

CURRENT TRENDS IN THE EXTRACTION OF PLANT BIOACTIVE COMPOUNDS BY ULTRASONICATION METHOD: A BRIEF REVIEW

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Received: 7 October 2022, Accepted: 27 October 2022, Published: 31 October 2022, Publisher: UTP Press, Creative Commons: CC BY 4.0

ABSTRACT

Bioactive compounds are chemical substances that have biological effects favourable to human health. Many foods contain bioactive compounds, such as fruits, vegetables, grains, and nuts, but animals only have extremely small levels of these substances. Many industries use bioactive compounds extensively, including poultry, agriculture, cosmetics, biofuel, and medical research. Techniques for extraction are necessary to get potential bioactive compounds. The ultrasound-assisted extraction (UAE) is identified as one of the most efficient methods, given its benefits for the extraction of bioactive substances. This review paper focused on the fundamental concepts behind the ultrasonication process as well as a recently published study on the use of ultrasound-assisted extraction (UAE) to extract bioactive compounds from a wide range of sources for various applications.

Keywords: *bioactive compounds; ultrasound-assisted extraction (UAE); oligosaccharides; prebiotic*

INTRODUCTION

Within the human body, numerous biological activities occur continuously and constantly. To support the many biological processes, bioactive compounds offer the necessary elements. Bioactive compounds are chemicals that have biological properties. They are dietary elements that affect an animal or human's cellular or physiological processes after ingestion. To be absorbed by the body, dietary substances are digested into extremely microscopic components. Food naturally contains biologically active compounds. Compared to other foods, fruits and vegetables have the most bioactive compounds.

Foods, including fruits, vegetables, cereals, nuts, and occasionally even animals, contain bioactive compounds which substantially

influence human health. Plant extracts contain other bioactive compounds besides phenolics, flavonoids, anthocyanins, tannins, betalains, carotenoids, plant sterols, and glucosinolates [1]-[2]. Some examples of bioactive chemicals are included in Table 1, along with their sources [2]. Most uses for bioactive compounds are in the sectors of food, medicine, cosmetics, poultry feed, and biofuel, but they also have benefits in other industries. The isolation, purification, structural characterisation, assessment, extraction, and use of bioactive compounds have all been the subject of numerous research.

Chemical composition and functional group structures affect the functional properties of bioactive substances. Some compounds demonstrate to offer protection against several diseases and metabolic disorders. For instance, flavonoids have anti-

Table 1 Bioactive compounds and their sources of origin

Type of Bioactive Compounds	Sources
Flavonoids	Berries, leeks, ginger, grapefruit, carrot, apple, onion, broccoli, cabbage, kale, tomato, lemon, parsley, buckwheat, and legumes. Coffee, tea, chocolate, a range of spices, herbs and red wine.
Anthocyanins	Acai, blackcurrant, blueberry, bilberry, cherry, red grape, and purple corn.
Tannins	Teas, coffee, pomegranates, persimmons, most berries (cranberries, strawberries, blueberries), grapes, red wine, chocolate (with cocoa content 70% and higher), and spices (cinnamon, vanilla, cloves, thyme).
Betalains	Red and yellow beetroot, coloured swiss chard, leafy or grainy amaranth, prickly pear, red pitahaya, and several cacti.
Carotenoids	Carrots, plums, apricots, mangoes, cantaloupes, sweet potatoes, kale, spinach, cilantro (coriander), collard greens, fresh thyme, turnip greens, and winter squash.
Plant sterols	Vegetable oils contain fewer nuts, legumes, grains, cereals, wood pulp and leaves.
Glucosinolates	wasabi (<i>Wasabia japonica</i>), broccoli, cabbage, kale, watercress, and garden cress.

Modified from Walia et al. [2]

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inflammatory, antioxidant, and beneficial effects on cardiovascular health. They also have anti-cancer properties. Anthocyanins are good for cardiovascular health, although more research is needed. Along with advantages for vision and skin, carotenoids also have preventive effects against various malignancies. Glucosinolates offer protection from cancer and dementia [2]. According to Lafarga, Ación-Fernández, and García-Vaquero [3], bioactive peptides and polysaccharides also possess antihypertensive, antioxidant, anticoagulant, anti-tumour, anti-inflammatory, and immunostimulatory properties.

Seaweeds, for example, contain a variety of bioactive substances, including polysaccharides (such as alginate, fucoidan, ulvan, and carrageenan), proteins (such as phycobiliproteins), carotenoids (such as beta-carotene and fucoxanthin), phenolic compounds (such as phlorotannins), vitamins (specifically vitamins A, B, C, D, and K), essential minerals (such as calcium, iron, iodine, magnesium, and potassium) and polyunsaturated fatty acids (specifically ω -3 fatty acids) [4]. These substances have been studied for their potential to cure several human pathologies and illnesses, including hyperglycemia, diabetes, metabolic disorders, cancer, pathogenic diseases, ageing, obesity, bone-related diseases, and cardiovascular and neurodegenerative diseases [4]. There have been several findings that seaweed's polysaccharides might be used as prebiotic components for both human and animal health purposes [5]-[7]. In a study by Wanyonyi et al. [8], the red seaweed *Kappaphycus alvarezii* was utilised as raw material to determine if it could be used as a supplement to whole meals to reduce the symptoms of metabolic syndrome in rats. *K. alvarezii* exhibited no adverse effects on any of the examined parameters. It was discovered that the entire *K. alvarezii* plant improved metabolic, hepatic, and cardiovascular functions in obese mice. In a separate investigation by Bajury et al. [9], it was discovered that the red seaweed *K. alvarezii* greatly boosted the growth of *Bifidobacterium* spp. and the amounts of acetate and propionate during in-vitro fermentation. Seaweed thus has the potential to be a unique and emerging prebiotic component.

Numerous bioactive chemicals are extracted from various sources and materials using ultrasound-assisted extraction, a green extraction method. This review paper intends to investigate the impact of innumerable parameters on UAE extraction methods. For this objective, papers published between 2017 and 2022 are reviewed. A brief explanation of the fundamental concepts of ultrasonication is also presented. There are also a few examples of how ultrasound might help improve the quality of food materials.

Ultrasonication Process: Mechanism and Operational Parameters

The techniques used to extract bioactive compounds are essential since a chemical reaction could modify the molecular structure of the molecules extracted, which could impact their usefulness. Several variables, including the extraction method, the raw materials used, and the extraction solvent used, affect the extraction of bioactive compounds [10]. The different extraction methods could be categorised as conventional or non-conventional. Soxhlet, maceration, and hydro-distillation are a few examples of conventional techniques. Modern methods, often referred to as non-conventional methods, are environmentally friendly or clean methods since they require less energy and organic solvents, both of which are good for the environment. Subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pulsed electric field extraction (PEF), high voltage electrical discharges (HVED), and enzyme-assisted extraction (EAE) are a few examples of modern approaches [11]-[12].

This review paper focuses on the UAE technique, including its description, mechanism, and optimised UAE settings for extracting several bioactive compounds from various sources. UAE is a revolutionary extraction method that has the potential to offer great repeatability in less time, larger yields of bioactive compounds, easier manipulation, lower processing temperatures, less solvent use, and lower energy input. This is primarily because of the breakdown of cell walls, decreased particle size, and improved mass transfer to the cell contents caused by cavitation bubble collapse [13].

Between sound and ultrasound, the wave's frequency is the key difference. A variety of sound wave frequency ranges can be divided into three general groups: (i) infrasonic waves have frequencies below the hearing range (<16 Hz), (ii) ultrasonic waves have frequencies above the audible range (>20 kHz), but below microwave frequencies (up to 10 MHz), and (iii) audible waves lie between 10 Hz to 20 kHz, which is where the human ear is most sensitive [14] (Figure 1).

The ultrasonic region of the spectrum is important from application and commercial perspectives. Ultrasound can also be broadly classified into two regions, namely: (i) high intensity ($10 - 1000$ W/cm²; low frequency, 20 to 100 kHz); and (ii) low intensity (< 1 W/cm²; high frequency, 100 kHz to 1 MHz) or also known as a signal or diagnostic ultrasonic region. The high-intensity sonication is utilised for extraction and processing applications.



Figure 1 The sound spectrum of audible range (20 Hz – 20 kHz), ultrasound range (20 kHz – 10 MHz) and microwave range (>10 MHz)

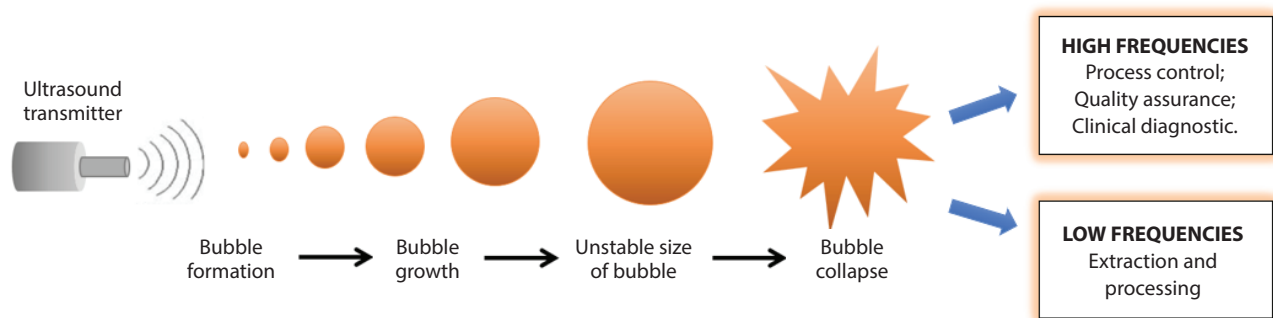


Figure 2 Bubble growth cycle during acoustic cavitation

At the same time, low intensity is typically used for process control and quality assurance, as well as employed as a clinical diagnostic technique [15].

Acoustic cavitation is the primary factor that drives the extraction effects of sonication. According to Kentish and Ashokkumar [16], acoustic cavitation bubbles formation, growth, and implosion when an ultrasonic wave travels through a solvent (Figure 2).

Any medium that transmits ultrasound experiences a series of compressions and rarefactions in the molecules. In a liquid medium, such alternating pressure fluctuations lead to the production of bubbles and, finally, their collapse [13]. Temperature and pressure variations (between 2000 and 10000 K and 100 and 1000 MPa, respectively) made by these implosions result in shear disruption, membrane thinning, and cell disruption. These changes enhance solvent penetration into cells and magnify the mass transfer of target compounds into the solvent [17]. Ultrasonic bath (Figure 3a) or probe-type (Figure 3b) ultrasound equipment are two examples of high-power ultrasound-producing machines and are the most used in different fields of analytical chemistry and food analysis. A transducer serves as the ultrasonic power source for both systems. The most popular and economical system, an ultrasonic bath, has a transducer that constitutes the bath beneath

the stainless-steel tank [18] (Figure 3a). In the bath system, the power generator typically generates energy at a frequency of 40 kHz, which is then dispersed as waves into the bath by one or more transducers. As opposed to probe-type equipment, there won't be any direct contact between the sample and the waves [18]. Nevertheless, the use of probe-type ultrasonication devices (Figure 3b) in various industries have grown recently. Compared to a bath system, these systems are more effective in irradiating the medium. The 20 kHz high-frequency electrical energy produced by the probe system's power generator is later transformed into mechanical energy by the transducer. The probe then releases the acoustic energy as waves after amplifying the mechanical energy [19].

For food and natural products, ultrasound-assisted extraction offers an environmentally friendly and commercially feasible substitute for conventional methods. The primary advantages include reducing extraction, processing duration, energy, the solvent used, unit operations involved, and CO₂ emissions [20]. Ultrasound power and frequency, intensity, shape, and size of ultrasonic reactors are the physical parameters that influence ultrasound-assisted extraction. In contrast, the medium parameters include the type of solvent used, the temperature level applied, the presence of dissolved gases and external pressure, and the type of matrix [20].

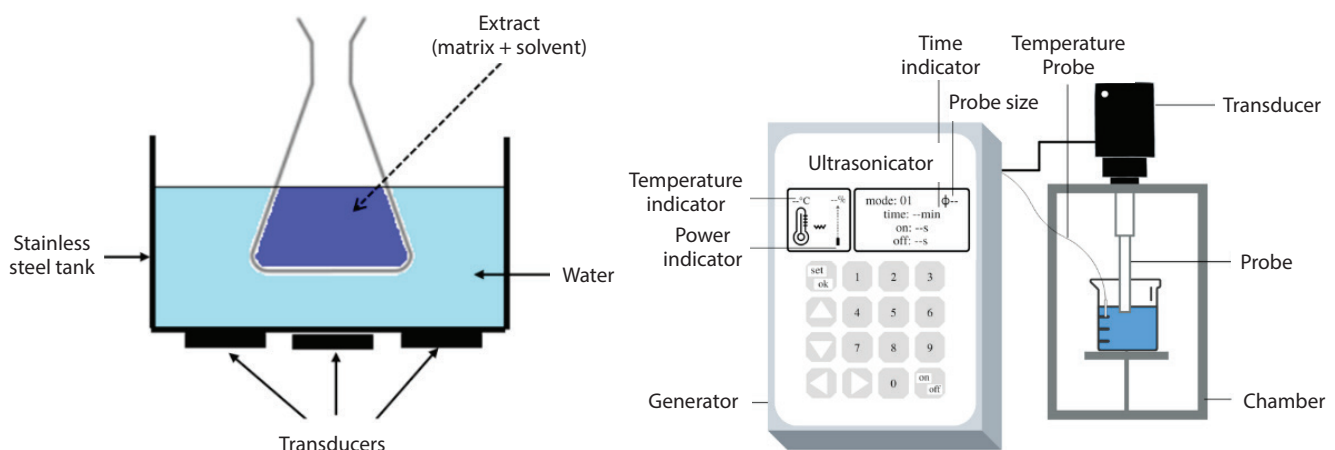


Figure 3 Ultrasonic systems. (a) Ultrasonic bath and (b) Ultrasonic probe

Applications of UAE in Extraction of Bioactive Compounds

China extensively cultivates *Flammulina velutipes* (FV) for food and medicinal purposes. Using the single factor test and response surface methods, Liu et al. [20] optimised the extraction of polysaccharides from the FV residue. When 1 g of residue in 50 mL of solvent was extracted using an ultrasonic system at 540 W for 20 minutes at a temperature of 75°C, an approximately 84 mg/g of polysaccharides was obtained. With a scavenging rate on DPPH of 55.6% and ABTS of 93.9%, the extract also demonstrated excellent in-vitro antioxidant activity.

By using the UAE process in distilled water for 30 minutes at 80°C, Nowacka-Jechalke et al. [21] extracted the crude polysaccharides from *Sparassis crispa*. The edible and therapeutic mushroom *S. crispa* grows in temperate areas of North America and Europe. The chemical analysis of the extract revealed that it was primarily composed of carbohydrates, with only trace amounts of proteins or phenolics. The extract also inhibited the growth of human colon cancer cell lines of Caco-2, LS180, and HT-29.

Response surface methodology was used by Wang et al. [22] to optimise the extraction of polysaccharides from the used *Lentinus edodes* substrate. According to their research, the optimal UAE settings (extraction temperature of 50°C, the liquid-solid ratio of 30 mL/g, and ultrasonic power of 120 W) allowed for producing 37.05 mg/g of total polysaccharide content. The polysaccharides also contained rhamnose polymers, which have strong antioxidant properties.

Fu et al. [23] found that different extraction techniques had a noticeable impact on the physicochemical properties and biological activities of polysaccharides from the leaves of the *Eriobotrya japonica* L., a semitropical fruit tree loquat. The extract obtained by UAE and MAE was shown to have strong antioxidant properties and inhibitory effects on α -amylase and α -glucosidase among the extraction techniques used. The main technique for combating metabolic changes associated with Type-2 diabetes is inhibiting these two enzymes' activity. Loquat leaves are popular traditional Chinese medicine that has been shown to have a variety of biological actions, including anti-diabetic properties [24]. Furthermore, substantial prebiotic effects were observed during the in-vitro fermentation process with UAE-treated leaf extract.

Among the five extraction methods used, Chen et al. [25] found that the UAE and EAE were the most effective methods in enhancing the prebiotic potential of polysaccharides fractions from bamboo shoots (*Chimonobambusa quadrangularis*), as these two fractions markedly increased the proliferation of probiotic strains and increased the production of short-chain fatty acids. While the molecular weight of the fractions was much smaller, it was observed that the fractions obtained using these two methods contained significantly more total carbohydrates.

The effects of four different extraction techniques on the relationship between the structure and prebiotic activity of fucoidan extracts were compared by Okolie et al. [26]. Although

conventional chemical, MAE, and UAE technologies all produced comparable results when extracting fucoidans, the MAE and UAE reported shorter extraction duration. The extracts from all four extraction techniques promoted the growth of *L. delbrueckii* spp. *bulgaricus*.

Guo et al. [27] isolated PPOS4 and PPOS5 as two different forms of oligosaccharides from sweet potatoes (*Ipomoea batatas* L.). The results of the single-factor studies demonstrated that the yield of PPOS4 first increased with increasing ultrasonic power but then declined as power and duration were elevated further. The yield of PPOS5 declined with increasing ultrasonic power and extraction duration, suggesting that PPOS5's stability may be poor. Nevertheless, degradation of the oligosaccharides may result from excessive ultrasonic power.

Melo-Silveira et al. [28] extracted xylan from corn cobs using an ultrasonic technique at a power of 200 W for 30 minutes at 60°C. By increasing the xylan dose from 0.5 to 2.0 mg/mL, it was discovered that the extracted xylan could inhibit the proliferation of human cervical cancer cells (HeLa) and human lung adenocarcinoma epithelial cells (A549) by 20% and 60%, respectively.

Research by Zhong et al. [29] used UAE to extract total flavonoids from *Dendranthema indicum* var. *aromaticum*, a novel plant with a strong aroma. The optimal extraction conditions were: a 40-minute extraction period, a solid/liquid ratio of 1:23 g/mL, and a temperature of 60°C. This information was a reference for pharmacological studies on *D. indicum* var. *aromaticum* and future bioactive compounds extraction from herbs.

Praveen and the research team [30] compared the extraction yield of seaweed polysaccharides by using different extraction techniques. The study shows a 4.67% yield by conventional solvent extraction (CSE), 9.56% yield by MAE, and 11% yield by UAE. Although EAE reported the highest extraction yield (50 to 70%), it was with limited applications due to its high price.

The operating conditions for the ultrasound-assisted extraction of polysaccharides from *Volvariella volvacea* (straw mushroom) were optimised by Cui et al. [31]. When processed with straw mushrooms at a 25:1 liquid-to-raw material ratio and a temperature of 57°C for 33 minutes of extraction, 8.28% of polysaccharides yield was attained. The UAE method produced a higher yield of polysaccharides from *V. volvacea* and was more effective than the hot-water extraction.

The polysaccharide from *Grifola frondosa*, often known as "the mushroom," was extracted using ultrasound technology [32]. The polysaccharides yield was 7.36% under optimal extraction conditions of 3.5 hours at 75°C and 370 W of ultrasonic power. Importantly, the strong antioxidant activity of *Grifola frondosa* polysaccharides showed that they could be exploited as a possible natural antioxidant in functional meals. Its anti-tumour activity on the suppression of HepG2 cell growth also indicated its potential to be exploited as an anti-tumour drug in the pharmaceutical industry.

The works by Gomes et al. [33] proved that the use of high-pressure processing (HPP) and ultrasound (US) processing were ideal for treating cranberry juice fortified with prebiotics. The juice was subjected to HPP for 5 min (450 MPa) and to ultrasonic treatment for 5 min (600 and 1200 W/L), followed by HPP for 5 min (450 MPa). Both non-thermal treatments preserved the fructooligosaccharides (FOS) content maintaining the prebiotic property of the juice. The increase of the content of a bioactive compound, anthocyanin, was observed when HPP followed ultrasound.

To produce pectin with modified functionality, Muoz-Almagro et al. [34] examined the effects of ultrasound on the structure of pectin from citrus and apples under various experimental settings. Foods can acquire certain rheological properties through the depolymerisation of pectin toward pectin derivatives with lower molecular weight, expanding their use as functional additives. Their research revealed that the treatment period, ultrasonic power, and amplitude all improved pectin depolymerisation. Their findings highlighted the significance of choosing the right ultrasound processing parameters to broaden the possible applications for modified pectin.

Rafiquzzaman et al. [35] discovered that ultrasonic power and sonication duration were the most important consideration for extracting carrageenan from seaweed using the UAE approach.

The influencing parameters were optimised, considering the type of chemical, raw material, solvent, and other factors. A low temperature was maintained with ice to prevent carrageenan from deteriorating throughout the UAE process.

The UAE approach was used to extract polysaccharides from the well-known Chinese traditional herbal medicine, *Angelica Sinensis* [36]. When the ratio of liquid to raw material increased from 10 mL/g to 40 mL/g, the polysaccharide yield increased before declining. When 40 mL/g of the ratio was utilised, the yield recorded was 20.24%. When the ultrasound duration was increased from 20 minutes to 25 minutes, the yield of polysaccharides increased linearly; however, when the duration was extended beyond 25 minutes, the yield of polysaccharides dropped. The 400 W ultrasonic power was used to get the best polysaccharide yield.

Yu et al. [37] applied the UAE to isolate total flavonoids (TF) from *Osmanthus fragrans* flowers. The results of single-factor experiments revealed that the solid-to-liquid ratio, solvent concentration, ultrasonic power, and extraction duration substantially impact flavonoids' yield. Wu [38] also reported that the major factors affecting the rate of UAE of polysaccharides from medicinal fungi (mushrooms) were the solid particle size, solid-liquid ratio, ultrasonication power intensity and temperature, plus the physical properties of solid materials being extracted. Apart from the extraction process's rate and yield, ultrasonication may impact the extract products' characteristics and bioactivities.

Ultrasonic-assisted extraction (UAE) of pomegranate seed oil (PSO) was studied by Tian et al. [39] to see how well different solvents performed. The most efficient oil extraction method was petroleum ether, followed by n-hexane, ethyl acetate, diethyl ether, acetone, and isopropanol. With petroleum ether and under the optimal conditions of ultrasonic power, extraction temperature, extraction duration, and S/S ratio at 140 W, 40°C, 36 min, and 10 mL/g, respectively, the highest oil yield of 25.11% w/w was obtained. The PSO yield obtained by the UAE was much greater than that obtained by Soxhlet extraction (20.50%) and supercritical fluid extraction (15.72%). The fatty acid compositions of the PSO recovered by Soxhlet extraction, SFE, and UAE were markedly different, with punicic acid (>65%) being the most abundant, utilising UAE.

The quality, texture, and flavour of cooked salted egg yolks (SEYs) with or without ultrasonic treatment were assessed by Wang et al. [40]. The experimental results showed that ultrasonic-assisted cooking might accelerate cooking (water) loss, oil exudation, and lipid oxidation and further encourage volatiles' formation in SEYs. The study's findings suggested that ultrasonic-assisted cooking might be employed as a possible method of manufacturing cooked SEYs with good flavour and a sandy, oily texture. Additionally, the quality and flavour of SEYs cooked with ultrasound assistance were improved.

CONCLUSIONS

It has been demonstrated and reported that using ultrasound-assisted extraction (UAE) to extract functional bioactive compounds is a cutting-edge green extraction technique. To fully exploit ultrasound's potential as a substitute for conventional extraction methods, this review paper emphasised the importance of selecting the appropriate ultrasonic processing settings (Table 2). A number of variables must be considered to obtain a substantial amount of bioactive compounds, including the ultrasound power, frequency of ultrasonication, extraction duration and temperature, solid-to-liquid ratio, raw material matrix, and extraction solvent. This highlights the UAE's potential to extract active compounds from various sources.

Sonochemistry, the principle of ultrasound, has often been considered a "clean technology" that has attracted interest recently due to its many benefits over traditional methods. The operation of UAE is easier than other extraction techniques like MAE. UAE is an energy-efficient technology because it offers excellent extraction yields due to its selectivity and energy savings because of its short operating duration. UAE is also a more cost-effective strategy since it only needs a small amount of solvent for extraction, producing less waste. In light of individual processes and shortcomings, UAE might be coupled with other extraction techniques to speed up the extraction of bioactive compounds and provide potentially higher-quality yields while avoiding potential heat treatment-related degradation. Last but not least, it is crucial to investigate the main challenges of the UAE process related to intensifying bubble generation via innovative reactor design and dispersing acoustic energy uniformly throughout the extraction solution.

Table 2 Summary of the applications of UAE (selected) in the recovery of bioactive compounds from various sources

Source	Compound(s)	Extraction technique/ Conditions	Findings	Ref.
<i>Flammulina velutipes</i>	Polysaccharides	UAE / 540 W, 20 min, 75°C	84 mg/g; DPPH: 55.6%; ABTS: 93.9%	[20]
Used <i>Lentinus edodes</i> substrate	Polysaccharides	UAE / 50°C, liquid-solid ratio of 30 mL/g, 120 W	Yield: 37.05 mg/g; strong antioxidant properties.	[22]
<i>Ascophyllum nodosum</i>	Fucoidan extract	UAE, MAE, chemical method	Shorter extraction duration (MAE and UAE); promoted the growth of probiotic strain.	[26]
Sweet potatoes	Oligosaccharides	UAE	Ultrasonic power could possess either positive or negative effects on the yield of oligosaccharides	[27]
Corn cobs	Xylan	UAE: 200 W, 30 min, 60°C	Inhibit the proliferation of HeLa and A549 cancer cells	[28]
<i>Dendranthema indicum</i> var. <i>aromaticum</i>	Flavonoid	UAE: 40 min, solid/liquid of 1:23 g/mL, 60°C	The reference point for pharmacological study on this plant	[29]
Seaweed	Polysaccharides	CSE, MAE, UAE, EAE	CSE: 4.76% yield MAE: 9.56% yield UAE: 11% yield EAE: 50 to 70% yield	[30]
<i>Volvariella volvacea</i> (straw mushroom)	Polysaccharides	UAE: liquid-to-raw material ratio of 25:1, 57°C, 33 min	8.28% yield	[31]
<i>Grifola frondose</i> (mushroom)	Polysaccharides	UAE: 3.5 h, 75°C, 375 W	7.36% yield Anti-tumour activity on HepG2 cancer cell line	[32]
<i>Angelica Sinensis</i>	Polysaccharides	UAE: liquid-to-solid ratio of 40 mL/g, 25 min, 400 W	20.24% yield	[36]
<i>Osmanthus fragrans</i> flowers	Total flavonoids	UAE	Influential factors are solid-to-liquid ratio, solvent concentration, ultrasonic power, extraction duration	[37]
Pomegranate seed	Oil	UAE and petroleum ether as solvent	25.11% yield, major fatty acid was punicic acid	[39]

ACKNOWLEDGEMENT

The authors would like to acknowledge the Ministry of Education (FRGS/1/2019/WAB09/KUTS/02/1) and the University of Technology Sarawak for research funding and technical support.

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