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Bioactives and bioactivity of selected underutilized fruits, vegetables and legumes grown in Sri Lanka: A review

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Abstract
Being a tropical country, Sri Lanka has many food crops and they have not been extensively studied for their phytochemicals, food-based applications or many industrial uses. A greater deal of research nowadays focus on the identification of bioactive compounds in dietary sources, separation, purification and formulation of functional food products which may utilize as supplements to the body's antioxidant defense system. The key purpose of the present review was to summarize the bioactivity of compounds available in selected underutilized food crops available in Sri Lanka to utilize them in functional food product development. There is much extent to research the bioactive potential of underutilized food crops. Evaluation of health benefits using ex-vivo studies should target the assessing the metabolic fate of bioactive compounds through cell metabolism since the biotransformation of bioactives through principal and peripheral metabolism in cells may either promote or elicit the desired bioactivity.

Keywords: Bioactive; antioxidant; under-utilized; functional food; extraction

1. Introduction
Extra nutritional components that typically present in fewer quantities and often the products from primary and secondary metabolism in plants are usually addressed as "bioactive" compounds or phytochemicals. These are vast in numbers and when taken categorically they can be identified as carbohydrates, lipids, nitrogen-containing compounds terpenoids, phenolics and alkaloids (Baxter *et al*., 1998) [8].

Every compound included in these categories is not known to be health benefits, but most of the compounds are identified as potential antioxidants which are highly effective in reducing the oxidative stress. Since qualitative and quantitative studies are mostly dependent on the extraction techniques, selecting a proper extraction method for bioactives from plant materials is very crucial (Azmir *et al*., 2013) [7]. There are many conventional extraction techniques such as Soxhlet extraction and solvent extraction. Modern extraction techniques include enzyme-assisted extraction, ultrasound-assisted extraction and microwave-assisted extraction (Zainal-Abidin *et al*., 2017) [74]. Identification and quantification of extracted bioactive compounds can be done using sophisticated techniques like Ultra-high performance Liquid Chromatography-Mass Spectrometer, Gas Chromatography-Mass Spectrometer and various other diagnostic equipments. However, the identification and characterization of compounds are very crucial for the development of functional food products and as well as pharmaceutical and nutraceutical products.

Functional foods and Nutraceuticals are of utmost interest in the modern world due to their favourable impact on reducing the risk of chronic diseases. Oxidative stress-related chronic diseases are able to be treated with diets enriched with bioactive compounds having functional effects, for which most scientists are nowadays experimenting, people are searching and industries are looking forward to market. According to WHO (2005), chronic diseases take decades to become fully established as their origins are at young ages of human life and they need to be treated long-term and with a systematic approach. When understanding the relationship between oxidative stress and chronic diseases, oxidative stress is the state when there is a disparity between the production of reactive oxygen species and antioxidant defense mechanism.

Atoms in molecules lose electrons becoming electrically charged particles (free radicals) and the process is known as oxidation. When an oxygen atom or oxygen-containing molecule

undergoes this process, Reactive Oxygen Species (ROS) are formed. The human body is a multicellular structure composed of numerous molecules between which chemical reactions take place to sustain growth, metabolism and reproduction. Free radicals and ROS, formed during these chemical reactions should be neutralized on many occasions and the molecules that can donate electrons and neutralize free radicals and ROS, which are also called antioxidants, are at the helm. When the state of normal redox is altered by ROS and the free radicals increasing the oxidative stress conditions, they can negatively affect major biological molecules including proteins, lipids and nucleic acids and can cause cell injury (Phaniendra *et al*., 2015) [56]. However, cells have evolved with antioxidant defense mechanisms which consist of both enzymatic and non-enzymatic mechanisms to prevent injury caused by these free radicals and ROS. There are circumstances in which the overproduction of these free radicals and ROS can overwhelm the antioxidant capacity of the cellular antioxidant defense system, leading to oxidative stress in the system.

Reactive oxygen species (ROS) and free radicals are highly reactive by-products of cellular metabolism, can help in cell signaling and regulation (Thannickal and Fanburg., 2000) [67] while been reported to cause several chronic diseased conditions such as cardiovascular diseases (atherosclerosis and hypertension), diabetes mellitus, neurodegenerative disorders (Parkinson's disease-PD, Alzheimer's disease-AD and Multiple sclerosis-MS), respiratory diseases (asthma), cataract development, rheumatoid arthritis and in various cancers (colorectal, prostate, breast, lung, bladder cancers) (Phaniendra *et al*., 2015) [56]. Cardiovascular diseases (CVD), cancer, hypertension and diabetes are such chronic diseases that are considered collectively as the prominent causes of death in most countries including Sri Lanka (WHO, 2019). The main reasons for these chronic diseases have been linked with lifestyle choices and the most important contributor is diet (Boyer and Liu., 2004) [9]. Therefore, it has been suggesting that dietary modification towards antioxidants richer meals is an effective strategy to prevent these chronic illnesses (Kaliora *et al*., 2006) [35].

Even though there are many logical and theoretical advantages of food-based approach for disease prevention, studies on specific foods or constituents has been limited by the variability in bioactive content and incomplete chemical characterization (Gu *et al*., 2013) [28]. Therefore, specific food products derived from fruits, vegetables and legumes have to be chemically characterized, highly desirable, easily incorporated into a diet and stable over time and storage conditions. The bioavailability, bioaccessibility and metabolic fate of bioactives in specific functional food formulations will be analyzed using various ex-vivo methods. Effect of these food formulations on the targeted biomarkers will be tested using human clinical trials. They will be much helpful in providing sound evidence to prove the favourable effects of food formulations. Being a tropical country, Sri Lanka has many fruits, vegetables and legumes that have not been fully studied for their phytochemicals, food-based applications, or for many industrial uses. Most of these food crops, not been utilized optimally due to the paucity of knowledge of suitable processing techniques and value addition have earned collective names such as 'neglected and underutilized' crops (Padulosi, 2012) [55]. Besides the fruit, bark, leaves, stem, root, twig, and sap of many food crops have been used as ingredients for traditional medicine in early civilizations to treat many health complications (Khoo *et al*., 2016) [37].

Presence of extraordinary hardiness in these food crops and their potential ability to adopt with severe growing and climatic conditions provide great promise in the era of climatic change (Ravi *et al*., 2010) [58]. Therefore, it is a positive fact that they would dominate the world crop sector in the future. Nevertheless, exploiting under-utilized agricultural commodities in the functional food sector, nutraceutical and pharmaceutical sector will be more income-generating not only to farmers but also to the country's economy through commercialization of novel research concepts. Hence the objective of this review paper is to discuss and review the bioactive potential of underutilized food crops.

2. Potential health benefits and Bioactive compounds

Fruit, vegetables and legumes are well-known excellent sources of compounds that exert numerous health benefits to the consumer. Health improving properties such as anti-inflammatory, antioxidant, anti-diabetic, anti-tumor, anti-Alzheimer's disease and anti-obesitylisted under the Table 01 are found in many crops which are considered under-utilized and have drawn the attention of scientists.

Table 1: Medicinal properties of under-utilized vegetables, fruits and legumes grown in Sri Lanka

Common Name/s	Scientific Name	Medicinal Properties
Fruits		
Beli	*Aegle marmelos*	(leaves and ripe fruits) for treatment of diarrhoea, dysenteries and diabetes mellitus (Kamalakkannan & Prince, 2003) [36]. Other actions like antifungal, antibacterial, antiprotozoal, antispermatogenic, leaves possess cardiotonic effect, antifungal, analgesic and antioxidant activities (Siddique *et al*., 2010) [63]
Anoda / Sugar apple / Custard apple	*Annona squamosa* L.	possess analgesic, anti-inflammatory, antipyretic, antiulcer (Chavan *et al*., 2010) [12], anti-fertility, anti-tumor and antimalarial activities, young leaves are used extensively for their antidiabetic activity (Shirwaikar *et al*. 2004) [62], cytotoxic, antitumoral and immunosuppressive activities (Mariod *et al*., 2012) [45]
Anoda / Soursop	*Annona muricata*	used as a natural medicine for arthritic pain, neuralgia, arthritis, diarrhea, dysentery, fever, malaria, parasites, rheumatism, skin rashes and worms, and also eaten to elevate a mother's milk after childbirth, anti-cancer properties (Coria-Téllez *et al*., 2018) [16], to treat diverse ailments such as respiratory and skin illness, internal and external parasites, bacterial infections, hypertension, inflammation, diabetes (Coria-Téllez *et al*., 2018) [16]
Bitter orange / Sour Orange	*Citrus aurantium* L.	used in Brazilian folk medicine and other countries to treat anxiety, insomnia and as an anticonvulsant suggesting depressive action upon the central nervous system (Carvalho-Freitas *et al*., 2002) [11]
Canistel / Lawulu	*Pouteria campechiana*	possess anti-oxidant, anti-inflammatory and anti-hyperglycemic properties (Aseervatham *et al*., 2014, Fuentealba *et al*., 2016) [6].
Mandarin	*Citrus reticulata*	anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities due to presence of

		bioactive compounds, such as phenolics, flavanone glycosides, hydroxycinnamic acids, vitamin C, and carotenoids (Abeysinghe *et al*., 2007) [1]
Ceylon olive	*Elaeocarpus serratus*	used in folk medicine in treatment of stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases (Das *et al*., 2017) [17]
Indian gooseberry / Nelli	*Phyllanthus emblica*	hypolipidemic and hypoglycemic activities and acts as an important constituent of many hepatoprotective formulations available (Liu *et al*., 2008) [44]
Vegetables		
African Eggplant / Thalanabatu	*Solanum macrocarpon*	in the treatment of gout, rheumatism and angina, childbirth anesthesia, to treat inflammatory tumours, cancerous tissues and in the treatment of Parkinson's disease (Oboh *et al*., 2005) [52]
Wild Eggplant / Thibbatu	*Solanum torvum*	used for cough ailments, useful in cases of liver and spleen enlargement and the ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment for pain (Sivapriya & Leela, 2007) [65].
Ceylon spinach/water leaf	*Basella alba* L.	Used in the management of cardiovascular diseases like stroke, obesity (Aja *et al*., 2010) [3], antioxidant and anticancer properties, lower age-related macular degeneration (AMD) and cataract formation (Lakshminarayana *et al*., 2005) [42]
Legumes		
Butter bean	*Phaseolus lunatus*	potential sources of bioactive peptides with antihypertensive, antithrombotic, anticancer, and antimicrobial activities. (Ciau- Solís *et al*., 2018) [15]
Tonga beans	*Vigna umbellata*	
Sword beans	*Canavalia gladiate*	

Bioactive compounds such as Vitamins E and C, polyphenols and their derivatives, flavonoids, lycopene, carotene, phospholipids, amino acids and peptides, phytic acid, pigments, sterols and coenzyme Q10 are known to have antioxidant properties (Kaliora *et al*., 2006) [35]. Compounds like flavonoids (quercetin, kaempferol, catechin) are present in onion, berries, olives and citrus fruits (Kris-Etherton *et al*., 2002) [40]. These are having a remarkable ability to reduce Low-Density Lipoprotein (LDL) cholesterol, platelet aggregation, tumor initiation and eicosanoid synthesis. Some phytoestrogens like lignans, isoflavones, and lycopene, found in clover, soybeans, legumes and tomatoes can lower LDL, total cholesterol, thrombosis and triglycerides. Soluble dietary fibers like β-Glucan, pectin, psyllium present in oats, barley, fruits and vegetables have exhibited desirable effects on triglycerides, total cholesterol and LDL levels. The major carotenoids identified in green leafy vegetables, lutein, â-carotene, violaxanthin, neoxanthin, and zeaxanthin are having good bioactive potential (Lakshminarayana *et al*., 2005) [42]. Phenolic antioxidants are primary antioxidants that act as free-radical terminators (Mariod *et al*., 2012) [45]. Not only phenolic compounds but also many protein and peptide compounds are known to be sources delivering bioactivity. Several food protein-derived peptides are potent *In vitro* ACE inhibitors (Ciau- Solís *et al*., 2018) [15]. The first ACE inhibitor peptides were isolated from a gelatin hydrolysate with the use of collagenase. Currently they are isolated from diverse protein sources including egg, corn, milk, fish, yeast, rice, and bovine blood plasma. Two purified rice-derived tripeptides (Val-Asp-Trp and Val-Trp-Pro) were characterized and exhibited high ACE inhibitor activity (Ciau- Solís *et al*., 2018) [15]. Legumes are high-quality protein sources that contain beneficial bioactive peptides. Different peptides derived from several legumes such as velvet bean-*Mucuna pruriens*, cowpea-*Vigna unguiculata*, and Jamapa bean-*Phaseolus vulgaris*. Lima bean (*Phaseolus lunatus*) contains a higher nutrient content and it is considered as a potential source of various bioactive peptides with functional properties such as antimicrobial, antihypertensive and antithrombotic and anticancer. *Aegle marmelos*is a plant containing many phytochemicals such as a egeline, agelinine, rutin, sterol-sitosterol, -D-glucoside, marmesinine, tannins, Phlobatannins, flavonoids, umbelliferone, quercetin and volatile oils (Eugenol and methyl eugenol) (Siddique *et al*., 2010) [63]. Two flavanones (naringin and hesperidin), phenolics (flavanone glycosides, hydroxycinnamic acids), vitamin C, carotenoids are bioactive compounds found in citrus fruits (Abeysinghe *et al*., 2007) [1]. *Elaeocarpus serratus* L. contains many significant bioactives such as saponins, tannins, cardiac glycosides, flavonoids, steroids (Das *et al*., 2017) [17]. The predominant bioactive compounds in *Annona muricata* is acetogenins. It also has other compounds like alkaloids, phenolic compounds, carotenoids, vitamins A, E and C (Coria-Téllez *et al*., 2018) [16]. *Pouteria campechiana* is having dominant carotenoid compounds such as neoxanthin, β-carotene, β-cryptoxanthin, violaxanthin (de Lanerolle *et al*., 2008) [19].

3. Extraction of Bioactive compounds

Based on the extraction method of bioactives from plants sources, the qualitative and quantitative evaluations may differ. There are many conventional extraction techniques such as maceration, percolation, Soxhlet extraction and solvent extraction. Modern extraction techniques include enzyme-assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, subcritical fluid extraction, supercritical extraction, and high pressure-assisted extraction (Zainal-Abidin *et al*., 2017) [74]. Solvent Extraction can be accomplished through various means such as maceration, infusion, effleurage and cold compression. Shortcomings linked with conventional methods are long extraction time, potential loss of interest molecules, high energy consumption. Novel techniques include enzyme-assisted extraction, supercritical fluid extraction, microwave-assisted distillation and ultra-sound assisted extraction. Novel techniques can lead to a compact, safe, efficient, energy-saving, sustainable extraction process. The majority of these extraction techniques are used to obtain bioactive extracts at an analytical scale. Only supercritical fluid extraction and soxhlet extraction methods are used in large scale industries to develop pharmaceuticals and nutraceuticals in developed countries.

Solvent extraction of bioactive compounds can be implemented by using the method described by Burbott and Loomis (1967) [10]. Plant/food material is ground in a mortar containing the appropriate solvent (e.g.: hexane, methanol, chloroform, liquid nitrogen) and anhydrous Na_2SO_4 and extracted few times with the same solvent to obtain the extract. Pigments can be removed by Norit A charcoal, using centrifugation at low speed. The clear solutions are then concentrated using a rotary evaporator. Major limitation of solvent extraction method is the specificity of solvents for the

solubility of different compounds. Since all soluble components are extracted, resins, fats, and fatty acids, waxes, or pigments are often co-extracted. During the extraction process, variation of pressure and temperature influences the solubility behavior of the compounds. Due to shorter extraction period, no high temperatures applied to the degradation of the compounds can be minimal.

Among many solvents used for extraction, distilled water is the primary solvent that can used for trapping bioactive compounds from the plant/food matrix. Apart from that ethanol, acetone and methanol are widely used. Thus extracted compounds are summarized under Table 2.

Accelerated solvent extraction (ASE) is an improved method of solvent extraction by applying high pressure, high temperatures and using different solvents in few extraction cycles (Richter & Schellenberg, 2007) [59]. Due to the increased solubility of the substances, the extraction time can be decreased. The phenolic constituents are extracted by using the method described by Abeysinghe *et al*. (2007) [1] with some modifications and the extract was used for the determination of total phenolics, total flavonoids and two flavanones (naringin and hesperidin).

Table 2: Bioactive compounds extracted from under-utilized vegetables, fruits, and legumes using different extraction methods

Extraction method	Solvent used	Common/ Scientific Name	Bioactive compounds	References
Solvent Extraction (percolation, maceration, infusion)	Ice-cold acetone, Hexane	*Basella alba* L.	Carotenoids	Lakshminarayana *et al*., 2005 [42]
	Water, Acetone	*Pouteriacampechiana*	Carotenoids- β-carotene, ζ-carotene, β-cryptoxanthin, violaxanthin, neoxanthin	De Lanerolle *et al*., 2008 [19]
	Methanol, water	*Citrus reticulata* *Citrus aurantium*	Phenolics (flavanone glycosides, hydroxycinnamic acids), Flavonoids, Flavanones (naringin, hesperidin), vitamin C, carotenoids	Abeysinghe *et al*., 2007 [1], Divya *et al*., 2016
	Methanol	*Aegle marmelos*	Phenolics	Siddique *et al*., 2010 [63]
	Acetone, Methanol, Water	*Solanum melongena*	Phenolic compounds, Chlorogenic acid	Hanson *et al*., 2006 [32], Silarova *et al*., 2019
	Ice-cold acetone, hexane	*Basella alba* L.	Carotenoids (lutein, β-carotene, violaxanthin, neoxanthin, and zeaxanthin)	Raju *et al*., 2007 [57]
	Methanol, Ethanol, water	*Elaeocarpus serratus* L.	Reducing sugars, steroids, phenol, flavonoids, phytosterol, cardiac glycosides,	Das *et al*., 2017 [17], de Lima *et al*., 2019 [20]
	Ethanol, BHT, Methanol	*Solanumtorvum*	Carotenoids (Provitamin A, Lutein)	Otu *et al*., 2017 [53]
	Methanol	*Phyllanthusemblica*	Phenolics, flavonoids, proanthocyanidins	Liu *et al*., 2008 [44]
	Distilled water	*Phyllanthusacidus*	Phenolics	Kumari *et al*., 2014 [41]
Soxhlet extraction	Chloroform	*Aegle marmelos*	alkaloids, reducing sugars, tannins and flavonoids	Mazumder *et al*., 2006 [46]
	Petroleum ether	*Annona squamosa*	Caryophyllene oxide	Chavan *et al*., 2010 [12]
	Ethanol	*Annona squamosa*	Phenolics	Shirwaikar *et al*., 2004 [62]
	Acetone	*Phyllanthusacidus*	Phenolics, Flavonoids	Padmapriya & Poonguzhali, 2015 [54]
Accelerated assisted solvent extraction	Acetone	*Citrus sinensis*	polyphenols	Nayak *et al*., 2015 [49]
Microwave-assisted extraction	Acetone	*Citrus sinensis*	polyphenols	Nayak *et al*., 2015 [49]
Subcritical water extraction	Distilled water	*Citrus* sp.	Phenolics, Polymethoxylated flavones	Kim *et al*., 2009 [38]
	Milli-Q-water	*Citrus* sp.	Flavanones –hesperidin, narirutin	Cheigh *et al*., 2012 [13]
Supercritical Carbon dioxide extraction	CO_2	*Citrus medica*	Bioactive essential oils	Menichini *et al*., 2011 [47]
		Citrus sp.	Carotenoids	Ndayishimiye & Chun, 2017 [50]
			flavonoids	Suetsugu *et al*., 2012 [66]
Ultrasound-assisted extraction	Methanol & 2-propanol, ethanol, water	*Solanum melongena*	Anthocyanin, Phenolics	Dranca & Oroian, 2016 [22], Uchida *et al*., 2017 [69]
	Methanol, water	*Annona squamosa*	Phenolic compounds	Mariod *et al*., 2012 [45]
	Acetone	*Citrus sinensis*		Nayak *et al*., 2015 [49]
	Chlorine chloride, oxalic acid	*Aegle marmelos*		Saha *et al*., 2019 [61]
Enzyme-assisted extraction	Alcalase-Flavourzyme (AF) enzymatic system, pepsin-pancreatin (PP) enzymatic system	*Phaseoluslunatus*	peptides	Ciau-Solís *et al*., 2018 [15]

Soxhlet extraction is done using standard apparatus, standard method (Furniss, *et al*, 1989) [27] for a specific period, with an appropriate solvent (Eikani *et al*., 2007) [24]. This is a time-consuming method and uses large amounts of solvents. Several chlorinated solvents (e.g., chloroform, carbon tetrachloride, tetrachloroethylene, and chlorobenzene) and non-chlorinated solvents (e.g., acetone, methanol, and acetonitrile) are normally used for extraction from various matrices, depending on the properties of the targeted molecules/compounds (Tiwari, 2015) [68]. Several drawbacks of this technique include low extraction yield, toxicity of certain solvents, presence of solvent residues in target compounds and safety risks, have influenced the industries to move towards developing green extraction technologies, minimizing or eliminating the use of organic solvents (Tiwari, 2015) [68]. Various compounds extracted using these methods are listed under the Table 2.

Microwave-assisted extraction (MAE) refers to the use of microwave heat to accelerate solvent extraction processes. With the use of water as the solvent, the solid and the slurry is treated either in open or in closed vessels (Flórez *et al*., 2015) [25] with microwave energy. Reduced extraction time and amount of organic solvents, improved efficiency and selectivity, the precision of analyte recoveries and good reproducibility, with limited degradation, decreased costs and contamination levels, avoiding additional sample clean-up and concentration steps before chromatographic analysis are some of the advantages of Microwave extraction over conventional methods. Selective heating, increased production, effective heating, elimination of process steps, reduced thermal gradients, reduced equipment size and faster start-up time are also favorable in Microwave-assisted extraction process (Jeyaratnam *et al*., 2016) [34], even though it is not much popular among industrial bioactive extraction.

Solid Phase Microextraction (SPME) is a comparatively new method usable for aroma compounds extraction. It is a very simple and efficient, solvent-free sample preparation method for routine laboratory analysis of organic compounds. A coated silica fiber is directly injected into the sample or the headspace above the sample. Thereby organic analytes are adsorbed to the respective fiber coating (Richter & Schellenberg., 2007) [59]. Different types of adsorbents have been used to extract different groups of analytes. Polar coatings, e.g. polyacrylate (PA) and Carbowax (CW), extract polar compounds, e.g. phenols and carboxylic acids, whereas non-polar coatings, e.g. polydimethylsiloxane (PDMS), retain hydrocarbons extremely well. The Carboxenpolydimethyl siloxane (CAR-PDMS) fiber has excellent capacity for concentrating volatile bioactive components from the headspace of foods heated to high temperatures, and even from raw foods and other matrixes to which application of heat is not desirable (Richter & Schellenberg, 2007) [59]. It has been successfully used for the analysis of the bioactive components of different plant materials.

Continuous subcritical water extraction (CSWE) is performed using the following protocol as described by Rodríguez-Meizoso *et al*., 2010 [60]. Preheated degassed Milli-Q water is passed through the extraction chamber, which contains the sample. The aqueous extract is cooled in the refrigerant at 25°C and, after passing through the variable restrictor, collected in a vial. The temperature of the extraction chamber is the key variable when subcritical water is used as an extract ant. The extraction yield is increased with temperature up to 150°C co-extraction of undesirable molecules or compounds (as it can be inferred from the fact that a lot of additional peaks appear in the chromatogram), such as paraffin and cuticular waxes, makes the solvent extraction step difficult and longer at the highest temperature. The pressure is a key variable in order to maintain the water under liquid state at temperatures over 100°C. One of the greatest advantages of the CSWE method is rapidity. Several researches have been conducted for extracting bioactive compounds from plant commodities using this method (table 2).

Supercritical Carbon dioxide extraction (SFE) is a technique that uses Carbon dioxide as an organic solvent. With SFE the extraction conditions can also be adapted by variation of temperature and pressure. Compared to solvent-extracted oleoresins, supercritical fluid extracts are free of any solvent, which is the main advantage for food industrial use (Richter & Schellenberg, 2007) [59]. SFE can be optimized in terms of several parameters: extraction temperature, extraction pressure, extraction time, restrictor temperature and capture solvent (n-hexane and n-heptane). Industrial usage in extracting bioactive compounds is reported for extracting bioactives from *Citrus* species as indicated in Table 2.

Ultrasound-Assisted Extraction (UAE) is the method use ultrasound as a pre-treatment during Solvent Extraction process. UAE offers environment-friendly, clean extraction with several advantages. Also, ultrasound is relatively easy to use, versatile, and flexible, and technique requiring low investment compared with other novel extraction techniques (e.g., SFE, pressurized solvent extraction, or ASE). Use of ultrasound is a relatively novel, clean, green extraction technology for extracting various compounds and other biomaterials such as proteins, peptides and bioactive molecules (Tiwari, 2015) [68]. According to Vinatoru *et al*. (2017) [72], Ultrasound is used in Supercritical fluid extraction and microwave and ultrasound, High pressure and ultrasound, during two instances: during the extraction process, where biomass and solvent are introduced and; in the evaporation process, to assist solvent removal at lower temperatures. Several recent studies have reported the successful ultrasound assisted extraction of bioactive compounds from various food crops.

Enzyme-assisted extraction is used to extract peptides-protein concentrates as described by Ciau- Solís *et al*, (2018) [15]. Single protein extraction is done of the Lima bean flour using an established wet fractionation method. Enzymatic hydrolysis is done with either an Alcalase®-Flavourzyme® (AF) enzymatic system or a pepsin-pancreatin (PP) enzymatic system. Here the temperature, enzyme to substrate ratio, and pH are some parameters that should be maintained properly for effective extraction. Pepsin mainly attacks peptide bonds containing aromatic amino acids, methionine, or leucine, while pancreatin has a higher specificity for the C terminal bonds of methionine and leucine residues. Fractionation by ultrafiltration is where the soluble fractions of the enzymatic hydrolysates are fractionated by ultrafiltration.

4. Evaluation of Bioactive compounds

Bioactive compounds and their potential health benefits can be identified using *In-vitro*, in-vivo and ex-vivo methods as discussed below. *In-vitro* methods are usually the analytical techniques performed using test tubes, culture dish, or elsewhere outside living organisms. In-vivo is always referred to as analysis taking place in a living organism. Studies using laboratory animals those in which the particular disease conditions are induced by administering several other compounds are considered as in-vivo methods. Ex-vivo means that which takes place outside an organism referring to

experimentation or measurements done in or on tissue from an organism in an external environment with minimal alteration of natural conditions.

Identification and quantification of extracted bioactive compounds can be done using sophisticated techniques like Ultra-high performance Liquid Chromatography-Mass Spectrometer, High-Performance Liquid Chromatography Diode Array Detection, Gas Chromatography-Mass Spectrometer, Nuclear Magnetic Resonance Spectroscopy and various other diagnostic equipment.

In-vitro antioxidant analysis techniques have been proposed based on different chemical reactions. According to Gülcin (2012) [29], a standardized method for antioxidant activity of a food component should meet the ideal requirements such as measuring chemistry occurring in potential applications, utilizing a biologically relevant radical source, been simple, using a method with a defined endpoint and chemical mechanism, chemicals and instrumentation which are readily available, having a good within-run and between-day reproducibility, been adaptable for assay of both hydrophilic and lipophilic antioxidants and using different radical sources, been adaptable to high throughput analysis for routine quality control analyses (Gülcin, 2012) [29].

Antioxidant analysis methods can be divided according to reaction, mechanisms in HAT (hydrogen atom transfer) and SET (single electron transfer) methods (Gülcin, 2012) [29]. When an antioxidant possessing the ability to transfer one electron to reduce any compound such as radicals, metals and carbonyls can be detected by SET-based methods. According to (Gülcin, (2012) [29], SET displayed through a change in colour as the oxidant is reduced and the ability of an antioxidant to quench free radicals with the donation of hydrogen is measured in HAT-based methods. Methods based on the HAT reaction include Oxygen radical absorbance capacity (ORAC), Total radical-trapping antioxidant parameter (TRAP), Inhibition of induced LDL oxidation, Chemi-luminescent assay, Crocin-bleaching assays, total oxyradical scavenging capacity assay, SET-based methods quantify the ability of a potent antioxidant. Trolox equivalence antioxidant capacity assay, Total phenolics assay by Folin-Ciocalteu reagent assay, Total antioxidant potential assay, Ferric ion reducing antioxidant power (FRAP) assay and other radical scavenging assays such as N, N-dimethyl-p-phenylenediamine radical (DMPD) scavenging assay, 2,2-Azinobis 3-ethylbenzthiazoline-6-sulphonic acid radical (ABTS) scavenging assay, Cupric ions (Cu^{2+}) reducing antioxidant power (CUPRAC) assay and 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay.

Apart from these there are other assays measuring scavenging ability for oxidants, that interact and damage the major macromolecules either in biological systems or in foodstuffs (Gülcin, 2012) [29] Superoxide anion radical scavenging assays, Hydrogen peroxide scavenging assays, Hydroxyl radical scavenging assays, Singlet oxygen quenching assays and Peroxynitrite ($ONOO^-$) scavenging assays. Various methods used to analyze the anti-oxidant activity of extracts from underutilized food crops are listed in Table 3. *In-vitro* Anti-hypertensive activitydetermines thereduction of arterial hypertension due to any compound or drug. The renin-angiotensin system is important in the physiopathology of arterial hypertension because it converts angiotensin I, via angiotensin I-converting enzyme (ACE), into angiotensin II. The ACE-inhibitory and renin-inhibitory activities of peptide fractions isolated by enzymatic hydrolysis of protein are conducted in order to assess the *In vitro* ACE-inhibitory and antihypertensive activities of peptide fractions extracted from Lima bean (*P. lunatus*) using two different sequential enzymatic hydrolysis systems (Ciau- Solís *et al*., 2018) [15].

The Ellman colorimetric method is based on determining the amount of thiocholine released when acetylthiocholine or butyrylthiocholine is hydrolysed by Acetyl Choline Esterase or Butyryl Choline Esterase (Adewusi *et al*., 2010) [2]. The released thiocholine is quantified by its reaction with 5,5´-bisdithionitrobenzoic acid (DTNB), which produces a yellow 5-thio-2-nitrobenzoate anion. One of the treatment methods against several neurological disorders including Alzheimer's Disease is Acetylcholine. Among the selected underutilized crops, methanollic extract of fruit pulp of *Aegle marmelos* (L.) Correa have been shown to have an inhibitory effect on AChE (Adewusi *et al*., 2010) [2].

Several studies report anti-inflammatory properties of extracts of *Aegle marmelos, Annona squamosa, Phyllanthus acidus* evaluated with in-vivo methods using selected rat species.

Ex-vivo or cell culture based methods are much popular for assessing the anti-inflammatory activity of bioactive compound extracts. Intracellular reactive oxygen species (ROS) assay can be used as described by Chingsuwanrote *et al*., (2016) [14] using Promonocytic non-differentiated U937 cells at complete phenol red-free medium. Cytotoxicity of fruit extract on non-differentiated U937 cells is assessed by sulforhodamine B (SRB) assay (Vichai and Kirtikara, 2006) [71]. Cytotoxicity is assessed based on Mariod *et al*., 2012 [45] using MCF-7 human breast carcinoma cells, HepG2 human hepatocellular carcinoma cells, HT-29 human colon adenocarcinoma cells and WRL-68 normal hepatic cells, maintained in Dulbecco's modified Eagle's medium (DMEM). MTT assay for Cellular viability is acolorimetric assay conducted according to the method described in Mariod *et al*. (2012) [45]. Different cell types are used to determine the inhibitory effect of samples cell growth using the MTT [3-(4,5-dimethylthiazole-2-yl) 2,5-diphenyltetrazolium bromide assay. The principle behind is the conversion of the yellow tetrazolium bromide (MTT) to purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells. Absorbance at 570 nm is measured and recorded. Results are expressed as a percentage of control giving percentage cell viability after 24 hours exposure to test agents. The potency of cell growth inhibition for each test agent is expressed as an EC50 value, defined as the concentration that caused a 50% loss of cell growth. The ratio (expressed as a percentage) of the absorbance of treated cells to untreated cells is known as viability. Mitochondrial dehydrogenase reduces MTT to an insoluble purple formazan. Cell viability is measured by a comparison of the purple colour formation. Dead cellsdo not form the purple formazan due to their lack of the enzyme. According to Table 3, several studies have investigated the cellular viability of plant extracts of selected underutilized crops.

Table 3: Methods of evaluating the bioactive potential of under-utilized fruits, vegetables and legumes

Assay	Technique	Under-utilized crop	Compounds/Properties analyzed	References
Qualitative phytochemical analysis	-	*Aegle marmelos* *Phyllanthusacidus*		Mujeeb *et al*., 2014 [48], Kumari *et al*., 2014 [41]
Tannins			Tannins	
Alkaloids			Alkaloids	
Saponins (Frothing test)			Saponins	
Cardiac glycosides (Keller-kilani test)			Cardiac glycosides	
Steroids (Liebermann-Burchard reaction)			Steroids	
Terpenoids (Salkowski test)			Terpenoids	
Flavonoids			Flavonoids	
Phlobatannins			Phlobatannins	
Anthraquinones			Anthraquinones	
Reducing Sugars			Reducing Sugars	
FRAP assay	Spectrophotometric method	*Citrus* sp.	Total Antioxidant Capacity (Trolox – TE/ g FW)	Abeysinghe *et al*., 2007 [1]
Determination of Total Phenolic content – Folin-Ciocalteu method			Phenolics (Chlorogenic acid equivalents-CAE/100g FW)	Abeysinghe *et al*., 2007 [1]
		Aegle marmelos *Annona squamosa* *Basella alba* L. *Pouteriacampechiana*, *Phyllanthusemblica*	Phenolics (gallic acid equivalents – GAE)	Siddique *et al*., 2010 [63], Mariod *et al*., 2012 [45], Dasgupta & De, 2007 [18], Aseervatham *et al*., 2013 [5], Liu *et al*., 2008 [44]
Determination of Total Phenols		*Aegle marmelos* *Solanum melongena*	Polyphenols	Mujeeb *et al*., 2014 [48], Hanson *et al*., 2006 [32], Silarova *et al*., 2019
Determination of Total Flavonoid content	Spectrophotometric method	*Citrus* sp. *Annona squamosa*	Flavanoids (mg Rutin Equivalents-RE/100g FW)	Abeysinghe *et al*., 2007 [1], Mariod *et al*., 2012 [45]
	gravimetry	*Aegle marmelos*	Flavonoids (mg/g)	Harborne protocol, Mujeeb *et al*., 2014 [48]
	Spectrophotometric method	*Solanumtorvum*	Flavonoids (mg Rutin Equivalents-RE/100g FW)	Arung *et al*., 2009 [4]
		Basella alba L.	Flavonoids (Catechin equivalents)	Dasgupta & De, 2007 [18]
		Pouteriacampechiana, *Phyllanthusemblica*	Flavonoids (Quercetin equivalents)	Aseervatham *et al*., 2013 [5], Liu *et al*., 2008 [44]
Determination of Vitamin C content	Reverse-phase HPLC	*Citrus* sp.	Ascorbic acid (mg/100g FW)	Abeysinghe *et al*., 2007 [1]
Total monomeric anthocyanin determination	Spectrophotometric method	*Solanum melongena*	Calculated based on Cyanindin-3-glucoside	Dranca & Oroian, 2016 [22]
Determination of Tannins (Method of Swain)	Spectrophotometric method	*Aegle marmelos*	Tannins	Mujeeb *et al*., 2014 [48]
Determination of Saponins (Brunner method)		*Aegle marmelos*	Saponins (mg/g)	Mujeeb *et al*., 2014 [48]
Determination of Naringin& Hesperidin content	Reverse-phase HPLC	*Citrus* sp.	Naringin & Hesperidin (mg/100g FW)	Abeysinghe *et al*., 2007 [1]
Determination of Alkaloids	gravimetry	*Aegle marmelos*	Alkaloids (mg/g of sample)	Harborne protocol, Mujeeb *et al*., 2014 [48]
DPPH radical scavenging activity	Spectrophotometric method	*Citrus* sp. *Annona squamosa* *Basella alba* L. *Pouteriacampechiana*, *Phyllanthusemblica*	*In-vitro* antioxidant activity	Mariod *et al*., 2012 [45], Dasgupta & De, 2007 [18], Aseervatham *et al*., 2013 [5], Liu *et al*., 2008 [44]
Determination of ABTS radical scavenging activity		*Pouteriacampechiana*	*In-vitro* antioxidant activity	Aseervatham *et al*., 2013 [5]
Determination of total antioxidant capacity		*Basella alba* L.	Antioxidant capacity – Ascorbic Acid equivalents and Gallic acid equivalents	Dasgupta & De, 2007 [18]
Nitric oxide radical scavenging activity		*Citrus* sp. *Pouteriacampechiana*	*In-vitro* antioxidant activity	Aseervatham *et al*., 2013 [5]
Assay of Hydroxyl radical scavenging activity		*Basella alba* L. *Pouteriacampechiana* *Phyllanthusemblica*	*In-vitro* antioxidant activity	Dasgupta & De, 2007 [18], Aseervatham *et al*., 2013 [5] Liu *et al*., 2008 [44]
Ferrous ion chelating activity		*Citrus* sp.	*In-vitro* antioxidant activity	Ebrahimzadeh *et al*., 2008 [23]
Superoxide anion radical scavenging activity		*Citrus* sp.	*In-vitro* antioxidant activity	Nishimiki *et al*., 1972
		Basella alba L.	*In-vitro* antioxidant activity	Dasgupta & De, 2007 [18]
		Phyllanthusemblica		Liu *et al*., 2008 [44]
Reducing power assay		*Citrus* sp. *Pouteriacampechiana* *Phyllanthusemblica*	*In-vitro* antioxidant activity	Aseervatham *et al*., 2013 [5], Liu *et al*., 2008 [44]

Lipid Peroxidation inhibition assay		*Citrus* sp. *Basella alba* L.	*In-vitro* antioxidant activity	Halliwell and Guttridge, 1987 [31], Dasgupta & De, 2007 [18]
ORAC (Oxygen Radical Absorbance Capacity) assay	fluorescence spectrophotometry	*Annona squamosa*	*In-vitro* antioxidant activity	Mariod *et al*., 2012 [45]
Cupric Reducing Antioxidant Capacity (CUPRAC)	Spectrophotometric method	*Phyllanthusacidus*	*In-vitro* antioxidant activity	Habib *et al*., 2011 [30]
In vitro ACE-inhibitory activity		*Phaseolus lunatus*	ACE inhibition activity of peptides	Ciau-Solís *et al*., 2018 [15]
Renin – inhibitory activity		*Phaseolus lunatus*	Renin inhibition activity of peptides	Ciau-Solís *et al*., 2018 [15]
Bioassay for acetyl- and butyryl-cholinesterase inhibition activity		*Citrus medica*	Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition	Menichini *et al*., 2011 [47]
Assay of antiproliferative activity	Cell culture	*Aegle marmelos*	Anti- proliferation activity against human tumor cell lines	Lampronti *et al*., 2003 [43]
MTT assay – cellular viability		*Annona squamosa*	Potency of cell growth inhibition	Mariod *et al*., 2012 [45]
		Phyllanthusacidus		Padmapriya & Poonguzhali, 2015 [54]

5. Utilization of bioactive potential of food crops

Functional foods and Nutraceuticals are of utmost interest in modern world due to their favourable impact on reducing the risk of chronic diseases. Identification of bioactive compounds in dietary sources, separation, purification and development of functional food products which may act as supplements to body's antioxidant defense system are trending topics nowadays. Referring to functional foods, "A food can be regarded as 'functional' if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease (Vicentini *et al*., 2016) [70]. In many countries, the demand for functional food and beverages is rapidly growing due to increasing health consciousness and lifestyle choices, along with an aging population; these have been the main market drivers for functional foods (Vicentini *et al*., 2016) [70].

There are several benefits of developing functional food products from underutilized food crops. Developed products are concentrated with bioactive compounds in a healthy manner. Since the raw products vary in bioactive content a dose cannot be decided particularly. Even the anti-nutrients in raw products may prevent absorption of nutrients. High profit can be made through product development as it will broaden the marketing aspects of food industry. Fast food chains can acquire new marketing trends with functional food products since the craving for fast food among population of all ages can be catered with much healthier products. Nevertheless, the perishable/seasonal fruits, vegetables, legumes can be made available throughout the year without being wasted. The products on shelf will make life easier for people having busy life style. It can be a mean of reducing the burden of health cost of population making an economically safe country.

6. Testing for bioactive potential in food crops-human clinical trials

Even though there are many logical and theoretical advantages of food-based approach for disease prevention, studies of specific foods have been limited by the variation in bioactive content and incomplete chemical characterization (Gu *et al*., 2013) [28]. Therefore, fruits, vegetables, legumes and value-added food products have to be chemically characterized, highly desirable, easily incorporated into a diet and stable over time and storage conditions. The bioavailability, bioaccessibility and metabolic fate of bioactives in specific food crops and food formulations will be analyzed using various ex-vivo methods and the effectiveness against the targeted biomarkers will be tested using human clinical trials. They will be much helpful in providing sound evidence to prove the beneficial effects of food products.

7. Factors influencing the bioavailability and bioaccessibility of bioactive compounds

The availability of bioactive compounds in consumed food crops for metabolism after digestion and absorption through the gastrointestinal tract should be examined to discover the efficacy of fresh food crops or crop-based value-added products. Not all bioactive compounds are absorbed by the gastrointestinal tract or not all thus absorbed compounds will be delivering intended health benefits. Biotransformation of compounds in intestinal and hepatic cells during the principal metabolism and in the vascular cells, skin cells, and other cells during peripheral metabolism may result in different other products which can be either beneficial or non-beneficial to the body. Even the internal factors of food matrix such as anti-nutrients which prevent the absorption may hinder the process of exerting ideal health benefits. Such factors will limit the utilization of a particular food crop. Hence it is critical to identify different methods that can be used to remove the effect of anti-nutrients from food crops before developing functional food products.

8. Conclusions & Future trends

The bioactive potential of underutilized food crops is discussed and reviewed to evaluate the appropriateness of utilizing them in functional food product development and popularizing the neglected crops which are to cater the food security in the future. Among the marked health benefits like antioxidant, anti-inflammatory, anti-cancer, anti-tumorogenesis, anti-proliferative, anti-obesity, anti-hypertensive, anti-diabetic, anti-thrombotic, antipyretic, analgesic, antiulcer, there are many properties which are not evidentially proven to exist, even though they are effectively used in traditional medicine. Hence there is much extent to research the bioactive potential of underutilized food crops. Evaluation of health benefits using ex-vivo studies should target the assessment of the metabolic fate of bioactive compounds through cell metabolism since the biotransformation of bioactive compounds through principal and peripheral metabolism in cells may either promote or elicit the desired bioactivity.

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