



THE NEWER DIMENSIONS OF NUTRACEUTICALS IN THE PROSPECTIVE OF ITS VALIDATION-A REVIEW

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ABSTRACT

A nutraceutical is a food or a part of a food substance that provides medical or health benefits, including the prevention and treatment of diseases. The food products used as nutraceutical are Probiotic, Prebiotic, Dietary fiber, Omega 3 fatty acid, and antioxidants. In this article an attempt has been made to outline the basic aspects of nutraceuticals such as its classification, and importance and complexity as dietary supplements. From various literatures few of the critical validation aspects of nutraceuticals like complexity of nutraceutical market, an emerging need for official dietary supplement testing methodology, and its role and applications as supplement in herbal medicine, regulatory acts and issues in India relevant to nutraceuticals, sample set validation of nutraceuticals, few of the research reports on validation of few selected nutraceuticals with current scenario and future prospects are compiled and reviewed, which would help to frame a fundamental idea on the validation aspects of nutraceuticals.

KEYWORDS: Nutraceuticals, diet supplements, complexity, validation, research aspects.

INTRODUCTION

“A nutraceutical is a food or a part of a food for oral administration with demonstrated safety and health benefits beyond the basic nutritional functions to supplement diet, presented in a nonfood matrix or nonconventional food formats, in such a quantity that exceeds those that could be obtained from normal foods and with such frequency as required to realize such properties, and is labeled as a ‘nutraceutical’.^[1] Nutraceuticals are diet supplements that deliver a concentrated form of a presumed bioactive agent from a food, presented in a non-food matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods.^[2,3,5] They also refer to natural functional/medical foods or bioactive phyto chemicals that have health promoting, disease preventing or medicinal properties.^[6,7] A nutraceutical is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food.^[8,51-53]

CLASSIFICATION OF NUTRACEUTICALS

Nutraceuticals are considered in two ways:

- Potential nutraceuticals
- Established nutraceuticals

A potential nutraceutical is one that gives a exact health or medical profit; potential nutraceuticals becomes established nutraceuticals only when it is having a enough clinical data. But the majority of the

nutraceutical products are in the 'potential' group, waiting to become established.

The food products used as nutraceutical are grouped as:

- Probiotic
- Prebiotic
- Dietary fiber
- Omega 3 fatty acid
- Antioxidant^[4,9,10]

PROBIOTICS

According to WHO Probiotics are living Microorganisms which upon ingestion in certain numbers, exert health effects, beyond inherent basic nutrition.

Probiotics could be part of the natural microbiota of both animals and humans, They have been used since the beginning of history as starter cultures. Probiotics are present in almost all fermented foods-vegetables, meat and dairy products and they have a long history of consumption and safe use. Lactic acid bacteria and Bifidobacteria are the best candidates for use as Probiotic culture. Lactic acid bacteria have the “General Recognised as Safe” (GRAS) status and Assigned by Food and Drug Administration(FDA). Bifidobacteria have the “Qualified Presumption of Safety”(QPS) status and Assigned by the European Food Safety Agency(EFSA).

Example

Lactobacillus spp, Bifidobacterium spp, Yeast, Enterococcus faecium, Bacillus spp.^[11]

PREBIOTICS

“A selectively fermented ingredient that allows specific changes, both in the composition and / or activity of the gastrointestinal microbiota that confers benefits upon the host wellbeing and health”^[12]

Examples

Lactulose, Inulin, Fructo-oligosaccharides(FOS), Galacto-oligosaccharides(GOS), Transgalacto-oligosaccharides(TOS)^[43]

DIETARY FIBER

Dietary fiber is an vital element of a healthy diet. It helps move food and waste efficiently through the digestive system. Fiber is the piece of plant foods that cannot be broken down by human digestive enzymes in the small intestine. Fiber is generally complex carbohydrates. The two types of fiber are soluble and insoluble. While they work differently, both are wanted for correct bowel function. Most fiber sources have both kinds of fiber in varying amounts.

Insoluble Fiber cannot be dissolved in water. This type of fiber attracts water to the intestines, making stools bulky and soft. It also speeds the movement of food through the digestive tract. Therefore, insoluble fiber may help avoid diverticular disease, colon cancer, hemorrhoids, and constipation. Cellulose, hemi cellulose and lignin are insoluble fibers. They produce the tough, chewy texture of wheat bran, whole grains, corn bran, and some vegetables.

Soluble fiber, or fiber that can dissolve in water, slows the progress of food through the body but does not increase fecal bulk. Soluble fiber helps maintain a healthy cholesterol level, normalize blood sugar levels in diabetics and may even help diminish blood pressure. Pectins and gums are examples of soluble fibers, and they are found in beans, oat bran, psyllium husks, and some fruits and vegetables.^[13]

Examples

Fruits, vegetables, cereals, grains and pasta etc.^[13]

Omega-3 Fatty acids (FAs)

Omega-3 FAs are a family of naturally occurring polyunsaturated fatty acids (PUFAs). Humans do not have the necessary metabolic pathways to synthesis the precursor FA (linolenic acid), which is essential for the production of the longer bioactive FAs. Therefore, these long-chain PUFAs must be obtained either from plant sources or by direct intake of EPA and DHA from marine or industrial products.^[14,15,16,17]

Examples

Leafy vegetables, nuts, and oils as a-linolenic acid.^[14,15,16,17]

ANTIOXIDANTS

Antioxidants are substances that may protect cells from the injure caused by unstable molecules known as free radicals. Antioxidants act together with and stabilize free radicals and may avoid some of the injure free radicals might otherwise cause. Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances. Antioxidants are essential and vital for plants and animals' sustenance. They are substances that protect cells from the injure caused by unstable molecules known as free radicals. The sources and origin of antioxidants which include fruits and vegetables, meats, poultry and fish were treated in this study. The types of antioxidants such as ascorbic acid, glutathione, melatonin, tocopherols and tocotrienols.^[18]

Examples

Meats, fish, Nuts, Grains and poultry.^[18]

Importance of Dietary Supplements and Herbal Medicines

A considerable amount of controversy surrounds the efficacy of dietary supplements, including herbal medicines, in the United States. Many of the dietary supplements have long-standing historical uses in several countries. Recently, the improved use and consumer acceptance of herbal supplements in the United States has prompted more research. In fact, the office of dietary supplements at NIH is funding \$1.5 million annually for university-based botanical research. One study, which will be performed at Purdue University (West Lafayette, Indiana), will involve research of grape polyphenols, tea catechins, and soy isoflavones. Clinical studies also have been undertaken; for example, St.-John's-wort was examined in a rigorous clinical study of patients with mild depression.^[19,22]

Researchers are accumulating a tremendous amount of information about dietary supplements; however, they still have very large gaps to fill regarding pharmacological components, dosages, contraindications, and biological pathways of action.

The innovation of the pharmacological components in herbal medicines such as ephedra alkaloids in *Ephedra sinica* (mahuang)^[19,23], valerianic acid in *Valeriana officinalis* L. (valerian)^[19,24], and allin–allicin in *Allium sativum* (garlic)^[19,25] have been well characterized and are called active constituents. However, in a large array of dietary supplements, the active substances are not known specifically. In these cases, a chemical group or a family of known ingredients are characterized and called marker compounds. The marker compounds can be used to point out the authenticity of a exact botanical substance. More importantly, the active components or

marker compounds potentially can be utilized to standardize the botanical substance.^[19]

Complexity of the Dietary Supplement Market

During the past 10 years or so, we have seen tremendous growth in the number of products and methods in the nutraceutical market. In addition to the traditional vitamin and mineral supplements, the Dietary Supplement Health and Education Act also encompasses botanical substances, specialty chemicals such as phytosterols, ω -(3,6)-fatty acids, and many more complex

mixtures of these products sold as dietary supplements. The below table-1, “The Complexity of the Nutraceuticals Market: A Summary of Nutraceuticals and Dietary Supplements,” lists the types of products on the nutraceutical market. Many different techniques and methods exist for quality control of this complex market. The major challenge that analysts face today is the standardization and validation of the analytical procedures utilized to evaluate nutraceuticals. Consumers will be neglected if a quality system is not defined for the entire industry.^[19]

Table: 1: The Complexity of the Nutraceutical Market: A Summary of Nutraceuticals and Dietary Supplements^[19]

Vitamins	Fat-soluble vitamins Nutritional factors Single vitamins Water-soluble vitamins
Multiple vitamins	Fat-soluble vitamin mixes Fat- and water-soluble vitamin mixes Water-soluble vitamin mixes
Minerals	Mineral chelates Mineral salts Single minerals Trace minerals
Multiple minerals	Amino acid mixes Calcium, magnesium, and zinc mixes Real salt
Multiple vitamins and minerals	Once daily multivitamins or minerals Three times daily multivitamins or Minerals
Botanical substances	Essential oils Mixed whole herbs Single whole herbs Tea mixes Traditional formulas
Herb extracts	Alcohol–water extracts Guaranteed potency herbs Supercritical fluid extracts Whole-extract herb mixes
Specialty	Antioxidants Carotenoids Diet aids Digestive aids Essential fatty acids Flavoglycosides Glandulars Isoflavonoids Lecithin Ω -(3,6)-fatty acids Phytosterols Probiotics Procyanidins Special formulas Topical creams USP-grade chemicals

EMERGING DRIVE FOR OFFICIAL DIETARY SUPPLEMENT AND ITS TESTING METHODOLOGY

Here in lays the challenge: if these materials can be standardized with valid methods, then the quality of dietary supplements will progress very much. The development then is furthered as new active constituents and marker compounds are separated and standardized. For the majority part, the quality testing of botanical substances has been established by individual companies, and several of these methods are proprietary and unvalidated, making it hard to establish a consistent technique, marker compounds, and reference standards.^[19,20,21]

The 1983 and 1990 editions of the British Herbal Pharmacopoeia have more than 300 monographs compiled by the British Herbal Medicine Association. These monographs offer regulatory information, Latin names, and both macroscopic and microscopic descriptions of the plant. They also offer chemical identification information, testing methodology, and quantitative standards for foreign matter, total ash, ash insoluble in acid, water-soluble extracts, and volatile oils, as appropriate. The primary quality technique is thin-layer chromatography (TLC), which allows chemical fingerprinting of whole botanical substances and can differentiate not only between species of plants but in some cases even between subspecies of plants.^[19,20]

Chemical fingerprinting poses an interesting challenge to method validation as outlined by USP and International

Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. Analysts can validate TLC chemical fingerprinting of botanical substances by validating the plant material used with standard botanical identification techniques before making the TLC chemical fingerprint. The chemical fingerprint then offers a quick, inexpensive method for the quality screening of whole herbs, without utilizing more costly and extensive methods such as high performance liquid or gas chromatography. This technique offers a basic means to identify an herb; however, it is not as useful in defining or quantifying active constituents and marker compounds.^[19,20] Blumenthal and colleagues catalogued a large number of herbs and their medicinal uses in The Complete German Commission E Monographs. These monographs name herbs and provide the regulatory status of each herb in Europe. Each monograph defines the name of a drug (herb), the composition of the drug, its uses, some pharmacokinetics, contraindications, side effects, dosage, mode of administration, and actions. This book is a rich source of information used by European physicians. The fundamental acceptance and treatment of botanical substances as drugs throughout Europe makes their manufacture and distribution tightly controlled. This practice, along with technology, also has affected the testing methodology for quality control of botanical substances. Quality is based chiefly upon identification and not upon quantifying active components of each herb. In contrast, the dietary supplements in the United States are treated as foods, and, as a consequence, are subject to very little control during manufacture and distribution.^[19,21]

Table:2: SELECTED FEW EXAMPLES OF MARKETED NUTRACEUTICALS

Marketed preparation of nutritional supplements ^[48,49]		
Product	Category	Contents
Coral calcium	Calcium supplements	Calcium and trace minerals
Weight smart	Nutritional supplements	Vitamins and trace elements
Omega women	Immune supplements	Antioxidants, vitamins and phytochemicals (eg. Lycopene)
Appetite Intercept	Appetite suppressants	Caffeine, tyrosine and phenylalanine
Chaser	Hangover supplements	Activated carbonate and vegetable carbon
Rox	Energy drink	Taurine, caffeine and glucuronolactone
Mushroom optimizer	Immune supplement	Mushroom, polysaccharides and folic acid
Biovinca	Neurotonic	Vinpocetine
Proplus	Nutritional supplements	Soy proteins
Snapple-a-day	Meal replacement beverage	Vitamins and minerals
Wellife	Amino acids supplements	Granulated-L-glutamine
PNer plus	Neuropathic pain supplements	Vitamins and other natural

Table: 3 TFEW SELECTED NUTRACEUTICALS AND THEIR APPLICATIONS ^[50]

CLASS/COMPONENT	SOURCE	POTENTIAL BENEFIT
Carotenoids 1. Beta-carotene 2. Lycopene	Carrots, various Fruits Tomatoes and processed tomato products	Neutralizes free radicals, which may damage cells; bolsters cellular antioxidant defenses. May contribute to maintenance of prostate health.
Dietary Fiber Insoluble fiber	Wheat bran	May contribute to maintenance of a healthy digestive tract
Fatty Acids Monosaturated fattyacids	Tree nuts	May reduce risk of coronary heart disease
Flavonoids Flavonols	Onions, apples, tea, broccoli	Neutralize free radicals, which may damage cells; bolster cellular antioxidant defenses
Isothiocyanates Sulforaphane	Cauliflower, broccoli, cabbage, kale, horseradish	May enhance detoxification of undesirable compounds and bolster cellular antioxidant defenses
Phenols Caffeic acid, ferulic acid	Apples, pears, citrus fruits, some vegetables	May bolster cellular antioxidant defenses; may contribute to maintenance of vision and heart health
Plant Stanols/Sterols Stanol/sterol esters	Fortified table spreads, stanol ester dietary supplements	May reduce risk of coronary heart disease
Polyols Sugar alcohols (xylitol, sorbitol, mannitol, lactitol)	Some chewing gums and other food applications	May reduce risk of dental caries (cavities)
Prebiotics/Probiotics Lactobacilli, bifidobacteria	Yogurt, other dairy and nondairy applications	May improve gastrointestinal health and systematic immunity
Phytoestrogens Isoflavones(daidzein, genistein)	Soybeans and soybased foods	May contribute to maintenance of bone health, healthy brain and immune functions; for women, maintenance of menopausal health
Soy Protein Soy protein	Soybeans and soybased foods	May reduce risk of coronary heart disease
Sulfides/Thiols Dithiolthiones	Cruciferous vegetables	May contribute to maintenance of healthy immune function

Table: 4 : Uses of traditional nutraceutical in chronic disease control ^[29-39]

Nutraceutical	Dose/ Duration	Effect
Allenic carotenoid Fucoxanthin(brown seaweeds)	2.4 mg /day	Improves insulin resistance and decreases blood glucose levels through the regulation of cytokine secretions from WAT (white adipose tissues)
n-3PUFAs (polyunsaturated fatty acids)	No data	Prevents several disorders affecting lungs and airways
ASU (unsaponifiable residues of avocado and soybean oils)	300mg / 3 years	Stimulates synthesis of aggrecan and extracellular matrix component as type II collagen and by reducing the production of catabolic (MMP-3) and pro-inflammatory (IL-8 and IL-6) mediators in OA (osteoarthritis)
CLA(Conjugated linoleic acids)	3 months	Significantly improves AHR (Airway hyper responsiveness) associated with a reduction in leptin/ adiponectin ratio in mild asthma
Siphonaxanthin, a marine carotenoid (green algae)	20µM / within 6h of treatment in	Induces apoptosis in HL-60 cells by decreasing Bcl-2, and increases activation of caspase-3

	HL-60 cells	
FPP(Fermented papaya preparation)	6g/day / 6 months	Unregulated TNF- α and thioredoxin (Trx) in liver cirrhosis
MUFAs (monounsaturated fats)	No data	Lowers CVD (cardiovascular disease) risk and MS (metabolic syndrome)
1,25(OH)2D, or Calcitriol	200–600IU/day	Regulates the levels of p21 and p27 and increases expression of BRCA-1 and -2 tumor suppressor genes contributing in the DNA repair mechanism
Resveratrol	No data	Chemosensitizes tumor by modulating drug transporters, cell survival proteins, cell proliferative proteins, and members of the NF- κ B and STAT3 signaling pathways
Fortified wheat flour	100 to 150 μ g/day	Reduces prevalence of NTDs (neural tube defect) at birth and increasing blood folate concentrations

The Push/ for Method Validation

The U.S. Pharmacopeia and National Formulary (*USP–NF*) is opening to list validated monographs for botanical substances. The most recent compendium of standards, *USP 24–NF 19*, includes dietary supplements and botanical substances. In all, the advisory panel on Analytical Methods for Identification and Characterization of Natural Products set priorities for 21 botanical substances.

The selection criteria as outlined in the *USP–NF* include absence of safety risk; extent of use as reported by trade sources; positive assessment by recognized pharmacognosists, usually on a presumption of helpful pharmacological action, history of use in traditional medicine, and the ability of the article to meet typical *USP–NF* requirements. This also meant that analytical methods had to be created and validated to meet *USP–NF* criteria^[19,26]. The only way that a material can be standardized is if the testing methodology can consistently and correctly quantify herb active constituents or marker compounds. This has been very complex, because botanical substances are in a complex matrix, and separation and quantification is daunting. Additionally, many of the reference standards and instrumentation are not available for everyone, making it complex to validate the method. This challenge is not new, and the *USP–NF* is not the only group determined to establish universally accepted testing methods. The International Nutraceutical Association has created botanical compound testing methods to split with the entire industry. It has established a protocol that designs a method, validates it at three independent laboratories, and collects the feedback. A USP panel then scrutinizes the method before final approval. The Association of Official Analytical Chemists International (AOAC

International) also has established a plan to develop and validate methods of analysis for botanical substances.

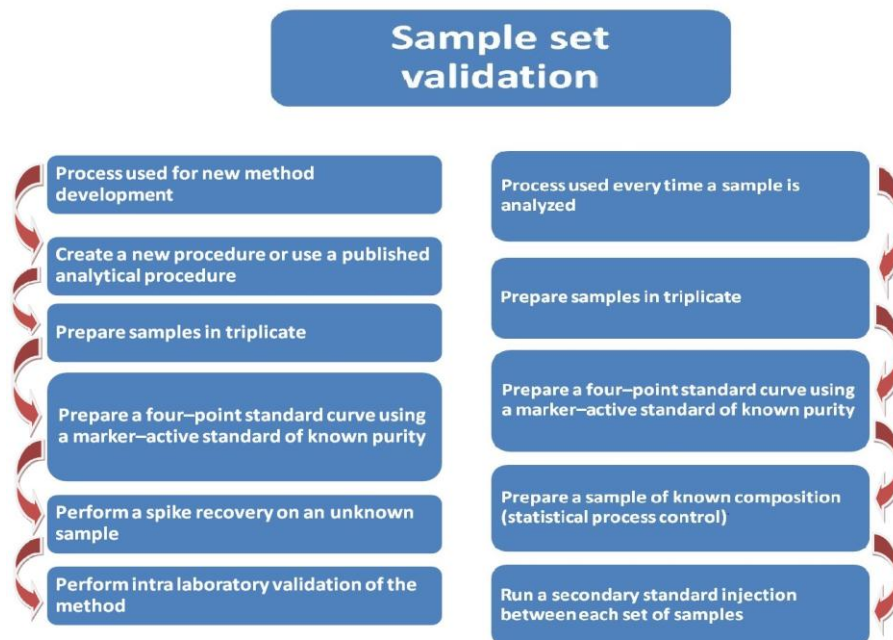
Validation can be interpreted differently depending, to a certain extent, upon the application and analytical technique used to make the determination^[19]. Lesnik stated “it can be a major source of confusion to laboratory analysts, quality assurance officers, and data users alike, because each group tends to have very dissimilar interpretations of what validation means.”

USP–ICH method validation guidelines are wide and produce methods that are perfect, specific, precise, and robust. These guidelines lead to data that can be trusted and defended. Analysts can find it somewhat hard to fully implement these guidelines into the quality control testing of nutraceuticals. Although testing single component products with completely validated *USP–ICH* methods may be possible, products such as herbs and herb blends can be difficult to analyze using *USP–ICH* guidelines. Sections 1.2 and 4.2 of the *ICH Harmonized Tripartite Guideline on Validation of Analytical Procedures: Methodology* states that impurities shall not interfere with the analyte determination or quantification^[19,28]. A complex aspect in testing herbs and herb blends occurs from analysts’ inability to define and identify all impurities. Although it may be easy to quantify known toxins in these products, defining an impurity in an herbal supplement is problematic. Plants can produce different levels of chemicals as a result of factors such as growing region, time of harvest, and soil quality.^[19]

Sample set validation

The sequence of steps involved in sample set validation of nutraceuticals is given below in the chart.

Chart: 1: SAMPLE SET VALIDATION

Table:5: Regulatory Acts and issues in India relevant to nutraceuticals^[39,40]

Regulatory Acts	Regulatory issues
The Food Safety and Standard Act (FSSA), 2006	Manufacture, sell or import of novel foods, GMF, irradiated food, organic food, and food for special dietary uses, functional food, nutraceuticals and health supplements
The Food Safety and Standard Authority of India (FSSAI), 2008	Single reference point for all matters relating to food safety and standards
Food Safety and Standards Rules and Regulations, 2009	More emphasis on science based and participatory decisions
The Food Safety and Standard Authority of India (FSSAI), 2010	Implemented

Table:6: FUNDAMENTALS ON REGISTRATION OF NUTRACEUTICALS^[41]

S. No.	India
Definition	FSSA define Nutraceutical as "Foods for Special dietary use"
Rules/Regulations for licensing and registration	The Food Safety and Standards Regulations
Regulations came into force in year	2011
Responsible Regulatory authorities for Registration of Nutraceuticals	Food Safety and Standard Authority of India (FSSAI)
Regulatory Requirements for Registration	A. Product evaluation B. Licenses C. Health & label Claim
Fees for registration	Rs. 100
Claims Approval by Authority	FSSA
Health Claims	1) Nutrient function claim, 2) Other function claims, 3) Reduction of disease risk claims

CURRENT SCENARIO OF NEUTRACEUTICALS IN INDIA

Indian nutraceuticals industry is expected to grow at 20 per cent to USD 6.1 billion by 2019-2020 due to rising awareness about health and fitness and changing

lifestyle, according to a report. At present, the nutraceuticals industry in India is about USD 2.2 billion and is mainly focused in the southern region, followed by the eastern region with three major states of Andhra Pradesh, Tamil Nadu and West Bengal, a report by

business consulting services firm, RNCOS said. "The nutraceuticals industry in India is one of the rapid growing markets in the Asia-Pacific region. Factors like rising awareness about health and fitness, ageing population, changing lifestyle are fostering this growth." The industry is anticipated to grow at around 20 per cent over the period to reach USD 6.1 billion by 2019-2020. Nutraceuticals business is divided into three segments - functional food, functional beverages and dietary supplements.

Further, the report said the nutraceuticals market penetration in urban India is at around 22.5 per cent, while it is comparatively low in rural India at 6.3 per cent. The urban penetration is more as demand for protein supplements is increasing among the urban youth due to rising desire towards maintaining fitness and building a strong physique. In India, mainly the FMCG and pharmaceuticals sectors dominate the nutraceuticals market. The Indian nutraceuticals market is divided into functional food and beverages (68 per cent) and dietary supplements (32 per cent). While oats, probiotics, nuts, tomato products, yogurt, sports and energy drinks, among others, fall under functional foods; vitamins, minerals, fibres and fatty acids, in the form of tablets and capsules, are part of dietary supplements.^[42]

FUTURE PROSPECTS OF NUTRACEUTICALS

Currently Indian nutraceuticals market is highly urban centric. However with the rise of rural market and if the growth trajectory remains the same, Indian nutraceuticals market is going to be more than double of current market within next five years and by the end of current decade may become fivefold since the beginning of decade. Currently functional food enjoys largest share of the Indian nutraceuticals market followed by dietary supplements. This trend will drive the market for fortified foods and pro-biotic. With the rise of life style related diseases in urban India and penetration in rural India, the nutraceuticals products going to remain in high demand. "Though having healthy size of health conscious consumer segment in India, still market has not adopted nutraceuticals for regular consumption. Indian consumers are still at 'awareness' or somewhat 'interest' stage of product adoption cycle.

Nutraceuticals players need to redefine their marketing strategies to bring customers at 'trial' and 'evaluation' stage to harness the immense growth potential of an Nutraceuticals market^[45,46]

Increasing awareness levels about fitness and health, spurred by media coverage are prompting the majority of people to lead healthier lifestyles, exercise more, and eat healthy. The expanding nutraceutical market indicates that end users are seeking minimally processed food with extra nutritional benefits and organoleptic value. This development, in turn, is propelling expansion in the nutraceutical markets globally. The emerging nutraceuticals industry seems destined to occupy the landscape in the new millennium. Its tremendous growth

has implications for the food, pharmaceutical, healthcare, and agricultural industries. Many scientists believe that enzymes represent another exciting frontier in nutraceuticals. "Enzymes have been underemployed they're going to be a hot area in the future." Fermentation technology using microbes to create new food products also represents potential. Global trends to healthy products cannot be reversed. Companies taking the lead by investing strategically in science, product development, marketing and consumer education will not go unrewarded.^[45,47]

RESEARCH REPORTS ON VALIDATION OF FEW SELECTED NUTRACEUTICALS

The research findings of few of the neuriceuticals and their validation have been taken from the relevant literatures and given an extractive of its fundamental methodology adopted as validation tool. We will be primarily emphasizing on neuriceuticals like green tea, broccoli, and rosemary,

VALIDATION OF GREEN TEA

The green tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide. The health benefits of tea and tea extracts have been well documented, especially with respect to chemo preventive effects on cancers, cardiovascular diseases and neurodegenerative diseases. Green tea contains an abundance of polyphenols with anti-oxidative capacities.^[27]

The increased popularity of this beverage all over the world currently may be due to evidence of a relationship between tea consumption and prevention of certain forms of human disease, such as cancer and cardiovascular disease. Many studies report reduced risk of carcinogenesis as a result of drinking green tea.^[44] The development of better analytical techniques to analyze the different components in the botanical dietary supplements will continue to be an important task to ensure the quality and safety of each of the products^[44]

A simple, specific, precise, accurate, rapid and reproducible RP-LC method has been developed^[54] by Samuel Saito et al. They quantified six catechins and caffeine in Brazilian green tea infusions (*Camellia sinensis* var. *assamica*).

According to their validation research report and concluding remarks, the developed method was an appropriate method for the quality control of major green tea contents that can be used by pharmaceutical and food industries. The composition of samples assayed suggested that the summer is the best season to extract a major content catechins and caffeine.

VALIDATION OF BROCCOLI

one of the fruit and vegetable which is currently under investigation in many research projects include organo sulphur compounds (glucosinolates and their degradation

products) from *Brassica* species^[55] *Brassica* vegetables contain glucosinolates, the metabolic breakdown products of which are potent modulators of xenobiotic-metabolizing enzymes that protect DNA from damage. A high intake of cruciferous vegetables is associated with a reduced risk of cancer, particularly lung and those of the gastrointestinal tract. The antioxidant activity and total phenolic content of broccoli extracts have been evaluated by using a model system consisting of carotene and linoleic acid. The total phenolic of the extracts was determined spectrophotometrically according to the Folin–Ciocalteu procedure. Broccoli found to have a high anti-oxidant activity correlated significantly and positively with total phenolics. A study conducted by D.A. Moreno *et. al* revealed^[56] that Plant foods can be improved as sources of essential micronutrients or micro minerals (generally occurring at relatively low concentrations in living tissues) either by increasing the concentrations of nutrients in the food or by increasing the bioavailability of micronutrients in the food, or both, and this subject has been reviewed for specific trace elements. These results emphasize the complex interactions of bioactive chemicals in food attempts to maximize one component may affect accumulation of another, and consumption of high amounts of multiple bioactive compounds may result in unexpected metabolic interactions within the body.^[57]

VALIDATION OF ROSEMARY: Silvia Moreno *et.al*^[58] have reported on the health benefits of Rosemary as nutraceutical. It is the only spice commercially available for use as an antioxidant in Europe and the United States. This specie has the advantage to contain different antioxidant molecules (lipophilic monoterpenes and diterpenes, as well as hydrophilic derivatives of caffeic acid asrosmarinic acid) that could be effective in both, aqueous fluids as well as in lipophilic parts of the body as a very effective antioxidant to scavenge free radicals. In addition, non-volatile extracts of rosemary can also be used to decrease 4.4 - to 17-fold the amounts of the synthetic butyl derivatives used as food or cosmetic preservatives^[59] The available evidence indicates that rosemary compounds might be of therapeutic benefit in bacterial infections and be an ideal candidate for nutraceutical health products. Non-volatile extracts of rosemary containing approximately an amount of 20µg/ml (18 µM) of carnosic acid as the key compound, killed several bacteria and represent therapeutic alternative against extracellular-intracellular *S. aureus* infections. This compound did not show pro-oxidant effects and its use is safe at least until a concentration of 250µg/ml (750 µM). The *in vivo* antibacterial efficacy of an ethanol extract of *Rosmarinus officinalis* L. containing high amounts of carnosic acid against the pathogenic bacteria *S. aureus* has been demonstrated previously in mouse^[60] Even though, prospective controlled clinical studies are still lacking.

CONCLUSION

Reports on general uses and importance of nutraceuticals for health are highly captured in litatures, but the validation aspects of nutraceuticals from point of quality assurance is still under developmental stage in our country. Hence this review will enable the reader to understand the changing face and newer validation aspects of nutraceuticals so as to enhance its potential as food for future.

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