

Enzyme assisted extraction of polyphenols from Niger seed meal: a review

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ABSTRACT

The changing global economy and the food surpluses around the world have now stimulated an interest in seeking a new alternative crop especially an Oilseed Crop. Niger seed (*G. abyssinica*) is drawing commercial and academic attention because of its nutritional value, biological activities and excellent antioxidant properties. Niger seed has diverse application and hence this seed is of great industrial importance. Presence of polyphenols in Niger seed has been reported previously. Phenolic compounds, naturally found in plants are of considerable interest and have enticed researchers around the globe in recent years due to their bioactive functions. These components are known as secondary plant metabolites and have antimicrobial, antiviral and anti-inflammatory properties along with their high antioxidant capacity. And thus, presence of such properties makes polyphenol a functional food. Enzyme-assisted extraction has attracted interest among researchers across industries like food, pharmaceutical and academia. This extraction method of polyphenols from plants is a suitable alternative to conventional solvent based extraction methods. The application of hydrolytic enzymes for extraction of natural functional compounds from plants is widely investigated in recent years for its advantages in easy operation, high efficiency, eco-friendly nature and preserving the bioactive properties of the extracts. This review summarizes the nutritional composition of Niger seed and importance of polyphenols which are known to be present in Niger seed. It also discusses the advantages of using eco-friendly extraction methods known as enzyme assisted extraction.

Keywords: Niger seed meal, Enzyme-assisted extraction, Phenolic compounds, *G. abyssinica*

INTRODUCTION

Non-traditional oilseeds are being considered because their constituents have unique chemical properties and may augment the supply of edible oils (Cherry and Kramer, 1989). Even today, no oil from any one source has been observed to be suitable for all edible applications. Since, oils from different sources mostly differ in their composition. This urges the need to search for new sources of novel oils.

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Up to this point, countless plants have been examined and some of these have been cultivated and developed as new oil crops. Among these new sources of oil crops, Niger (*Guizotia abyssinica* Cass.) seed oil is of interest and may play an important role in human nutrition. As per M F Ramadan, Niger is an oilseed that has been cultivated for approximately 5000 years. South India and Ethiopia are the two major producers of Niger seeds. It belongs to the same botanical family as that of sunflower and safflower (*Compositae*) (Baagø, 1974). It is a dicotyledonous herb, moderately to well branched, and grows up to 2m in height (Getinet and Teklewold, 1995). Niger is cultivated in both temperate and tropical climates, being considered a temperate-region plant that has adapted to a semi-tropical environment. It prefers moderate temperatures for growth, from about 19°C to 30°C.

Polyphenols have been discovered in plants since their early appearance. These compounds, also called secondary metabolites, are indeed crucial for many important functional aspects of plant life, including structural roles in different supportive or protective tissues, involvement in defence strategies, and signalling properties, particularly in the interactions between plants and their environment. Collectively, higher plants synthesise several thousand different known phenolic compounds, and the number of these which have been fully characterized is continually increasing. There is a huge demand for novel natural compounds that rocketed the growth and development of plant based compounds called as bioactives that either are beneficial for health or are toxic upon consumption. Increased release of such bioactives from plant cells either by cell disruption or by extraction through the cell wall can be enhanced using a particular enzyme or mixture of different enzymes. However, in biotechnology the use of enzymes is not commonly exploited to its greatest potential within the food industry. Here, we discuss the application of nature friendly enzyme-assisted extraction of bioactive compounds. In various parts of the world there is an excess production of some conventional crops and also there is a quest for new crops, these also includes plants that produce beneficial oils. Till date, we know that the agricultural industry has produced raw materials that were employed mostly for the food industries. Yet, later on use of a greater amount of its products will be coordinated for chemical applications.

Niger seed (*Guizotia abyssinica* Cass):

Botanical details, production, composition and uses:

Niger (*Guizotia abyssinica* Cass) is one of the main oilseed crops having commercial importance. It has been under cultivation for long period of time spanning approximately 5000 years. Niger is a dicotyledonous herb, which is moderately to well branched, and attains up to two meters of height (Getinet and Teklewold, 1995). Niger seed belongs to the *Compositae* family and there are six species of which *G. abyssinica* is the only cultivated species (Baagø, 1974). India and Ethiopia are the major producers of Niger oil seed, with 90 kilotons of production reported in India during the year 2011-2012 (IOPEPC 2012).

In India, Niger is grown mainly in the states of Maharashtra, Orissa, Madhya Pradesh, Karnataka and Andhra Pradesh. In India, Ootacamund, N-5, RCR-18 and IGP-76 are the different varieties of Niger seed that are grown chiefly (Bhagya and Sastry, 2003). The crop is known to grow mainly on poorly drained, heavy clay soils (Alemaw and Wold, 1995; Francis and Campbell, 2003) in both temperate and tropical climates. However, it prefers moderate

temperatures for growth, from about 19°C to 30°C (Quinn and Myers, 2002). In literature, Niger seeds are reported to contain 483 calories, 2.8–7.8% moisture, 17–30% protein, 34–39% total carbohydrate, 9–13% fiber, 1.8–9.9 g ash, 50–587 mg/100 g calcium, 180–800 mg/100 g phosphorus, 0.43 mg/100 g thiamine, 0.22–0.55 mg/100 g riboflavin, and 3.66 mg/100 g niacin (Bhardwaj and Gupta, 1977; Kachapur et al., 1978; Rao, 1994). Table-1 also gives a comparative picture of composition of Niger oil seeds.

Table-1. Nutrient composition of Niger oil seed (Ref Rao, 1993)

Components	Value
Starch g/100g	6.1
Phosphorus mg/100g	801
Calcium mg/100g	587
Magnesium mg/100g	323
Iron mg/100g	30
Zinc mg/100g	6.6
Manganese mg/100g	8.5
Copper mg/100g	5.7
Chromium mg/100g	0.4
Tannins mg/100g	158
Nicotinic acid mg/100g	0.57
Riboflavin mg/100g	0.22

Niger seeds are used after frying or used as a condiment, are milled into flour, or pressed with honey into cakes. Apart from this they are used for livestock feed after oil extraction while the plants are used as green manure (a type of cover crop grown primarily to add nutrients and organic matter to the soil) (Riley and Belayneh, 1989; Bhagya and Sastry, 2003). In countries like USA, Europe, and Japan, Niger seed is used as bird feed (Dutta et al., 1994; Marini et al., 2003; Shahidi et al., 2003). Niger seed is also used in human food mainly as a spice in the frying of vegetables (Bhagya and Sastry, 2003).

Oil- Content, composition and uses:

The oil content in Niger seed is in the range of 30–50% (Getinet and Teklewold, 1995; Dange and Jonsson, 1997) and is having pale yellow colour with a nutty taste. It is very similar to sunflower and safflower oils with respect to linoleic acid content (Francis and Campbell, 2003). Recently, Bhatnagar and Gopal Krishna (2013) reported 31.8–41.3 g/100 g oil content of Niger seeds obtained after extraction with solvents of different polarities. In India, 20,000 tons of oil production was reported in 2009-10.

The fatty acid composition of Niger seed oil is typical for seed oils belonging to the *Compositae* family (e.g., safflower and sunflower) with linoleic acid being the dominant fatty acid. Getinet and Teklewold, (1995) reported fatty acid composition of the seed to be 7–8% palmitic and stearic acids, 5–25% oleic acid, and 55–80% linoleic acid. The Indian

varieties of niger seed contains 25% oleic and 55% linoleic acids (Nasirullah et al., 1982), and have lower linoleic acid percent when compared with seeds grown in Ethiopia (75%) (Nasirullah et al., 1982). The variation in fatty acid level in different varieties of Indian and Ethiopian origin is reported in Table 2.

Table-2. Variation in the fatty acid levels (% of fame) of Indian and Ethiopian Niger seed oil (Ref, Ramadan 2012)

Fatty acids	Ethiopian seed oil	Indian seed oil
Palmitic acid (C16:0)	8.0-9.7	9.4-16.9
Behenic acid (C22:0)	0.3	0.4-0.5
Palmitoleic acid (C16:1)	0.2	0.2
Stearic acid (C18:0)	5.6-8.1	6.5-6.8
Oleic acid (C18:2)	5.9-11.0	8.3-9.2
Linoleic acid (C18:2)	70.7-79.2	63.0-71.1
Linolenic acid (C18:3)	0.4	0.3
Arachidic acid (C20:0)	0.4	0.5

Bhatnagar and Gopal Krishna (2013) have characterized Niger seed oil components and reported the following parameters: linoleic acid (69.4–73.2 %) was the major fatty acid and trilinolein (31.2–33.4 %) was the major triacylglycerol; color (40.0–95.0 Lovibond units), free fatty acids (3.6–12.3 g/100 g), peroxide value (3.2–7.8 mequiv O₂/kg), iodine value (137.6–140.3 cg I₂/g), saponification value (177.3–185.9 mg KOH/g) and unsaponifiable matter (1.3–4.3 g/100 g); total tocopherols (171.9–345.8 ppm with α -tocopherol 154–276 ppm as major component); total phenolics (247.1–2,647.7 ppm; vanillic acid with 176–1,709 ppm as major component); total sterols (1,249.6–6,309.3 ppm) and total carotenoids (18.9–181.0 ppm).

Niger seed or its oil also used in perfumes as a carrier of the scents and fragrances. Niger seed oil is used as a substitute for olive oil and as a pharmaceutical substitute for sesame oil. It also finds usage in the manufacture of soap and as a lubricant or lighting fuel. The oil is also documented to find use though to a limited extent in paints (being slow-drying), for which the Ethiopian seed is superior to the Indian seed as it has higher linoleic acid content.

Meal- Content, composition and uses:

The utilization of Niger seed proteins in human food is very limited due to the presence of a high fiber content (more in Ethiopian varieties when

compared to Indian variety) and an unacceptable dark cake colour (Seegeler, 1983). The Niger meal contains approximately 30% protein and 23% crude fiber after oil extraction and the oil, protein, and crude fiber contents are dependent on the hull thickness with thick-hulled having less oil and protein and more crude fiber (Getinet and Teklewold, 1995). The meal was reported to contain more crude fiber than most oilseed meals and is free from any toxic substance. Earlier, Bhagya and Sastry, (2003) reported an integrated method of processing to dehull the niger seed with a dehulling efficiency of 96–98%. Further, the oil extracted from dehulled seeds was of good quality and the cake was high in protein and low in fiber. The amino acid composition of Niger protein was deficient in tryptophan (Table-3).

Table-3. Amino acid Composition of Niger oil seeds (Ref Rao, 1993)

Constituents	Values
Lysine	4.0
Histidine	2.1
Arginine	3.3
Aspartic acid	9.6
Threonine	5.3
Serine	5.5
Glutamic acid	27.0
Proline	-
Glycine	4.8
Alanine	3.7
Valine	4.0
Methionine	1.2
Isoleucine	3.7
Leucine	6.5
Tyrosine	2.7
Phenylalanine	2.8
Tryptophan	1.0
Chemical score	33
First limiting amino acid	Methionine

The lipoprotein contained 4% moisture, 12% ash, 46% protein, 20% fat, 7% crude fiber, and 11% soluble carbohydrate (Eklund, 1971a; 1971b; 1974). The antioxidant potential of defatted niger seed and its extracts was reported by Shahidi et al. (2003). The meal remaining after oil extraction was tested as a source of antioxidants. Three solvent systems, A [80:20 (vol/vol) ethanol/water], B [80:20 (vol/vol) acetone/water], and C (water) were evaluated as extraction media. Extract A, which exhibited superior antioxidant activity over B and C, was studied further by using column chromatography and HPLC. Out of four fractions obtained (I–IV) were obtained, Fraction IV was also highly effective in scavenging the 2, 2-diphenyl-1-picrylhydrazyl radical but was less active when used in a bulk oil model system. UV spectroscopy suggested that the major active component present was a chlorogenic acid-related compound (Table 4).

Table-4. Phenolic acids in Niger seed crude extract (mg/g extract) (Shahidi et al., 2003)

Phenolic Acids	Chlorogenic	Caffeic	p-Coumaric	Ferulic	Sinapic
Free	2.6	0.6	Trace	-	0.1
Esterified	-	40.7	0.2	0.2	Trace
Glycosylated	-	2.1	-	-	-
Total	2.6	43.4	0.2	0.2	0.1

Furthermore, HPLC analysis established that chlorogenic acid was dominant in the free phenolics fraction (2.6 mg/g). It was concluded in the study that that defatted niger seed and its extracts can provide a natural source of antioxidants (Shahidi et al., 2003). The use of niger seed was advisable in applications where the functionality of seed components, such as their fat- and moisture binding ability and textural effects, are important in addition to their antioxidant activity.

Polyphenols:

Occurrence, structure and types

Polyphenols are secondary metabolites found in plants, and characterized by the presence of more than one phenol unit or building block per molecule. They play important roles in defense against plant pathogens and animal herbivore aggression and as response to various abiotic stress conditions, such as rainfall and ultraviolet radiation. Fruits, vegetables, and beverages like berries, tea, beer, grapes/wine, olive oil, chocolate/cocoa, coffee, walnuts, peanuts, borojo, pomegranates, popcorn, yerba mate, and other fruits and vegetables (Vinson et al., 2001; 2002) are the main sources of phenolic compounds in the human diet (Kapur and Kapoor, 2001; Dimitrios, 2006) (Table-5).

Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. In general polyphenols are divided into flavonoids and non-flavonoids. Flavonoids share a common carbon skeleton of diphenyl propanes, two benzene rings (ring A and B) joined by a linear three-carbon chain. The central three-carbon chain forms a closed pyran ring (ring C) with A benzene ring. They are subdivided into many subclasses: flavonols, flavones, flavanones, anthocyanidins, flavanols, and also isoflavones (Daglia, 2012). The main groups of nonflavonoids include phenolic acids, which are subdivided into derivatives of benzoic acid, like gallic acid and protocatechuic acid, along with derivatives of cinnamic acid, that consist chiefly of coumaric, caffeic, and ferulic acid. Stilbenes, constitutes the second group whose main representative is resveratrol, that exists in both cis and Trans isomeric forms, while the third group comprises of lignans which are produced by oxidative dimerization of two

phenylpropane units. The sensorial food properties such as flavor and color are influenced by polyphenols, and they also contribute to the aroma and taste of numerous food products of plant origin (Lule and Xia, 2005). The contribution of polyphenols towards aroma is mainly due to the presence of volatile phenols. Polyphenols apart from above-mentioned diversity are also present in plant tissues mainly as glycosides and/or associated with various organic acids and/or as complex polymerized molecules with high molecular weights, such as tannins.

The term "polyphenol" includes more than 8,000 compounds with great structural diversity (although each has at least one aromatic ring with one or more hydroxyl groups). They can be divided into 10 different classes depending on their basic chemical structure. Table 1 shows the main families of phenolic compounds, most of which are found in nature associated with mono- or polysaccharides (glycosides) or functional derivatives such as esters or methyl esters. Moreover, the main sources where phenolic compounds are found have been classified.

Biological effects of polyphenols:

Polyphenols have been studied in the past two decades for their potential involvement in the prevention of many chronic diseases, like cardiovascular disease, cancer, osteoporosis, diabetes mellitus, and neurodegenerative diseases. Many of the effects that dietary polyphenols exhibit are likely through impacts on transcriptional networks or signalling cascades that modulate gene expression, promoting anti-inflammatory mediators, and NO production (Christy C. Tangney et al., 2013). The protective activity of polyphenols have been attributed to their role as an anti-oxidant, free radical scavenger, and metal chelator, or to due to inhibition of telomerase, cyclooxygenase, or lipoxygenase, interaction with signal transduction pathways and cell receptors, acting as anti-microbial agents, anti-allergic and effect on inflammation and endothelial dysfunction by in part affecting the recruitment or "homing" of inflammatory cells from the circulation and down-regulating the production of adhesion molecules by the endothelium, thereby hindering cellular migration into the subendothelial space and reducing atherosclerotic plaque formation (Christy C. Tangney et al., 2013) (Table-5).

Anti-microbial activities of polyphenol:

Friedman et al., (2006) reported inhibition of food-borne pathogenic bacteria, such as *Bacillus cereus* by tea catechins, such as (-)-gallic catechin-3-gallate, (-)-epigallocatechin-3-gallate, (-)-catechin-3-gallate, and (-)-epicatechin-3-gallate, at nanomolar levels. It was reported that many of these compounds were more active than antibiotics, such as tetracycline or vancomycin, at comparable concentrations. The study indicated the tested tea catechins could exert a positive effect against gastrointestinal diseases.

The pre-treatment of a human cultured cell line (HL cells), with flavonols at polyphenol concentrations ranging from 0.5 to 50 mM decreased the infectivity of *Chlamydia pneumoniae* by 50% as compared to untreated controls (Alvesalo et al., 2006). Proanthocyanidins derived from berries inhibit the growth of several pathogenic bacteria, such as uropathogenic *Escherichia coli*, cariogenic *Streptococcus mutans*, and oxacillin-resistant *Staphylococcus aureus* (Cote et al., 2010).

Proanthocyanidins also showed antiviral effects against influenza A virus and type-1 herpes simplex virus (HSV) by preventing the entry of the virus into the host cell, which is the first critical step in primary HSV-1 infection (Geschera et al., 2011). Lignans obtained in hexane extract from *Aristolochia taliscana* roots, a plant used in traditional Mexican medicine, contains neolignans, among which Licarin A was found to be the most active against four mono-resistant variants and 12 clinical isolates of *Mycobacterium tuberculosis* strains with MICs ranging from 3.12 to 12.5 mg/ml (Leon-Díaz et al., 2010).

The proliferation inhibition activities on *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were also measured for evaluating the antimicrobial activity of polyphenols from tobacco leaf. The diameters of inhibition zones were 20.23 ± 0.42 , 17.66 ± 0.86 and 12.89 ± 0.29 mm, respectively (Wang et al., 2008).

Enzyme assisted extraction of bioactives from plant sources:

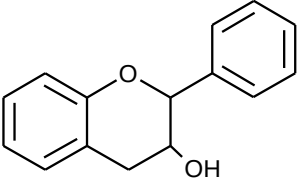
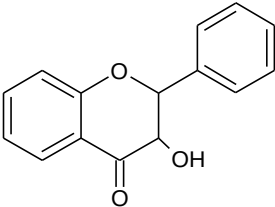
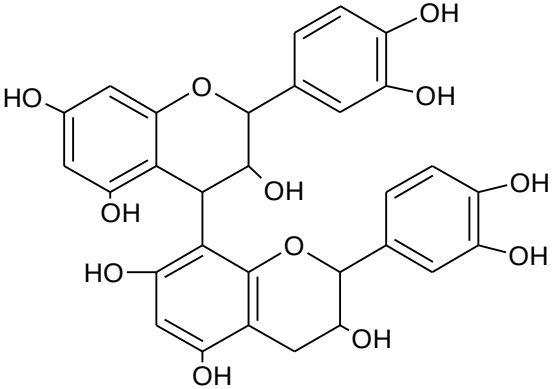
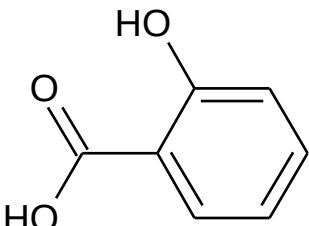
Table-5. Plant, fruit and vegetable-based biological compounds classified as polyphenols and separated according to differences in chemical structure (Myburgh, 2014)

Groups	Classes	Examples and Derivatives	Excellent sources
Phenolics	Cinnamic Benzoic	Caffeic acid Gallic acid	Coffee beans Tea leaves
Flavonoids	Flavonols	Quercetin ; Kaempferol Quercetin glucosides	Yellow onions, deep green vegetables. Cherry tomatoes Intensely flavored vegetables and herbs(celery,parsley)
	Flavones		Soy products
	Isoflavones		Dark blue, red and deep red fruit and vegetables; red wine
	Anthocyanins		Citrus fruit and pith of peel, Concentrated tomato(paste)
	Flavonones		
	Flavonols	Naringin; Hesperetin; Eriodictyol; Naringenin Catechins and derivatives Epicatechin Gallic catechin Epigallocatechin Epigallocatechin- gallate Proanthocyanidin	Tannins, grapes, seeds, cocoa.
Stilbenes	Resveratrol Piceid	Trans and cis resveratrol Trans and cis piceid	Wine, red grapes Red grape juice
Lignans		For example enterolignans Allicin	Seeds, legumes, wholegrains Bulbs, certain fruits

Phenols are classified as cell-wall phenols (which are bound to polysaccharides) and non-cell-wall phenols (associated with vacuoles and the cell nucleus) in higher plants (Pinelo et al., 2006 EA 2). The retention of phenols in the cell wall depends on physical traits, compositional and structural characteristics of cell wall polysaccharides as the latter contains hydrogen groups as well as aromatic

with polyphenols (Le Bourvellec et al., 2004 EA 3). As cross-linking between cell wall polysaccharides act as the main barrier for the release of intracellular substances, degradation of cell-wall polysaccharides is one of the key steps in releasing phenols from cell wall. Conventional methods such as cold pressing, super-critical fluid and solvent extraction which are used to extract bioactives from plants sources suffers

Table-6. Chemical structure of Polyphenol classes and microorganisms sensitive to them (Daglia, 2012).

 Flavon-3-ol	Antibacterial	<i>V.cholerae</i> - <i>S.mutans</i> - <i>C.jejuni</i> <i>C.perfringes</i> - <i>E.coli</i> - <i>B.Cereus</i> <i>H.pylori</i> - <i>S.aureus</i> - <i>L.acidophilus</i> <i>A.naeslundii</i> - <i>P.oralis</i> - <i>P.gingivalis</i> <i>P.melaninogenica</i> - <i>F.nucleatum</i> <i>C.pneumonia</i>
	Antiviral	Adenovirus-Enterovirus-Fluvirus
 Flavonol	Antifungal	<i>Candida albicans</i> <i>Microsporum gypseum</i> <i>Trichophyton mentagrophytes</i> <i>Trichophyton rubrum</i>
 Condensed Tannin	Antibacterial	<i>S.mutans</i> , <i>E.coli</i> , <i>S. aureus</i>
	Antiviral	Influenza A virus, type-1 Herpes Simplex virus
 Phenolic acids	Antibacterial	<i>S.aureus</i> , <i>L.monocytogenes</i> <i>E.coli</i> , <i>P.aeruginosa</i>

and glycosidic oxygen atoms that have the ability to form hydrogen bonds and hydrophobic interactions

from several drawbacks, including low selectivity, low extraction efficiency, safety hazards, high energy

input, low product quality, environment risk and toxicological effects [5 from enzyme assisted]. As an alternative technology, enzyme-assisted extraction has enticed considerable interest among researchers across industries like food, pharmaceutical and academia. The application of hydrolytic enzymes for extraction of natural functional compounds from plants is widely investigated in recent years for its advantages in easy operation, high efficiency, eco-friendly nature and preserving the bioactive properties of the extracts. A variety of enzymes like cellulases, pectinases and hemicellulases, which attack cell wall components, are used to disrupt the structural integrity of the plant cell wall, thereby enhancing the extraction of bioactives like polyphenols from plants (Table-7). These enzymes increase extraction yields of bioactives by increasing the cell wall permeability. Various microorganisms like bacteria, fungi, animal organs or vegetable/fruit extracts are the sources of these enzymes and it is imperative to understand their catalytic property and mode of action, optimal operational conditions and which enzyme or enzyme combination for extraction applications.

α -L-rhamonidase treatment have been shown to increase flavonoid release from plant material while minimizing the use of solvents and heat (Kaur et al., 2010). In the processing of pectic polysaccharide from mangosteen for enhancing extraction of an antioxidant, utilization of pectinase has been reported (Gan et al., 2010). The enzyme at 0.1% w/w increased extraction from 1.7 to 7.4 g/kg of raw material dry weight. Dobozi et al. (1988) reported that treatment of mustard seeds with cellulolytic enzymes results in an increase (20–30%) in the yield of oil. Santamaria et al. (2000) showed that enzyme preparations of cellulase, hemicellulase and pectinase when used for the treatment of chilli and an increase in the yield of carotenoids (11%) and capsaicinoids (7%). Chowdhary and Satyanarayana (2007) reported significant increase (206%) in lycopene extraction from tomato tissues using cellulases and pectinases versus control experiments. Similarly, a 2.5-fold increase in yield of lycopene from tomato skin was reported by using pancreatin digestion. Deng et al. (2014) reported extraction of *Fructus Mori* polysaccharides (FMPS) from *Fructus Mori* using four kinds of enzymes.

Table-7. List of bioactive compounds of industrial importance obtained by enzyme-assisted extraction from plants (Puri et al., 2012).

Product type	Product	Source	Enzyme used	Maximum yield %
Oils and carotenoids	Oil	Grape seed	Cellulase, protease, xylanase and pectinase	17.5
	Carotenoids	Marigold flower	Viscozyme, Pectinex, neutrase, corolase and HT-proteolytic	97
	Volatile oil	Mandarin peel	Xylan-degrading enzymes	15
	Carotene	Carrot pomace	Pectinex Ultra SP-L	0.0064
	Lycopene	Tomato	Pancreatin	2.5-fold
		Tomato	Cellulase and pectinase	206
	Capsaicin	Chilli	Cellulase, hemicellulase and pectinase	N.D.
	Anthocyanin	Grape skin	Pectinex BE3-L	N.D.
Glycosides	Sugar	Grapefruit peel waste	Cellulase and pectinase	0.6377
	Oligosaccharide	Rice bran	Cellulase	39.9
	Starch	Cassava	Pectinase enzyme	45.6
	Pectin	Pumpkin	Xylanase, cellulose, b-glucosidase	14.0
Others	Polyphenols	Grape pomace	Pectinolytic	98.0
	Flavonoid (naringin)	Kinnow peel	Recombinant rhamnosidase	N.D.
	Phenolics	Citrus peel	Celluzyne MX	65.0
	Proteins	Lentils and white beans	Glucoamylases	50.3
	Catechins	Tea beverage	Pepsin	80.0

Glucose oxidase offered a better performance in enhancement of the extraction yields of *FMPS*, antioxidant and activate alcohol dehydrogenase activities. Miron et al., (2014) reported enzyme-assisted extraction (EAE) and pressurized liquid extraction (PLE) for extraction of phenolic compounds from lemon balm (*Melissa officinalis*) by using cellulase, endo- β -1,4 xylanase and pectinase. The results showed that EAE enhanced the total phenolic content and the antioxidant capacity compared to a non-enzymatic control. Among the bioactive phenolic compounds identified in lemon balm, rosmarinic acid was the main component, although other important compounds were also identified, such as caffeic acid derivatives (salvianolic acids, lithospermic acid) and rosmarinic acid derivatives (rosmarinic acid hexoside, sagerinic acid, sulfated rosmarinic acid). The study confirmed that EAE can be considered alternative method for the extraction of natural compounds with biological activity from natural sources.

Niger seed has huge diverse applications and it can be used as an alternative to traditional Oilseed crop. It has been extensively studied for its antioxidant properties. Polyphenols which are known to be present in Niger seed exhibit wide range of biological activity such as anti-microbial, anti-inflammatory, prebiotics and antioxidant activity. Polyphenols may play an important role in the prevention and treatment of highly widespread diseases such as cancer and cardiovascular diseases. Use of enzymes to extract bioactives from plants is a suitable alternative for solvent based extraction. Application of enzymes is still not widely known, but there is a huge scope for the eco-friendly extraction methods for production of variety of bioactives.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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