A critical look at challenges and future scopes of bioactive compounds and their incorporations in the food, energy, and pharmaceutical sector

Sanidhya Pai · Akshatha Hebbar · Subbalaxmi Selvaraj

Received: 29 October 2021 / Accepted: 21 February 2022 / Published online: 2 March 2022
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Abstract
Bioactive compounds refer to secondary metabolites extracted from plants, fungi, microbes, or animals. Besides having pharmacological or toxicological effects on organisms leading to utilization in food and pharmaceutical industries, the discovery of novel properties of such compounds has led to the diversification of their applications, ranging from cosmetics and functionalized biomaterials to bioremediation and alternate fuels. Conventional time-consuming and solvent-intensive methods of extraction are increasingly being replaced by green solvents such as ionic liquids, supercritical fluids, and deep eutectic solvents, as well as non-conventional methods of extraction assisted by microwaves, pulse electric fields, enzymes, ultrasound, or pressure. These methods, along with advances in characterization and optimization strategies, have boosted the commercial viability of extraction especially from agrowastes and organic residues, promoting a sustainable circular economy. Further development of microfluidics, optimization models, nanoencapsulation, and metabolic engineering are expected to overcome certain limitations that restrict the growth of this field, in the context of improving screening, extraction, and economy of processes, as well as retaining biodiversity and enhancing the stability and functionality of such compounds. This review is a compilation of the various extraction and characterization methods employed for bioactive compounds and covers major applications in food, pharmacy, chemicals, energy, and bioremediation. Major limitations and scope of improvement are also discussed.

Keywords Bioactive compounds · Extraction · Industrial applications · Pharmaceutical · Characterization

Introduction
Bioactive compounds engender bioactive properties in the human body without adding any nutritional benefit and fall under secondary metabolites in plants. They express pharmacological or toxicological effects in humans and other animals (Câmara et al. 2020; Azmir et al. 2013). These compounds are procured from foods such as vegetables, fruits, and whole grains. Natural bioactive compounds are classified as polyphenols, triterpenes and phytosterols, terpenoids, polysaccharides, capsaicinoids, carotenoids and tocopherols, alkaloids, saponins glucosinolates, and others (Makkar et al. 2021). They are also extracted from fungi, animals, and bacteria (Câmara et al. 2020), as well as from agro-industrial residues such as avocado peel, mango seeds, and grape peels (Shirahigue and Antonini 2020).

They manifest antioxidant, anti-allergic, anti-inflammatory, antimicrobial, anticarcinogenic, and antimutagenic properties and are essential for the human body. Moreover, they are also vital in the pharmaceutical, food, and chemical industries (Câmara et al. 2020). Bioactive compounds account for numerous health benefits and help to prevent various diseases and metabolic abnormalities which were proved in several pharmacological studies (Chhikara et al.
In the food and fermentation industry, bioactive compounds such as essential oils, flavonoids, tannins, phenolic acids, carotenoids, organosulfur compounds, phytosterols, and tocopherols are used in the processing of vegetable oils, meat and seafood products, bakery products, dairy products, etc. (Shirahigue and Antonini 2020). Bioactive compounds are found to have anti-aging properties which are desirable for the cosmetic industry (Câmara et al. 2020).

Diverse types of extraction strategies have been employed over the years, considering properties such as the nature of the source matrix, relative solubility, structure, and chemical properties of both the bioactive compound and solvents used, as well as the effect of temperature, pressure, pH, etc. on the time, yield, and selectivity of extraction (Azmir et al. 2013). Conventional methods of extraction include maceration and decoction, which have been widely used in the extraction of essential oils and other bioactive compounds at a household scale (Muala et al. 2021), as well as the model laboratory-scale Soxhlet extraction method that uses slightly elevated temperatures to recirculate solvent within an apparatus and to aid the extraction of compounds from samples placed in a thimble (Raynie 2019).

However, these methods come with limitations, in terms of high extraction times and bulk solvent use, lower efficiencies when relative yields and specificity of extracted compounds are taken into consideration. For example, maceration requires 2 to 7 days for a satisfactory extraction, and the ratios of solvent to crude extract could vary from 4:1 to even around 50:1 in the case of decoction (hot water extraction) (Li et al. 2020). Often, there are requirements of pure and expensive solvents which are toxic. Elevated temperatures used in some methods are unsuitable for the extraction of thermally unstable compounds (Raynie 2019).

Various methods have been developed to overcome the above limitations, in addition to the development of several new types of solvents that are “green,” less toxic, cost-effective, and more specific (Pal and Jadeja 2020). Ionic liquids, deep eutectic solvents, and aqueous two-phase systems, for instance, have been incorporated to increase yield and speed of extraction, as well as aid in the extraction of compounds that were too difficult to extract with conventional solvents (Priyadarshi et al. 2020). The solvent–bioactive substance interactions could be compared and optimized using several parameters, such as the Hansen parameters and solvation parameter models (Lefebvre et al. 2021).

The basic principle of unconventional extraction methods is assisted extraction using ultrasound, pressurized liquids, microwaves, and pulsed electric fields, with the main aim of cell wall rupture or deterioration, thus enhancing mass transfer and facilitating effective mixing due to the exposure of cytoplasmic contents to the solvent (Lefebvre et al. 2021). Ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), supercritical fluid extraction (SCFE), and pulse electric field-assisted extraction (PEFAE) are a few unconventional methods following the above principles. Enzyme-assisted extraction (EAE) has also been considered as an option to extract substances associated with the cell wall, rather than the cytoplasm, by employing enzyme-driven cell wall digestion (Azmir et al. 2013).

Supercritical fluid extraction is one of the most popularly used extraction strategies, usually employing supercritical CO2 due to favorable thermodynamic properties and the renewable nature of the solvent (Gan et al. 2020). The success and efficiency of any method are also determined based on the choice of solvent; the effect of temperature and pH, which could potentially alter cell wall organization, specificity; chemical structure of the compound to be extracted; and feasibility of the process, among other effects. A suitable combination of parameters is arrived at by implementing optimization strategies specific to the process, as shown by Catarino et al. (2019).

Previous reviews have focused on individual aspects of the extraction of bioactive compounds. Sources, applications, and extraction strategies have been documented separately. We aim to provide a comprehensive review explaining bioactive compounds in the food, chemical, and pharmaceutical industry, their sources, extraction methods, and their limitations, characterization, applications, and future scope.

### Methods of extraction of bioactive compounds

The diversity of primary and secondary metabolites of plants and microbes in nature and their innumerable applications in various fields necessitates the use of a vast array of extraction methods, optimized according to their properties (Zhang et al. 2018). Extraction is the process of obtaining a compound of interest from a raw source. It is broadly classified into two types: conventional and non-conventional. Conventional methods include maceration, decoction, and Soxhlet extraction. Most industrial-scale extraction units rely on solvents (notably, hexane), most of which are products of the petrochemical industry. High energy consumption and bulk use of such solvents have adverse environmental impacts (Pal and Jadeja 2020). Non-conventional methods thus focus on using various physical or enzymatic means to enhance extraction (ultrasound, pressure, pulsed electric fields, microwaves, etc.) while utilizing lesser amounts of solvents or specialized “green” solvents (supercritical fluids, deep eutectic mixtures, etc.) (Anticona et al. 2020). Figure 1 represents the classification of extraction methods, along with specific examples under each category.
Conventional methods

Maceration

Maceration is a table-top extraction method commonly used for the extraction of medicinal plants. Some examples with sources and process conditions are mentioned in Table 1. It involves crushing the raw source coarsely and placing it in a container. The solvent is poured to cover the crushed source completely and is allowed to stand for 3 days with frequent agitation until the soluble matter is dissolved. The mixture is strained and decanted to complete the extraction process (Majekodunmi 2015). Eventually, the extract is separated using evaporation in a water bath. This method is apt for thermolabile plant extracts as it does not require elevated temperatures. However, this extraction process is time-consuming (Zhang et al. 2018).

Soxhlet extraction

Soxhlet extraction is a model extraction technique, used to extract compounds, traditionally, lipids, from solid or

| Principle                  | Sources                          | Compounds extracted                        | Process conditions                                      | References       |
|----------------------------|----------------------------------|--------------------------------------------|---------------------------------------------------------|------------------|
| Heating, infusion          | Chokeberry fruit                 | Phenols, Anthocyanins                     | 50% ethanol, 1:20, particle size of 0.75 mm              | Zhang et al. 2018 |
|                            | Dried root of *S. baicalensis*   | Baicaelein                                 | 70% ethanol                                              | Xie et al. 2019  |
|                            | *Georgi*                         |                                            |                                                        |                  |
|                            | *Salvia officinalis*             | Rosmarinic acid, Carnosol                 | Boiling water or 50% hydroethanolic solution, 5 days, room temperature | Vieira et al. 2020 |
|                            | *Piper betle* (betel) leaves     | Eugenol, Eugenol acetate                  | 100% acetone, 1:5, 72 h, room temperature               | Das et al. 2019  |
|                            | *Anthemis cotula* L. (stinking chamomile) | Anthecotuloid, Caffeoyl quinic acid and quercetin | 96% ethanol, 1:20, room temperature, overnight         | Sut et al. 2019  |
|                            | Blackcurrant leaves              | Polyphenols (TPC), Flavonoids (TFC), and Proanthocyanidin oligomers (OPC) | Water, 1:100, 500 rpm, 7 h, 30 °C                      | Cao-Ngoc et al. 2020 |
|                            | *Red algae Gracilaria gracilis*  | Allophycocyanins, Phycoerythrins, and Phycocyanins | M phosphate buffer, 10 min; 1:50                      | Pereira et al. 2020 |
|                            | *Papery skin of Allium cepa L.* var. ascalonicum* (fractions of Maja Cipanas onion) | Anthocyanin, Alkaloids, Polyphenols, Tannins, Flavonoids | 70% ethanol + HCl (2 N), 1:10 pH: 1, 24 h, 40 °C | Saptarini and Wardati 2020 |
semi-solid matrices (Talekar et al. 2018). A porous, usu-
ally disposable thimble made of cellulose (Raynie 2019) is
placed inside an extraction chamber, to hold the sample. The
solvent used for extraction is heated in a round-bottomed
flask, which is connected to the extraction chamber. Vapor
flows into a condenser, and the condensate is directed to the
extraction chamber, where extraction occurs. A siphon redi-
 rects the solvent along with the extracted compounds back
into the flask below. This process is repeated until extraction
is complete (Weggler et al. 2020). Table 2 mentions some of
the sources and compounds extracted by Soxhlet extraction
as well as the corresponding solvents and process conditions
involved. This method is used as a benchmark to compare
and develop newer methods. It is also relatively easy to auto-
mate, without requiring a lot of supervision (Raynie 2019).
However, exceptionally long extraction times (12 to 24 h),
high energy consumption, and problems in selectivity and
efficiency limit the scope of this technique (Weggler et al.
2020; Mussatto 2015; Wianowska and Wiśniewski 2015).

Table 2 Principle involved, along with various sources, compounds extracted, solvents used, and the corresponding process conditions used in Soxhlet extraction

| Principle | Sources | Compounds extracted | Process conditions | References |
|-----------|---------|---------------------|--------------------|------------|
| Heating, condensation, extraction, and reflux of S within a Soxhlet apparatus | Spent coffee (silverskin) | Caffeine | Hexane, dichloromethane, ethanol, 1:50, 6 h, the temperature was a solvent boiling point | Mussatto et al., 2015 |
| | | Chlorogenic acid | 60% isopropanol (60%), 1:10, 27 °C | |
| | Waste Punica granatum L. (pomegranate) seeds | Oils (PUFAs, punicic acid) | Hexane, 1:15, 4 h, 60 °C | Talekar et al., 2018 |
| | Rosmarinus officinalis L. (rosemary) leaves | Rosmarinic acid, Carnosic acid, Carnosol | 96% food grade ethanol, demineralized water, 1:12, 8 h | Hirondart et al. 2020 |
| | Piper betle (betel) leaves | Eugenol, Eugenol acetate | 100% acetone, 1:5, 56 °C, 8 h | Das et al., 2019 |
| | Agaricus bisporus L. | Ergosterol | Hexane, ethanol, or limonene (150 mL for 4.5 g of sample), 4 h, | Heleno et al., 2016 |
| | Anthemis cotula L. (stink- ing chamomile) | Anthecotuloid, Caffeoyl Quinic acid, and Querce- tin | 96% ethanol, 1:20, 6 h | Sut et al, 2019 |
| | Allium cepa L. var. ascalonicum | Alkaloids, Polyphenols, Tannins, Flavonoids | 70% ethanol + HCl (2 N),1:10, pH: 1, 2 h | Saptarini and Wardati, 2020 |
| | Silybum marianum L. Gaertner fruits | Silymarin | n-hexane (defatting), methanol (for Silymarin extraction), 2:75, 6 h (defatting)+5 h (actual extraction) | Wianowska and Wiśniewski, 2015 |
| | Miscanthus sinensis (runo) stem | Runo dye | 50% ethanol and 4 h | Pinzon et al., 2020 |

Decoction

A decoction is a method of extraction of heat-stable bio-
active compounds, obtained by boiling in water, which is
usually used as a solvent (Hmidani et al. 2019). It is widely
used in traditional medicine in the form of oral formulat-
ions containing extracts of certain medicinal herbs, due
to its capability of a rapid therapeutic action (Wang et al.
2019). Common sources include hard8 solids such as roots,
bark, and seeds, which are ground and heated with water in
a closed vessel. The extract is then cooled and filtered from
the insoluble residue (Perera et al. 2017). Table 3 gives a
general overview of the various compounds extracted by
decoction, along with corresponding sources and process
conditions. A decoction is characterized by noticeably short
extraction times of around 5 to 10 min. However, this might
be a disadvantage if the bioactive compounds that must be
extracted are not so soluble in water. In addition to this, there
is a large solvent to solid ratio involved (Zhang et al. 2018).
Advanced methods of extraction

Ultrasound-assisted extraction (UAE)

UAE is performed on both laboratory scale and industrial scale. It is carried out using ultrasonic waves to cause cavitation which leads to the implosion of bubbles in the medium. Cavitation induces collisions, macroturbulence, and disruption of the solid particles. It creates pores and enlarges them which in turn increase the mass transfer rate and the penetration of solvents into the biomass (Gonzalez et al. 2020). UAE can be set up in different configurations depending on the requirements of the extraction process. The transducer is directly dipped in the bulk. It makes the process effective but increases the chances of contamination (Esclapez et al. 2011). Consecutively, different methods of extraction are used to determine the quantity of the desired contents. UAE can be performed with a variety of solvents such as water, ethanol, methanol, acetone, and ethyl acetate, but it must be carried out at a lower temperature to maintain the integrity of thermosensitive compounds (Roohinejad et al. 2017). A few compounds that are extracted using UAE along with sources, solvents, and process conditions are listed in Table 4.

Microwave-assisted extraction (MAE)

This is a technique that assists extraction by irradiating microwaves (frequency range: 300 MHz to 300 GHz) onto the sample (Rehman et al. 2020). Energy associated with these microwaves is converted to thermal energy as the moisture present within the cells starts to evaporate due to ionic conduction as well as dipole rotation, two phenomena associated with microwave technology (Zghaibi et al. 2019). As a result, the cells experience a pressure build-up, eventually causing them to rupture, thus releasing the bioactive compounds (Pinzon et al. 2020). This technique has gained a lot of interest due to its ability to use extraordinarily little quantities of solvents and rapid extraction times, as well as greater reproducibility and control of process conditions such as temperature and pressure (Milani et al. 2020). The principles involved in this technique also facilitate a homogeneous temperature distribution, which also aids in higher yields and favorable heat and mass transfer from the sample to solvent (Pinzon et al. 2020). Table 5 provides an overview of the sources and various compounds extracted using this technique, along with the necessary parameters required for optimal yields.

Pressurized liquid extraction (PLE)

PLE involves the use of solvents at elevated temperatures, lower than their respective critical points to maintain them in the liquid state. This process exploits the mass transfer properties at elevated temperatures and pressures (Zakaria et al. 2020). The process involves moistening the sample with the solvent. The desired compound desorbs from the sample and gets absorbed in the extraction solvent.
The temperature being the key parameter of PLE is used to modify the physicochemical properties of the solvent (Anticona et al. 2020). There are two types of setups used for PLE: static and dynamic, as well as a combination of both. The dynamic system includes a continuous pumping of aliquots of the solvent, the rate being around 0.5–2.5 ml/min. In the static method, the extracted solvent is collected every 5–10 min (Vázquez-Roig and Picó 2015). PLE is used in the contamination analysis in complex matrices such as food. It can be used to identify tenacious organic pollutants (Ridgway 2012). Table 6 lists out a few compounds that are extracted using PLE as well as the sources, required solvents, and process conditions.

Enzyme-assisted extraction (EAE)

Enzyme-assisted extraction is useful when it comes to the extraction of phytochemicals associated with the cell wall. The presence of cellulose, hemicellulose, and lignin in higher concentrations makes it difficult to implement other popular extraction techniques (Nadar et al. 2018). EAE overcomes this problem by employing enzymes such as cellulase, pectinase, and alpha-amylase involved in the digestion of the cell wall (Azmir et al. 2013). This method has several advantages in terms of environmentally friendly methods and lower consumption of energy and equipment compared to other techniques, reduced usage of toxic solvents, and efficient extraction of thermally sensitive and volatile compounds used as fragrances, flavors, pigments, etc. (Nadar et al. 2018). This method has immense potential and several bioactive compounds of industrial and pharmaceutical importance have been successfully extracted (Table 7) However, enzymes are too expensive to be utilized for extraction of large volumes of substances. Designing efficient ways to scale up such processes is also a challenge (Franco et al. 2020).

Pulse electric field-assisted extraction (PEFAE)

This extraction technique works on the principle of electroporation. This occurs when cells are exposed to high-intensity electric field pulses that charge cell membranes, eventually creating pores due to increased repulsive forces between membrane constituents, usually after the transmembrane potential crosses a value of 1 V (Gorte et al. 2020). Static bench-scale equipment usually consists of a high voltage power generator, a digital oscilloscope (to monitor voltage, current, frequency, etc.) and a treatment chamber, where the sample is placed (Bozinou et al. 2019). This method is an alternative to other
Table 5 Various bioactive compounds extracted by microwave-assisted extraction, along with the principle, respective sources, and process parameters

| Principle                                                                 | Sources                        | Compounds extracted                  | Process conditions                                      |References         |
|---------------------------------------------------------------------------|-------------------------------|--------------------------------------|---------------------------------------------------------|-------------------|
| Microwave irradiation, intracellular moisture evaporation, pressure build-up, and rupture of cells | *Anthemis cotula L.* (stinking chamomile) | Anthecotuloid, Caffeoylquinic acid, and Quercetin | 96% ethanol, 1:20, 30 min, 600 W | Sut et al. 2019 |
|                                                                           | *Miscanthus sinensis* (runo) stem | Runo dye                             | 50% ethanol, 15 s, 540 W                                | Pinzon et al. 2020 |
|                                                                           | *Phyllostachys pubescens* (bamboo) | Polyphenols, Favoroids               | Methanol, 6.25 g/mL, 105 °C, and 4 min | Milani et al. 2020 |
|                                                                           | *Nannochloropsis sp.* (microalgae) | Lipids (PUFAs and omega-3 fatty acids) | 10% brine 1:20, 100 °C, and 30 min | Zghaibi et al. 2019 |
|                                                                           | Hemp nut                       | Cannabinoids (cannabidiol, cannabinol, tetrahydrocannabinol) | Methanol, 375 W, 109 °C, and 30 min | Chang et al. 2017 |
|                                                                           | *A. nodosum*                   | Fucose sulfated polysaccharides      | 1000 W, and 5 min                                      | Garcia-Vaquero et al. 2020 |
|                                                                           | *Fucus vesiculosus*            | Fucose sulfated polysaccharides      | Water, 120 psi, 1 min, and 1:25                       | Rodriguez-Jasso et al. 2011 |
|                                                                           | *Mangifera indica L.* (mango) peel | Mangiferin                           | Deep eutectic mixture of lactic acid, sodium acetate, and water (3:1:4), 436.45 W, 19.6 min, and 59.8 mL/g | Pal and Jadeja 2020 |
|                                                                           | Coriander                      | Heneicos-1-ene                       | Ionic solvents (BMIM-BF4) (0.1 M), 800 W, 90 °C, 2 min, 1:10 | Priyadarshi et al. 2020 |

Table 6 Principle, sources, compounds, and process conditions used in pressurized liquid extraction

| Principle                                                                 | Sources                        | Compounds extracted                  | Process conditions                                      |Reference         |
|---------------------------------------------------------------------------|-------------------------------|--------------------------------------|---------------------------------------------------------|-------------------|
| Extract targeted analytes from a sample matrix into a small amount of S using high Ts and pressures | *Rosmarinus officinalis L.* (rosemary) leaves | Rosmarinic acid, Carnosic acid, Carnosol | 183 °C, 130 bar, and 3 min                                | Hirondart et al. 2020 |
|                                                                           | *Silybum marianum L.* Gaertner fruits | Silymarin                            | Acetone, 125 °C, 10 min, and 60 bar                     | Wianowska and Wiśniewski 2015 |
|                                                                           | Feijoa leaf                    | Gallic acid, Catechin and Isoquerectin | Ethanol–water, 80 °C                                   | Santos et al. 2021 |
|                                                                           | Orange peel                    | Hesperidin, Naringin, Narirutin, tangeretin, naringenin, hesperidin | 75% ethanol, 65 °C, 40 min, and 10 MPa                  | Anticona et al. 2020 |
|                                                                           | underutilized chia seeds       | Omega 3-rich oils (ALA and Linoleic acid) | Ethanol, 60 °C, and 10 min                              | Villanueva-Bermajo, 2019 |
|                                                                           | *Moringa oleifera* leaves      | Phenolic compounds                   | 35% ethanol, 128 °C, 20 min                            | Rodríguez-Pérez et al. 2016 |
|                                                                           | *Neochloris oleoabundans*      | Carotenoids                          | Ethanol, 100 °C, 20 min, 1500 psi, 0.6 g algae + 2 g sea-sand | Castro-Puyana et al. 2017 |
|                                                                           | *Fucus vesiculosus*            | Gallic, Protocatechuic, and Gentisic acids | 58.65% ethanol, 137.18 °C, and 4.68 min,                | Sumampouw et al. 2021 |
|                                                                           | *Chlorella sp.* microalgae     | Phenolic compounds                   | Water, 100 °C and 250 °C, and 5 to 20 min              | Zakaria et al. 2020 |
| Principle                                      | Sources                                      | Compounds extracted                                | Process conditions                                                                 | References                  |
|------------------------------------------------|----------------------------------------------|----------------------------------------------------|-----------------------------------------------------------------------------------|-----------------------------|
| Cell-wall digesting enzymes                    | Capsaicinoids and Carotenoids                | Enzymes: R. nigricans enzymatic extract (cellulase, hemicellulase, pectinase), 30–36°C. Concentration: 113.039 µg/mL, 70 min for carotenoids and 45 min for capsaicinoids. | R. nigricans enzymatic extract (cellulase, hemicellulase, pectinase), 30–36°C. Concentration: 113.039 µg/mL, 70 min for carotenoids and 45 min for capsaicinoids. | Salgado-Roman et al. 2008  |
| C. annuum baydgi                               |                                              |                                                    |                                                                                   |                             |
| Chondrus crispus and Codium fragile           | Neutral sugars, Uronic acid, Proteins and Sulfates | Enzymes: Cellulase, beta-glucanase, Ultraflo, Neutrase (a protease) (0.5%), 50 °C (water bath), 3 h followed by enzyme denaturing (90 °C, 15 min) | Cellulase, beta-glucanase, Ultraflo, Neutrase (a protease) (0.5%), 50 °C (water bath), 3 h followed by enzyme denaturing (90 °C, 15 min). | Kulshreshtha et al. 2015    |
| Waste Punica granatum L. (pomegranate) seeds | Oils (PUFAs, punic acid), Proteins, Insoluble fibers (Cellulose, Hemicellulose, Lignin) | Enzymes: Protease, 45 °C, Concentration: 50 U/g, 14 h, pH 7.2 | Protease, 45 °C, Concentration: 50 U/g, 14 h, pH 7.2. | Talekar et al. 2018         |
| Fucus distichus, Saccharina latissima (brown macroalgae) | Fucoids | Enzymes: Cellic®CTec2 (commercial cellulase), alginate lyase (Sphingomonas), 40 °C, pH: 6 | Cellic®CTec2 (commercial cellulase), alginate lyase (Sphingomonas), 40 °C, pH: 6. | Nguyen et al. 2020          |
| Helianthus annuus L. (sunflower) wastes (petals, florets) | Carotenoids (lutein, zeaxanthin, Antheraxanthin, violaxanthin) | Enzyme: Viscozyme® (a multi-enzyme complex) + d,l-menthol/ d,l-lactic acid (2:1), 40 °C, 2 h | Viscozyme® (a multi-enzyme complex) + d,l-menthol/ d,l-lactic acid (2:1), 40 °C, 2 h. | Ricarte et al. 2020         |
| Scutellaria baicalensis Georgi                | Baicalin                                      | Enzyme: HG-5 enzyme from Bacillus spp.             | HG-5 enzyme from Bacillus spp.                                                     | Ma et al. 2021              |
| Essential oils                                 |                                              | Concentration: 2 wt% (both enzymes), 3 h           | Concentration: 2 wt% (both enzymes), 3 h.                                          | Shimotori et al. 2020       |
| Mentha arvensis L. (Japanese peppermint)      |                                              | Enzyme: Cellulase T + hemicellulase 90. Concentration: 2 wt% (both enzymes), 3 h | Cellulase T + hemicellulase 90. Concentration: 2 wt% (both enzymes), 3 h.          | Shimotori et al. 2020       |
| Haematococcus pluvialis                       | Astaxanthin                                   | Enzyme: Flavourzyme® + beta-glucanase, S: water, 50 °C. Concentration: 5% v/w, 1 h, pH: 7. | Flavourzyme® + beta-glucanase, S: water, 50 °C. Concentration: 5% v/w, 1 h, pH: 7. | Poojary et al. 2017         |
| Mushrooms—Lentinus edodes, Agrocybe aegerita, Pleurotus ostreatus, Agaricus bisporus | Umami (mainly Monosodium glutamate)          | Enzyme: Pectinase (7 U/g) in a 0.2 mol/L sodium acetate buffer, 50 °C. Concentration: 0.08% (w/w), 2.5 h, pH: 5. | Pectinase (7 U/g) in a 0.2 mol/L sodium acetate buffer, 50 °C. Concentration: 0.08% (w/w), 2.5 h, pH: 5. | Zhao et al. 2019            |

**Table 7** Principle and various sources, bioactive compounds extracted, corresponding enzymes utilized, and optimum process conditions involved in enzyme assisted extraction
Characterization of bioactive compounds

A plethora of bioactive compounds exist in multi-component states which make the isolation and separation and characterization process a crucial task. Characterization is a process that is performed to obtain a pure form of the target bioactive compound which helps in determining the amount, structure, and biological activity of the compound (Mahato et al. 2019). It plays a key role in the identification of potentially bioactive compounds with novel functionalities, including but not limited to drugs and antimicrobials. This is important in areas where these substances were traditionally used, but their exact chemical structure and properties were left undocumented (Ayalew 2020). Various chromatographic techniques have been developed to fractionate various kinds of compounds present in a single extract, such as ion exchange chromatography (IEC), thin layer chromatography (TLC), size exclusion chromatography (SEC), high-speed counter current chromatography (HSCCC), and high-performance thin layer chromatography (HPTLC). More advanced methods such as nuclear magnetic resonance (NMR), mass spectrometry (MS), Fourier transform infrared spectroscopy (FTIR), and so on are more selective and enable an analysis of bioactive compounds at molecular levels (García-Vaquero and Rajauria 2018). Table 10 describes various methods of characterization that are employed, in addition to the principles involved and compounds that have been identified by each method.

Applications of bioactive compounds

In this study, the applications of bioactive compounds in five major sectors such as food, pharmaceutical, bioremediation, energy, and chemical along with their major sources and important compounds extracted were discussed (Fig. 2).

Food sector

Bioactive compounds add a substantial value to the food industry. Food and nutrient supplements (Talekar et al. 2018), food coloring (Chhikara et al. 2019), meat and meat products (Pogorzelska-Nowicka et al. 2018), etc. all contain bioactive compounds necessary for the human body as mentioned before. They are added as a food enhancer as well, for example, carotenoids, curcumin, and anthocyanins are used as coloring agents; ascorbic acid is used widely as an additive to prevent oxidation in foods; vanillin and cinnamaldehyde are used as flavoring agents. Fermentation is one of the main areas under the food industry that produces a lot of bioactive compounds (Sadh et al. 2018).
| Principle | Sources | Compounds extracted | Process conditions | Reference |
|-----------|---------|---------------------|--------------------|-----------|
| Electroporation | *Arthrospira platensis* | Water-soluble proteins (WSP), C-phycocyanin | Water (aqueous microalgae suspensions 2% w/w, monopolar pulses, 20 kV/cm, and 100 kJ/kg suspension at room temperature) | Carullo et al. 2020 |
| | *Saitozyma podzolica* (yeast) | Lipids | Ethanol-hexane-water (18:7.3:1), Cell concentration: 20 g/L, electric field: 40 kV/cm, energy: 150 kJ/L suspensions, pulse duration: 1 µsec | Gorte et al. 2020 |
| | *Moringa oleifera* dry leaves | Phenol and Antioxidants | 20 mL of double distilled water per gram of ground leaves, 40 min, pulse duration: 20 ms, pulse interval: 100 µsec; field strength: 7 kV/cm | Bozinou et al. 2019 |
| | *Acanthophyllum squarrosum* roots | Saponins | Electric field: 6.4 kV/cm; pulse number: 80 | Shahi et al. 2021 |
| | *Oryza sativa* (brown rice) | Gamma oryzanol, Tocopherols, several polyphenols, and fatty acids | Acetone (40%), electric field: 2 kV/cm, S concentration: 5 mL/g, pulse duration: 100 µsec, frequency: 5 Hz | Quagliariello et al. 2016 |
| | *Annona squamosa* (custard apple) leaves | Purpureacin 2, Rutin | Ethanol (70%), electric field: 6 kV/cm, pulse number: 300, energy: 142 kJ/kg, 5 min | Shiekh et al. 2021 |
| | Sea bream and sea bass residues (gills, head, bones) | PUFAs (docosahexaenoic acid, omega-3, etc.), minerals (Ca, P, etc.), Amino acids (arginine, Leucine, Llysine) | Distilled water, 1 mL/mg solids, pulse width: 20 µsec, frequency: 10 Hz, pulse number: 100, electric field: 7 kV | Franco et al. 2020 |
| | *T. chuii* and *P. tricornutum* | Carotenoids, Chlorophyll A, Chlorophyll B | Energy: 100 kJ/kg, pulse duration: 100 ms, pulse frequency: 2 Hz | Kokkali et al. 2020 |
| | *Tetraselmis chuii* | Carotenoids | 24-h extraction using DMSO, subsequent PEFAE-electric field: 1 kV/cm, pulse number: 400 | |
| | | Chlorophyll B | Water, electric field: 3 kV/cm, pulse number: 45; extraction time: 24 h | |
| | *Phaeodactylum tricornutum* | Carotenoids, Chlorophyll A | DMSO (50%), electric field: 3 kV/cm, pulse number: 45; extraction time: 4 h | |
such as single-cell proteins used as an alternative source for protein (Ritala et al. 2017); lactic acid used for acidulation and preservation (Miller et al. 2011); xanthan used as an emulsifier, thickener, and stabilizer (Habibi and Khosravi-Darani 2017); laccase used for baking and in the beverage industry as a stabilizer (Mayolo-Deloisa et al. 2020); astaxanthin used as a coloring agent (Gwaltney-Brant 2021); citric acid used as a food preservative and flavoring agent (Kazmi and Clark 2012); fumaric acid used as an acidulant (Karaffa and Kubicek 2021); and others. The main sources of such bioactive compounds are fruits, wastes produced in the wine industry, plant, fruit, and vegetable waste like peels, seeds, and pomace (Shirahigue and Antonini 2020). Common fruits such as apple, mango, plum, banana, and citrus fruits contain phenolic acids, flavanols, carotenoids, anthocyanins, and lipids. Common vegetables like potato, carrot, beetroot, and broccoli contain carbohydrates, phenolic acids, carotenoids, and flavonoids. A few other sources of carotenoids are Neochloris oleoabundans, C. annuum bay-dgi, Helianthus annuus L. (sunflower) wastes (petals, florets), T. chuii, and P. tricornutum. Chokeberry fruit, papery skin of Allium cepa L. var. Ascalonicum, strawberry, raspberry, blueberry, blackberry, and Tannat grape pomace are a few sources of anthocyanins. Phenols and polyphenols are extracted from Thymus atlanticus (Moroccan thyme), bitter gourd, Tannat grape pomace, Phyllostachys pubescens (bamboo), and Oryza sativa (brown rice) to name a few sources. The sources mentioned above are listed in the extraction tables (Tables 1–9).

**Pharmaceutical and therapeutic sector**

The development of completely novel compounds for therapeutics is a daunting and time-consuming task (Sinha and Härder 2021). This has helped steer major advances in the utilization of a vast diversity of bioactive compounds already found in nature, as well as disciplines like ethnopharmacology, involved in the systematic research and exploration of sources that have traditionally been used as medicine (Suntar 2020), including higher plants (Silva et al. 2019), microalgae, seaweeds (Rodriguez-Jasso et al. 2011), microorganisms (Ramkissoon et al. 2020), fungi (Heleno et al. 2016), and marine organisms (Franco et al. 2020). Plants such as Aloe vera, consisting of pseudoprototinosaponin-AIII and prototinosaponin-AIII (Shrinet et al. 2021), alkaloids, triterpenes, thiocyanates, cardiac...
glycosides, and cyanogenic glycosides, among others, extracted from *Terminalia catappa* (Behl and Kotwani 2017) as well as Steviol glycosides in *Stevia rebaudiana* leaves (Zlabur et al. 2015), have antidiabetic properties. Baicalein, a flavone obtained from the dried roots of *S. baicalensis Georgi*, is known for its anti-cancer and anti-inflammatory activities and has been used to treat several gastrointestinal ailments such as gastric ulceration, liver fibrosis, and so on (Xie et al. 2019). Comparable properties have been observed in silymarin (treatment of liver disorders as well as antitumor activity), extracted from *Silybum marianum L. Gaertner* (Wianowska and Wiśniewski 2015). The extracts of *Anthemis cotula L.* (stinking chamomile) were found to have potential in the treatment of Alzheimer’s disease and skin hyperpigmentation conditions (Sut et al. 2019). Nutraceuticals such as quercetin and kaempferol have also been employed for managing similar neurodegenerative disorders (Makkar et al. 2020).

Other extremely important sources of bioactive compounds include algae as well as marine organisms. Fucose-sulfated polysaccharides, extracted from brown algae such as *A. nodosum* and *Fucus vesiculosus*, have been proven to be beneficial antioxidants and anticoagulants (Garcia-Vaquero et al. 2020), in addition to being anti-inflammatory and antiviral (Rodriguez-Jasso et al. 2011). *Fucus vesiculosus* is also rich in phlorotannins, used in the treatment of goiter, obesity, rheumatoid arthritis, asthma, etc. (Catarino et al. 2019). The extracts of *Chondrus crispus* and *Codium fragile* have successfully exhibited activity against the *Herpes simplex* virus (HSV) (Kulshreshtha et al. 2015).

| Principle | Sources | Compounds extracted | Process conditions | References |
|-----------|---------|---------------------|--------------------|------------|
| Separating extract from the matrix using supercritical fluids like scCO₂ | *Mentha spicata* | Flavonoids (luteolin) | scCO₂, pressure: 200 bar, 60 °C, and 60 min | Puri et al. 2012 |
|          | *Feijoa leaf* | Gallic acid, catechin, and isoorquercetin | scCO₂, 15% ethanol–water (as cosolvent), 210 min, pressure: 30 MPa, and 55 °C | Santos et al. 2021 |
| Underutilized Chia seeds | Omega 3-rich oils (ALA and linoleic acid) | scCO₂, pressure: 45 MPa, 60 °C, 240 min, and 40 g/min | Villanueva-Bermejo 2019 |
| Leaves of *piper amalago* | Pyrrolidine | scCO₂, Co-solvents: ethanol, methanol, and propylene glycol 5% (v/v), pressure: 150, 200, and 250 bar, 40, 50, and 60 °C, 20, 40, and 60 min, CO₂ flow rate: 3 mL/min, and particle size: 0.757 mm | Uwineza and Waskiewicz 2020 |
| *Catharanthus roseus* | Vinblastine and vincristine | scCO₂, Co-S: ethanol 2, 5 and 10% (v/v), pressure: 300 bar T: 40, 50, and 60 °C | |
| *Artemisia annua L* | Artemisinin | scCO₂, pressure: 100 bar, 40 °C, CO₂ flow rate: 13.3–20 g/min | Lefebvre et al. 2021 |
| Dried ivy leaves | Chlorophyll | scCO₂, Co-S: ethanol (80/20 v/v), 25 °C, pressure: 15 MPa, and 30 min | |
| *Haematococcus pluvialis* | Astaxanthin | scCO₂, 50 °C, pressure: 50 MPa, 175 min, and flow rate: 2 L/min | Álvarez et al. 2020 |
| *Rana chensinensis ovum* | *Rana chensinensis ovum* oil (eicosapentaenoic acid, α-linolenic acid, docosaheaxaenoic acid, arachidonic acid, linoleic acid, and oleic acid) | scCO₂, pressure: 29 MPa, flow: 82 L/h, 50 °C, and 132 min | Gan et al. 2020 |
| Name                                           | Basic principle                                                                 | Sources                                    | Compounds identified/separated                                                                 | Reference                     |
|------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------------------------------------------------|--------------------------------|
| Thin layer chromatography (TLC)                | The compound having polarity like that of the solvent will get adsorbed faster than other compounds | Citrus fruits                              | Tangeretin, 5′-demethyltangeretin, nobiletin, 3′-demethylnobiletin, 4′-demethylnobiletin, 3′-demethylnobiletin, 5-demethylnobiletin, 5,3′-demethylnobiletin, 5,4′-demethylnobiletin, 5,3′,4′-demethylnobiletin, naringenin, hesperetin | Santiago 2013                  |
|                                                |                                                                                 | Streptomyces misionensis V16R3Y1 Bacteria extracts | Hexahydro-3-(2-methylpropyl) pyrrolo [1,2-a] pyrazine-1, 4-dione followed by N-valeryl-l-proline decyl ester, benzene, acetamide, 2-(ethylhexyl)-hexylsulfate, 5-isopropylidene-3,3-dimethyl-dihydrofuran-2-one | Saadouli et al. 2020           |
| Ion exchange chromatography (IEC)              | Separation of ionized molecules based on their charge. Exchangers having positive/negative charged species retain unlike charges in a column, allowing like charges to pass through | Brown algae                                | Fucoidans (fucose, uronic acids, galacturonic acid, glucuronic acid, sulfates)                  | Masoodi et al. 2021            |
|                                                |                                                                                 | Crotalus durissus terrificus venom Walterinema aegyptia (Egyptian black snake) | Bordonein L. (L. amino acid oxidase) Walterospermin | El-Aziz et al. 2020a, b         |
| Size exclusion chromatography (SEC)            | Molecules in the extract are separated according to their sizes (molecular weights) | Bothrops atrox venom Hazelnut and walnut shells Euglena cantabrica | Batroxase Triglycerides, fatty acids, steryl esters, monosaccharides, phenols Paramylon, glycans | Mahato et al., 2019            |
|                                                |                                                                                 |                                            | El-Aziz et al. 2020a, b                         | Herrera et al., 2020           |
|                                                |                                                                                 |                                            |                                                   | Muñoz-Almagro et al., 2020      |
| Name                                                                 | Basic principle                                                                 | Sources                                                                 | Compounds identified/separated                                                                 | Reference                  |
|----------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|----------------------------|
| High-speed counter-current chromatography (HSCCC)                     | Fully liquid phase chromatographic technique. The separation is achieved without using any solid phase. The stationary liquid phase is retained on the column by gravitational or centrifugal forces alone | *Polygonum multiflorum* roots                                            | Gallic acid, Catechin, Epicatechin, Polydatin, Piceatannol, Rutin, Resveratrol, Isoforrapontigenin, Hyperoside, Rhein, Emodin, 2,3,5,4′-Tetrahydroxy stilbene-2-O-β-D-glucoside | Garcia-Vaquero and Rajauria 2018 |
|                                                                     |                                                                                 | *Lycium barbarum* fruits                                               | Zeaxanthin, Zeaxanthin monopalmitate, Zeaxanthin dipalmitate                                         | Gong et al. 2020           |
|                                                                     |                                                                                 | *Dipsacus asper* roots                                                 | Iridoid glycosides, Triterpenoid saponins                                                            | Yu et al. 2020             |
| High-performance thin layer chromatography (HPTLC)                   | The principle is like TLC but with different particle size distribution and thickness of sorbent layers | *Abelmoschus moschatus*                                               | Behenic acid, Arachidic acid, Stearic acid, Oleic acid, Linoleic acid, Palmitic acid                 | Maimaiti et al. 2020       |
| High-performance thin-layer chromatography-heated electrospray ionization-high-resolution mass spectra (HPTLC-HESI-HRMS) |                                                                                 | *Vernonia anthelmintica*                                              | 3,4-O-dicaffeoyl Quinicacid, 3,5-O-dicaffeoyl quinic acid, 4,5-O-dicaffeoyl quinic acid              | Maimaiti et al. 2020       |
|                                                                     |                                                                                 | *Musa acuminata* peel                                                | Quercetin, Catechin, Rutin                                                                          | Vijay et al. 2019          |
| Nuclear magnetic resonance (NMR)                                     | An atom placed under a strong magnetic field will respond with its nuclear spin at a certain frequency | *Pseudomonas aeruginosa UWI-1*                                        | Tris(1H-indol-3-yl) methylium, bis(indol-3-yl) phenylmethane, indolo (2, 1b) quinazoline-6, 12 dione | Ramkissoon et al. 2020     |
| Name                          | Basic principle                                                                 | Sources                                                                 | Compounds identified/separated                                                                 | Reference                      |
|-------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|--------------------------------|
| *Solidago gigantea* Ait. root extract |                                                                                   | Kingidiol, Epoxy-hemiacetal, Clerodane lactone (hautriwiac lactone), Solidagoic acid A, Solidagoic acid B |                                                                                                 | Móricz et al. 2021             |
| *Ardisia elliptica* |                                                                                   | Quercetin, Kaempferol, Myricetin derivatives, α-amyrin, β-amyrin, squalene oxide, Ardisianoside, Friedelane derivatives, Ardisenone, Ardisphenol B, Ardisinol II, Ardisianone derivatives, Embelin, Gallic acid |                                                                                                 | Wong et al. 2021               |
| Fourier transform infrared spectroscopy (FTIR) | Generates a sample-specific FTIR spectrum representing the composition of various molecules depending on the extent of infrared radiation absorbed |                                                                                                      |                                                                                                 |                                |
| *Lantana camara* leaf oil |                                                                                   | Alcohols, Carboxylic acids, Alkanes, Ketones, Primary amines, Phenols |                                                                                                 | Ayalew 2020                    |
| *Glycosmis pentaphylla* |                                                                                   | Carbonyl, Amide, Imines, Phenyl ether, Furan groups |                                                                                                 | Murugan et al. 2020            |
| *Microalgae (S. platensis)* |                                                                                   | Methylene (carotenoids), Carbonyl (phytosterols), Flavone phenyl ring, Ketones (flavonoids), Aromatic groups, Phenyl ether linkages |                                                                                                 | Lopez-Hernandez et al. 2020    |
| Mass spectrometry (MS) | Measuring charge to mass ratio of ionized molecules                              |                                                                                                      |                                                                                                 |                                |
| Along with UHPLC-Q-TOF–MS (the ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry) |                                                                                                      |                                                                                                      | Alsenani et al. 2020          |
| *I. galbana* |                                                                                   | Pheophytin A, Trilinolenic glyceride                                                                 |                                                                                                 |                                |
| *Scenedesmus* sp. NT8c |                                                                                   | A-Linolenol, Stearic acid, Hexadecanoic acid                                                                 |                                                                                                 |                                |
| *Chlorella* sp. FN1 |                                                                                   | Pheophytin A, Ester                                                                                   |                                                                                                 |                                |
Research on bacterial and fungal bioactive compounds has explored antimicrobial and anti-cancer properties (Sinha and Hader 2021). Fungi are sources of the very first antibiotics, such as penicillins, carbapenems, and cephalosporins. Compactin and lovastatin have been very instrumental as cholesterol-lowering agents (Hoeksma et al. 2019). Bioactives obtained from fungal sources were found to exhibit antibacterial, antiviral, anti-cancer properties in addition to being immunostimulants (Poojary et al. 2017). Several indole alkaloid compounds have been extracted from bacteria such as *Pseudomonas aeruginosa* UWI-1 having antibiotic potential (Ramkissoon et al. 2020). Sclerotiderm A, a compound isolated from *Scleritoderm A nodosum*, was found to be effective in the treatment of human colon, breast, and ovarian tumors (Sinha and Hader 2021).

Various bioactive compounds of pharmaceutical importance have been extracted from animal sources as well. Franco et al. (2020) suggested using residues of sea bream and sea bass (gills, head, bones) to extract high-value antioxidants. *Rana chensinensis* ovum oil was found to have beneficial unsaturated fatty acids, instrumental in the prevention of cardiovascular as well as cerebrovascular diseases (Gan et al. 2020). Advances in venomics have helped in the extraction and isolation of animal (snake) venom, useful in the drug discovery and development of antivenom (El-Aziz et al. 2020a, b). A range of sources along with relevant compounds, many of which are of pharmaceutical interest, have already been listed in Tables 1–10.

**Bioremediation sector**

Bioactive compounds have found a range of applications in bioremediation sectors as well, in the form of coagulants (Ibrahim et al. 2021), biofilms (Mugge et al. 2021), bioactive extracts (Zerrifi et al. 2018), and so on. A wide range of bioactive compounds obtained from various sources has shown promising activity against harmful algal blooms (HAB), including α-linolenic, oleic, and palmitic acids from *Botryococcus braunii*, diethyl phthalate from *Stoechospermum marginatum*, and so on (Zerrifi et al. 2018, 2021). Similarly, bioaccumulation of nutrients, as well as metals (Cd, Cu, Zn, Pb, Cr) by *Sargassum*, has triple benefits in terms of reducing eutrophication and coastal metal pollution in addition to sequestering metals and useful bioactive compounds which could then be used in pharmaceutical, cosmetic, food, and fertilizer industries (Saldarriaga-Hernandez et al. 2020). d’Errico et al. (2020) reported the capability of a fungal endophyte, *Drechslera* (strain 678) to have dual functions as a biopesticide, due to the presence of compounds such as monocerin, as well as for bioremediation of methyl tert-butyl ether, a soil contaminant, usually used as a gasoline additive.

Tannins have also been widely used in wastewater treatment plants. Tannin-based coagulants have been employed
to remove turbidity and flocculate suspended solids (Ibrahim et al. 2021). Condensed tannins sourced from *Acacia mearnsii* and tannic acid have been used to remove both cationic and anionic dyes from water (Grenda et al. 2020). Das et al. (2020) have also reviewed the use of tannin cryogels and wool tannins in the removal of heavy metals and methylene blue, respectively, from contaminated water.

Biofilms formed by marine and intertidal bacteria have a lot of potential in bioremediation. Mugge et al. (2021) studied the changes in bacterial populations and biofilm compositions in surface and deep-sea water when exposed to crude oil or chemical dispersants, which is promising in the management and clean-up of oil spills. Lipopeptides obtained from *Bacillus subtilis* CN2 showed interesting properties about the degradation of polymeric aromatic hydrocarbons and recovery of motor oil from contaminated soil (Bezza and Chirwa 2015). Hence, bioactive compounds are promising when it comes to areas like wastewater treatment and hydrocarbon degradation.

**Energy sector**

With the upsurge in human population, renewable energy like biofuels and other sustainable practices has become essential. Five billion tonnes of biomass waste are produced in the food and agroforestry industry. It has ample potential in the production of bioactive compounds which can be utilized as biofuels to reduce biomass waste. Ethanol and vegetable oil are widely produced as biofuels in biorefineries (Ferreira-Santos et al. 2020). Gorte et al. (2020) demonstrated pulse electric field treatment to extract lipids from fresh oleaginous yeast cells. Single-cell oils or microbial oils that are extracted from yeasts, fungi, microalgae, and bacteria can be utilized as alternative fuels, although the extraction process is expensive and not efficient. These remain as the major drawbacks still left to tackle. *C. camphora* is a potential renewable energy source, and the biomass production along with the volatile constituents (camphor, eucalyptol, limonene, β-pinene) has been studied (Zhang et al., 2020a, b). Additionally, sugar-based waste (sugar cane, sugar beets), animal waste (cow, swine, poultry), food industry waste, starch-based wastes (corn), lignocellulosic waste (switchgrass, micanthus, corn stover, corn fiber), and glycerine serve as sources of bioenergy and biofuels such as ethanol, methanol, and butanol (Swain 2017). Microbial fuel cells (MFCs) use microbes to generate electricity. They have shown enhanced power density with electron shunting capabilities of a few secondary metabolites such as epigallocatechin-3-gallate, gallic acid, gallocatechin, and anthocyanin. The addition of fungal and algal metabolites in the MFCs improves electricity production (Nath and Ghangrekar 2020). Condensation of β-pinene is processed to form dimers which is an excellent option for a renewable and high energy–density jet fuel and can also be used as diesel (Jung et al. 2016). Effects of a dual biofuel blend consisting of different concentrations of jatropha biodiesel and turpentine oil were studied in a single-cylinder diesel engine and are a cost-effective alternative for fossil fuels. There was a reduction in carbon monoxide, hydrocarbon, and nitrous oxide emissions by 13.04%, 17.5%, and 4.21%, respectively, but an increase in CO₂ emissions by 11.04% (Dubey and Gupta 2018).

**Chemical sector**

Bioactive compounds have applications in various chemical industries, including but not limited to polymers and biomaterials (Nogueira et al. 2020), dyes and textiles (Agnhage et al. 2017), leather processing (Das et al. 2020), perfumes, and cosmetics (Sharmeen et al. 2021). Certain bioactive compounds (oils, fatty acids) have traditionally been used (in the oil and soap industries) (Ng et al. 2021), whereas the toxicity and unsustainability of conventional chemicals, processes, or end-products have recently accelerated the usage of bio-based substitutes in novel materials, catalysts, and certain raw materials in the industry (Chin et al. 2021; Basak et al. 2021).

Spiridon et al. (2020) developed a biomaterial with cellulose, collagen, and polyurethane as its constituents, to facilitate the controlled release of antioxidants such as tannin and lipoic acid, with a potential for biomedical and cosmetic applications. Similar approaches of encapsulation of antioxidants were carried out using Aloe vera agrowastes, by incorporating them into electro spun nanofibers made from polyethylene oxide (Solaberrieta et al. 2020). These technologies have also enabled the development of biodegradable and even edible food packaging films with enhanced antimicrobial, antioxidant, and mechanical properties, achieved by a combination of biopolymers (starch, chitosan, gluten) and bioactive (essential oils, polyphenols, carotenoids) (Nogueira et al. 2020). Other interesting properties, such as flame retardancy, antibacterial, and UV light protection in textiles have been achieved by using tannin-based macromolecules (Basak et al. 2021). Dyes, such as the run dye from the core stem tissues of *Miscanthus sinensis* Andersson (Pinzon et al. 2020) and naturally sourced anthraquinone dyes from the roots of *Rubia tinctorum* L. (Agnhage et al. 2017), have also been produced.

Various essential oils such as lavender, carvone, linalool, limonene, citronellol, and eucalyptus are popular choices in the perfume and cosmetic industry. In addition to fragrance, they also act as preservatives and active ingredients and have beneficial effects on the skin (Sharmeen et al. 2021). Phyto-tannins, polysaccharides (laminarin, carrageenan, etc.), astaxanthin, and several bioactive peptides present in seaweed and microalgae have been reported as excellent sources for treatment.
cosmeceuticals due to their anti-aging, anti-acne, antimicrobial, skin glow enhancement, moisture retention, UV protection, and anti-allergic properties (Jesumani et al. 2019).

The industrial extraction of oils (palm, coconut, and castor oils) is another important sector of the chemical industry, due to a wide range of applications, ranging from edible oils and hair care products (Ng et al. 2021) to the manufacture of soaps and grease (Patel et al. 2016). Moreover, several petrochemical industries in Malaysia are looking into the possibility of using palm oil as well as glycerol sourced from vegetable oil as feedstocks in the manufacture of lubricants (Chin et al. 2021), shifting resources from fossil fuels to bioactive compounds. Similarly, castor oils are used in the manufacture of biodegradable polyesters as well as lubricants and paints (Patel et al. 2016).

Tannins, on the other hand, are widely abundant and industrially important bioactive compounds that have been used in the leather processing and wood (adhesive and preservation) industry for centuries (Das et al. 2020). In addition to this, several novels and sustainable alternatives to 3D printing (Liao et al. 2020) using tannins have been explored. Bioactive compounds thus have a high potential for creating a sustainable chemical industry if measures are taken for efficient extraction and conservation of biodiversity.

**Limitations**

Conventional extraction processes are helpful; however, they are inefficient and time-consuming. To overcome the said limitations, non-conventional extraction methods have been designed. However, they have certain demerits as well. Susceptibility of thermosensitive compounds, non-uniformity of extraction in large-scale industries, and high maintenance costs and CO₂ consumption leading to high-value compounds are the challenges faced in UAE, MAE, and SFE, respectively. For example, in the case of SFE, astaxanthin and phycobiliproteins in microalgae are high-value compounds due to the expensive extraction and purification processes (Roohinejad et al. 2017). Various other limitations specific to each extraction method have already been discussed.

A major impediment to the widespread use of bioactive compounds is the varying stability and loss of activity, especially in foods, as most experiments verifying beneficial properties are done under controlled conditions. Variation among individuals is also a crucial factor that must be considered while studying the nutritional and therapeutic benefits of bioactive compounds. Differences in processes such as absorption and metabolism, as well as diversity in age, gender, and lifestyles, could result in varied effects of such compounds in a population. From the ecological perspective, meeting the growing demands for bioactive compounds is bound to exert a lot of pressure on biodiversity, land, and marine resources, which could threaten the survival of exceedingly rare species.

Challenges that are frequently overlooked about bio-analytical methods of characterization are interference to a considerable extent, clean-up during sample preparation, low sensitivity, accuracy, and unreliable methods to name a few. Other aspects, such as bioavailability, bio accessibility, safe and “green” production practices, safety, and toxicology, must be considered as well, especially when downstream processes account for 50–80% of the production value (Cuellar-Bermudez et al. 2015).

**Future scope**

The advent of non-communicable diseases such as cancer, obesity, diabetes, and so on over the last few decades, the instances of antibiotic resistance in pathogenic microorganisms, the increased awareness for sustainable products, bioremediation efforts, and the recent pandemic have led to a spike in demand for healthier, natural, immune-boosting and bio-fortified foods, novel antibiotics and pharmaceuticals, bio-based raw materials for various processes, and biomaterials with novel functionalities. A report by Grand View Research, Inc. (2016) states that the global market size for bioactive ingredients is expected to cross USD 51.71 billion by 2024, with functional foods and beverages contributing to 25% of the market, sourced from plants and marine organisms (https://www.grandviewresearch.com/press-release/global-bioactive-ingredients-market). This surge in demand necessitates further research into sustainable and effective methods of screening, extraction, characterization, processing, and commercialization of good-quality bioactive compounds. Fu et al. (2019) have suggested a shift to multi-targeted approaches to screening multiple bioactive compounds simultaneously with the help of biosensor and microfluidic chip-based technologies, as opposed to conventional chromatographic methods. The mechanism of action of bioactive compounds in certain cases is best understood during in vivo screening methods; hence, the scope of discovery of these compounds is limited when screening is confined to in vitro assays (Ahamefule et al. 2020). Designing effective in vivo assays, especially for antibiotic activity, could help in screening such compounds utilizing novel mechanisms. Ways to improve selectivity and yield of extraction, such as modeling solvent-compound interactions and affinities, as well as optimization of physical parameters in case of non-conventional methods must be explored further to decrease costs and facilitate scalability.

Metabolic engineering is a potential tool to facilitate microbial production of bioactive compounds such as terpenoids, omega-3 PUFAs, and so on, to tackle the
exploitation of rare and limited marine resources for commercial production. Other approaches of finding optimum sources from by-products of food processing industries and agricultural residues can help in implementing sustainable methods of waste recycling and high-value product recovery, boosting a circular economy.

Nanoencapsulation strategies have a good potential when it comes to retaining bioavailability, enhancing stability, and facilitating the controlled release of bioactive compounds while being delivered into functional food items. Encapsulation within naturally assembling structures and biopolymer films in food packaging are a few approaches. The challenge lies in making them economically viable alternatives to conventional solutions.

**Conclusion**

This article has reviewed bioactive compounds, their extraction methods, characterization methods, applications, limitations, and future scope, all together. The popular conventional and non-conventional extraction methods to extract bioactive compounds along with tables containing the basic principles, sources, latest compounds extracted, solvent or enzymes used, and process conditions were reviewed and listed. Bio-analytical characterization methods were elucidated with the help of a table. The applications of bioactive compounds in the food, pharmaceutical, bioremediation, energy, and chemical sectors were documented diffusely. Moreover, the limitations and challenges faced in the extraction and characterization processes were compiled. The prospects of the bioactive compounds were put together considering the ongoing pandemic situation although it needs further investigation. This gives an insight into the value of bioactive compounds from the perspective of human health and the sustainability of global resources. As the technology ameliorates, the potential of bioactive compounds in various sectors is bound to escalate, thus unlocking new possibilities.

**Acknowledgements** The authors thank the authorities of Manipal Institute of Technology, Manipal, Karnataka, India, for their support in carrying out this work.

**Author contribution** SP and AH contributed to the literature collection, analyzed data, and wrote the original draft. SS designed and supervised the study and was also involved in drafting the manuscript. All authors read and approved the final manuscript.

**Funding** Open access funding provided by Manipal Academy of Higher Education, Manipal.

**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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