RESEARCH WORK

Sun Pharma Award for the paper "Multi-elemental Analysis and In Vitro Evaluation of Free Radical Scavenging Activity of Natural Phytopigments by ICP-OES And HPTLC", published in Frontiers in Pharmacology.

The undersigned researcher, wishes to give a broader aspect of the sophistication of cutting edge tools, their working, their utilization in daily scientific domains as well as highlight the applicability of modern State of Art tools with acceptability of end results in accordance to global regulatory standards like USP-1064, testing for articles of botanical origin and utilization of advanced planar chromatographic system like High Performance Thin Layer Chromatography (HP-TLC), and United States Pharmacopeia (U.S.P)-203 standards for detection of metal content in plant products by spectroscopic techniques like Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

The research work proposed for doctoral thesis encompasses a comparative approach for establishing safety profiles of Natural and toxicity profiles of Synthetic food dyes / colors that are consumed in daily lifestyle or diverse food cultures of Asian continents specifically India and their heterogenicities in regards to chemical composition and diverse cooking styles in every household.

Researchers in past, have portrayed through comprehensive, systematic and exhaustive review highlighting pivotal role of natural (Phyto) pigments as most preferred health beneficial, bio-therapeutic agents enriched with phyto-constituents with potent sensorial and microbiological functionalities. Use of carotenoids, copper, anthocyanins have been centre of research. However, the lacuna of existing documented evidences lacks elemental as well as micro-macro

nutrient profile estimation of other plant-based pigments like Annatto, Anthocyanins, Betanins, Paprika Oleoresins, Turmeric oleoresins etc., one of prime reasons being natural pigments been exempted from certification by food councils across the world. The pigments obtained from natural source particularly the plant (phyto) source may contain traces of heavy metals. Considering the above factual overview, a novel method utilizing optical spectroscopy was put to task for estimating the content of Arsenic (As), Lead (Pb), Cadmium (Cd) and Mercury (Hg), along with determination of essential elements like Iron (Fe), Calcium (Ca), Magnesium (Mg) as micro-macro nutrient rich profiles.

The target parameter to assess the safety profiles of natural plant pigments was heavy metal analysis, which was done utilizing most recent regulatory approved, ICP-OES instrumentation. Test samples of selected natural pigments whose shade and tone match with those of routinely approved artificial food dyes were prepared using Titan MPS microwave digestor from Perkin Elmer.

The phyto pigments were subjected for elemental estimation by ICP-OES (AVIO-200 model) technique in accordance with pharmacopeial dossiers USP 231 and USP 233. The elemental characterization of selected natural pigments utilizing Perkin Elmer TITAN MPS digestor equipped with nebulizer and dual axial plasma assisted fast elemental analysis. The heavy metal content was found to be less than "**1ppm**", which was within limits specified by Food Safety Standards Authority of India ("**F.S.S.A.I**") The developed protocol was validated by performing calibration using heavy metal reference standards of Mercury (Hg), Lead (Pb), Cadmium (Cd), Arsenic (As), Selenium (Se), Chromium (Cr), Copper (Cu), Cobalt (Co) and Antimony (Sb). The amounts determined for heavy metals were within 0.1ppm (0.00001) limits as specified by FSSAI.

The elemental characterization of natural pigments like Annatto, Anthocyanins, Betanin, Turmeric Oleoresins, Paprika Oleoresins by ICP-OES (Avio-200) and

microwave digestion technique using optical plasma spectroscopic approach, provided scientific evidence labelling them free from heavy metals and safe for daily human consumption. The ICP-OES characterization of food colors from organic sources also highlighted natural pigments specially 'Annatto', 'Anthocyanins', 'Betanins' and 'Paprika' as "Phyto-pigments" with bio-therapeutic activities and medicinal properties e.g. preliminary treatment in anaemia or other blood related disorders, hypocalcaemia, anticancer agents etc. The anti-oxidant activity of natural food pigments by HP-TLC-DPPH assay demonstrated novel findings compared to earlier works.

Besides, the Natural pigments were found to be enriched with essential phyto-minerals like Iron (Fe), Calcium (Ca), Magnesium (Mg). Anthocyanins pigment reported highest Iron (Fe) content of 20.52 mg/L. Betanin pigment reported highest Magnesium (Mg) content of 17.85 mg/L, along with considerable amount of Iron (Fe) and Calcium (Ca), of 5.097mg/Land 5.368mg/L respectively. Paprika Oleoresin Pigment reported Calcium (Ca) content of 5.958mg/L. The ICP-OES tool thus, proved as novel and effective tool in labelling them not only non-toxic for human consumption but also useful as biotherapeutic and phyto-pharmaceutical agents.

The HP-TLC-MS served as modernized tool for fast classical yet novel identification of illegal dyes as additives and the determination of primary aryl amines as contaminants from commonly used azo food colorants. The study besides exhibiting diverse applications of cost effective and eco-friendly HP-TLC technique, such as effect direct bioactivity for prediction of radical scavenging property of Natural pigments, also highlights other cutting-edge, State of Art tools and their applications in pharmacokinetics like Liquid Chromatography-diode array (LC-DAD/MS) tandem mass spectroscopy and Gas chromatography equipped with triple quadrupole mass analyzer (GC-MSD) for accurate determination of PAA's as contaminants from daily consumed foods and related

matrices. The elemental characterization of Natural pigments like Annatto, Anthocyanins, Betanin, Turmeric oleoresins, Paprika by ICP-OES technique using optical plasma spectroscopic approach, provided scientific evidence labelling them free from heavy metals and safe for daily human consumption.

Besides heavy metal evaluation, the rapid micro / macro nutrient profiling firmly established the biotherapeutic application of natural pigments as phyto-minerals enriched with essential elements in treatment of insomnia, blood disorders like anaemia, nutraceuticals as well as phytopharmaceuticals agents in immunity related complications, hypocalcaemia, anticancer agents etc.

Finally, yet importantly, the in-vivo studies carried out suggested that with prolonged consumption of contaminated foods with azo Synthetic colors / dyes, associative linkage is possible with complexities eventually leading to carcinogenesis of vital organs like Liver, Kidney, Heart and Brain,

Thus, the proposed research concluded with a streamlined, innovative chromatographic fingerprint approach enables scientific evidence-based toxicity and safety profiles of synthetic food colors/dyes, like Sudan, Sunset Yellow 5 & 6, Carmosine, Citrus Red 2 & 3, F D & C Red -17, D& C Red-17 as additives, in diverse food merchandizes either adulterated / approved for application of colors. On an very interesting note, the Reflectance Spectrophotometer values (**da*, db* & dc*)** measured provided an idea of comparative fastness of Synthetic and Natural food dyes and established strong valid evidence of how the illegal adulteration of forbidden food dyes go unnoticed from Food councils and Regulatory bodies in-spite of stringent norms imposed on approved food colors. The Pharmacokinetic studies performed, is one of the novel work where, the rat blood plasma was isolated and subjected for HP-TLC-MS, LC-MS & GC-MS for fast and rapid estimation of carcinogenic aryl amines like Benzidine, 2,4-Xylidine, 2, 6-Xylidine, Para amino-azobenzene(4-AZB), Ortho toluidine (OT), Ortho amino-azo toluene(O-AAT), etc. which are secondary metabolites of some of

selected routinely approved food dyes in addition to illegal adulteration of Forbidden dyes like Malachite green, Metanil yellow and Sudan Dyes screened and estimated in amounts (> 1000-4500 ppm), suggesting the toxicity in very near future as well as in long run eventually leading to Hepatocarcinoma, Nephron-toxicity etc. The outcomes revealed that Spectro- densitometric and Optical plasma spectroscopic tools in combination of Pharmacokinetics and in-vivo strategic evaluation would set the trend for future analysis of Super foods as well as Foodomics.

Thank you

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