species of bacteria was found to be through the formation of biofilms of exhibiting quorum sensing phenomena and showing swimming or swarming motility for spreading of infection. Three different assays were conducted using the polyherbal formulation and it depicted inhibition of quorum sensing phenomena and both swimming and swarming motility in all three assay techniques.

The process of revealing the mode of action of a natural or unknown preparation was always the toughest hurdle in every drug discovery experiment. The primary chemical analysis of the polyherbal formulation revealed presence of mixtures of several different classes of organic compounds belonging to several different classes like flavonoids, alkaloids, glycosides, polyphenols and others. Some of these compounds could work synergistically to exhibit the efficacy already shown by the polyherbal formulation. However, qualitative chemical analysis wasn't found sufficient for elucidation of the mode of action of the preparation. Hence, a relatively newer but effective method named Bacterial Cytological Profiling was adopted for the revelation of the mode of action against bacteria. It was already found that each species of bacteria produces different phenotypic features of both the cellular structure and nuclear material when treated with antibiotics working through different modes of action. Based on these basic observations, a couple of bacterial isolates were treated with ten different antibiotics to capture their fluorescence microscopy guided signature cytological profiles belonging to any particular pathway among the five major pathways or modes of actions through which antibiotics work. Both the bacteria were undergone by the treatment of the developed formulation under similar conditions as other

antibiotics with known mode of action and the generated resulting bacterial cytological profile was matched through statistical principal components analysis and analysed cluster map revealed that the poly herbal formulation treated bacterial profiles matched very closely with the profiles generated by Kanamycin and Amikacin. Therefore, it was concluded that the poly herbal preparation developed with the support of the knowledge of Ayurveda was successfully exhibited anti-microbial action by inhibiting the bacterial protein synthesis. Scanning electron microscopy also revealed its action upon the outer membrane of Gram-positive and oligotrophic Gram-negative bacteria.

This study examined and tried to establish the potential of an Ayurveda based poly herbal formulation in inhibiting multiple antibiotic resistant gastric pathogenic bacterial infections and also in reducing the severity of diarrhea. This study warranted the need of more detailed multicentric and multidisciplinary study to explore the full potential of the preparation and development of a proper dosage form for human use and benefit.