# *ABSTRACT*

A global looming situation over food safety and quality is that the synthetic food colourants more than the permissible level are being added in foods, causing concern over human health. This study has focused on the impacts of synthetic food colourants stratified through a survey conducted among the selected random samples of food vendors (N=100) and home makers (N=100) in Northern region of Coimbatore city, Tamilnadu, along with which, supermarkets and shops (N=150) were surveyed for popular brands of synthetic food colourants. Equivalent natural food colouring sources were studied, where annatto seeds (*Bixa Orellana*), eucalyptus bark *(Eucalyptus grandis)*, madder roots (*Rubia cordifolia*), roselle petals (*Hibiscus sabdariffa*) and tamarind seeds (*Tamarindus indica*) were selected. The natural sources were subjected to the processes of aqueous extraction and powdering of the natural substance. Tamarind seeds did not leach out any colour and was ruled out. The shelf life of the natural colourants were monitored in room temperature of 27ºC till tenth day and refrigeration of 5ºC till fifteenth day, where a slimy layer was found at the bottom of the glass bottles in the aqueous extracts which were confirmed to be microbial growth. Microbial assay for fungal and bacteria identification of species were done through Rose Bengal Chloramphenicol Agar Medium and Nutrient Agar Medium, respectively. Then, primary toxicity study was done with brine shrimp assay for all the selected natural food colourants, in which roselle petals showed higher toxicity and was omitted from further analysis. Chemical analysis for antioxidants and phytonutrients were analysed in the samples of annatto seeds, eucalyptus bark and madder roots from which all the substances showed less antioxidant properties and presence of maximum of six to nine metabolites out of 17 metabolites of phytonutrients were present. The aqueous extracts were unstable and started to degenerate the colours, thus further processing was done with powdered substances. Inductive Coupled Mass Spectrometer (ICPMS) analysis was done to detect the heavy metals toxic to human health. In ICPMS, madder root powder had high amount of lead (3.28560 µg), cadmium (0.05162 µg) and chromium (5.48116 µg). Thus, it was not further analysed. During characterization, annatto seeds powder was amorphous in nature with less loss of mass in it, whereas eucalyptus bark powder was crystalline in nature and had a heavy loss of mass whenever there was increase in temperature, while the colours of both the substances were stable. The characterised powders were optimized to determine the level of consumption through an eight weeks (56 days) *in vivo* study, which revealed that annatto seeds powder can be consumed 3 mg/kg of body weight and eucalyptus bark powder of 6 mg/kg of body weight. The animals were sacrificed, the vital organs were harvested and no

significant changes were found in histology and hematological parameters, when compared with the reference ranges. Then to analyse their safety in foods, the selected natural food colourants were incorporated into selected recipes of sweets, snacks and fruit preservatives in accord with the optimized level. Then the natural colour incorporated recipes were checked for discolouring and were evaluated organoleptically which showed an overall of good acceptability ranging between three to four points in five points hedonic scale rating, rated by the selected panel members. Keeping the rating in mind, the researcher has developed a sensor that detects the toxicity in the food colours which hinders the safety of the foods, with the RGB values in the Colour sensor was operated to detect the level of permissible limit prescribed by the FSSAI for each colourant to reveal the level of toxicity present in the solid foods added with commercial food colourants.