PHYTOCHEMICALS OF BIOACTIVE COMPOUNDS OF *BRASSICA JUNCEA* (BROWN MUSTARD) SEEDS

Odangowei Inetiminebi Ogidi

Department of Biochemistry, School of Applied Sciences, Federal Polytechnic Ekowe, Bayelsa State, Nigeria

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Summary

Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals. Secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes for compounds associated with plant growth and development, and are regarded as products of biochemical "side tracks" in the plant cells and not needed for the daily functioning of the plant. Several of them are found to possess various types of important capabilities to perform functions in the living plants such as protection, attraction or signaling. Most species of plants seem to be capable of producing such compounds.

Brassica juncea (brown mustard) seed is an economically important cruciferous plant used as a food spice that has been well-known in many countries for centuries for its medicinal and nutritive values. Mustard contains numerous phytochemicals such as: vitamins, minerals, dietary fiber, chlorophylls, glucosinolates (and their degradation products), polyphenols and volatile components (allyl isothiocyanate, 3-butyl isothiocyanate, etc.). The content and exact chemical composition of these phytochemicals is affected by the plant variety, growth environment, extraction process and food processing methods. In addition, mustard may possess a plethora of pharmacological activities including anti-oxidation, anti-inflammation, and bacterio-static and antiviral activity. Mustard has also been used to combat several illnesses such as cancer, obesity, depression, diabetes, cataracts, and also used in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for treatment of various gastrointestinal, respiratory and skin diseases. This chapter provides an overview of plant characteristics, origins, distribution, processing methods of mustard and mustard seed products, as well as its phytochemicals and bioactivities of the isolated components. This chapter may serve as references for the development and utilization of mustard resources and sustainable source of affordable drugs which is potentially a good source for obtaining pharmacologically standardized phytotherapeutics.

1. Introduction

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Researchers have great interest in screening of medicinal plants for therapeutics. The active principles of many drugs found in plants are secondary metabolites. In folk medicine, medicinal herbs and plant products were used in treating

a wide range of infections and other diseases. Today, a great number of different medicinal plant products are available in the market (including cosmetics and pharmaceuticals), which contains biologically active substances. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have reported to be safe and without side-effects.

Brassica juncea is an economically important plant that has been well known in many countries for centuries for its medicinal and nutritive values. Various parts of the plants are edible and used in a range of folk medicines and spices. The seeds are usually about 1 to 2 mm in diameter. The mustard plant belongs to the *Cruciferae (Brassicaceae*) family. Mustard used in food is often a mixture of seeds from two or more species of *Brassicaceae*, for example *Sinapis alba L*. (white or yellow mustard), *Brassica nigra* (black mustard) and *Brassica juncea L*. (Brown or oriental mustard). Mustard plant at different types have been widely cultivated and used as spice, medicine and a source of edible oil since ancients' times. Mustard is also used in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for treatment of various gastrointestinal, respiratory and skin diseases.

There are various polyherbal formulations of mustard discussed in Ayurveda medicine. A decoction of *Moringa oleifera* root (1 in 20) with the addition of bruised mustard seed is useful in doses of 1-2 ounces in excess abdominal fluid (ascites) due to liver and spleen diseases. According to Ayurveda medicine, mustard preparations are mild laxatives, diuretics, and liver-bile stimulators and have been also documented to purge the toxins out of the body. The leaves and seeds of these plants are edible and diverse medicinal uses of seeds are also well known in other countries. In China, mustard seed is a folk remedy for arthritis, foot ache, lumbago and rheumatism.

It is also used in the treatment of tumors; leaves are used in soups for bladder infections, inflammation or hemorrhage. In Korea, the seeds are used for abscesses, colds, and lumbago, rheumatism and stomach disorders. The seeds are also reported to be hypoglycemic, antioxidant, anti-diabetic, hyperglycemic, anxiolytic, and goiterogenic.

2. Origin, Types, Characteristics and Distribution of Mustard Seeds

2.1. Origin Mustard Seeds

Mustard has been cultivated in many parts of Eurasia for centuries and is suitable for subtropical and temperate climate cultivars. Its main origin is considered to be Central Asia (northwestern India), followed by eastern India, central and western China, Myanmar, and Iran and its Near East. China is theorized to be the original region where varieties differentiated, with Sichuan Province possessing the highest degree of differentiation. The plant appears in some form in the diets of Japan, Africa, India, Bangladesh, China, Korea, Italy, Nepal, Pakistan, and African Americans.

2.2. Types of Mustard Seeds

Brassica is the most important genus of the *Brassicaceae* family and consists of 37 different species. This genus comprises six interrelated species, three diploids (*B. Nigra, B. Oleracea and B. Rapa*) and three Amphidiploids (*B. Carinata, B. Juncea and B. Napus*) species. This genus is categorized as oilseeds, fodder, spices and vegetable plants using buds, inflorescences, leaves, roots and seeds of plants. *B. oleracea* and *B. rapa* contain the majority of *Brassica* plants and they have many options from all species of edible forms. In *B. oleracea* there are vegetables such as kale, collards, cabbage, savoy cabbage, Brussel sprouts, cauliflower, broccoli, kohlrabi and Chinese kale, while in B. Rapa includes turnip, Chinese cabbage, pak choi, komatsuna. Other *Brassica* species include mustard greens, Chinese broccoli, Japanese mustard (mizu-na), gai-lohn, horseradish, wasabi, daikon, arugula, watercress and radish. *Brassica* vegetables are rich in sulfur-containing compounds which are responsible for the rich glucosinolate content, the pungent smells and the spicy flavor.

2.3. Characteristics of Mustard Seeds

The mustard plant is a member of the *Brassicaceae* family, also known as crucifers or the cabbage family, which bears characteristic four petal yellow flowers as well as tiny round edible seeds. Different varieties of mustard include white or yellow mustard (*Sinapis alba*); Oriental, brown or Indian mustard (*Brassica juncea*), and black mustard (*Brassica nigra*). Mustard seeds have been used as condiments for more than 5,000 years in ancient cultures such as Romans, Egyptian, Sumerian and Chinese. With almost 529,000 tons production per year, mustard is considered today as a major spice in international trade. Food crops such as rapeseed, canola, cabbage, broccoli, turnip, cauliflower, radish, horseradish and wasabi are also members of the *Brassicaceae* family. Mustard plants can reach 150 cm in height. The young stems and leaves are bristle, the stems are erect, the lower leaves are smaller, the edges are notched or teethed, the upper leaves are narrow-lanceolate, and the edges are not sparsely toothed or entire. Mustard is an annual cool season plant that requires long days and a relatively short growing season. Depending on the seeding time and growing condition, the yellow variety of mustard seeds mature in 85 to 95 days, whereas Oriental and brown types require 95 to 105 days. Mustard is usually cultivated in rotation with small grains such as cereal crops. In comparison to other *Brassicaceae* members such as canola, mustard seedlings have a higher tolerance to harsh growing conditions such as drought, frost and heat. However, excessive moisture and heat stress while flowering can result in a lower seed yield in *Brassica* crops. Mustard seeds can grow under both rain-fed and irrigation systems but are mainly produced under irrigated conditions in western Canada. Considering the favorable growing condition, mustard is mainly cultivated in the northern hemisphere. Canada is the most important producer of mustard with 28% of world production, followed mainly by countries such as Nepal, Myanmar, Ukraine and Russia.

Figure 1. *Brassica juncea* (Brown Mustard) Plant

Figure 2. *Brassica juncea* (Brown Mustard) Seeds

2.4. Distribution of Mustard Seeds

Canada is also the dominant exporter of mustard seed with an average of 57% of the international spice market share and almost 128 million dollars annual economic value. Canadian mustard seed is mainly exported to the United States with an average of 43%, followed by Germany and Belgium at 19% each, and also Japan and the Netherlands at 4% each. Asian countries use mustard seed mainly as oilseed crops, while European countries, Canada and the United States use it primarily for the condiment and spice.

Although Canada is a major producer of pure mustard seed globally, it is a minor crop accounting for only 3% of all specialty crop production in Canada with an average of 160,000 tonnes annual production. Yellow, brown and Oriental are three types of

condiment mustard that are mainly grown in Canada and comprise almost 57%, 22% and 16% of the total production area, respectively. The Prairie Provinces of Saskatchewan and Alberta are Canada's primary mustard seed producers.

2.5. Processing of Mustard and Mustard Seed Products

In the production process of commercial mustard seed products, after harvesting, mustard seeds will be dried to an appropriate moisture content of 9% and then stored at temperatures lower than 20°C. The drying process is essential to prevent mold growth, increase shelf life and guarantee long-time storage. Partial drying of the crop starts immediately on the farm and then is completed at the processing plants. Drying temperature should not be higher than 43°C, otherwise it might damage the seed.

Prior to milling and processing the mustard seeds into various products such as flour, bran and ground mustard, the crop is usually partially defoliated to facilitate the crushing process. Mustard flour is a fine powder obtained from the seed kernel (endosperm). It is prepared by successive milling and sifting to remove the bran (testa and aleurone layer) from the interior part (embryo and cotyledons) of the seed. Mustard flours are used primarily to give mustard flavor to a product and for their functionality as emulsifiers. They are commonly used as binding agent in dressings, sauces, pickles and processed meat products, especially sausages. Mustard bran is the by-product from flour production, which is widely used as a natural thickener in sauces and dressings. The majority of the functional properties of mustard bran such as water-banding, emulsifying, and stabilizing are mainly attributed to the presence of water-soluble polysaccharides in its structure. Ground mustard is another commercial product that is produced from grinding the whole mustard seed including the kernel and bran parts. It is widely used in processed meat products such as, salami, bologna, and frankfurters for flavoring, emulsifying, water-binding and also as a bulking agent for the inexpensive replacement of meat with vegetable protein sources. Ground mustard is also used in the production of pickles, sauces and dressings. Altogether, the mucilaginous compounds of the bran part play an important role in the functional properties of ground mustard.

Mustard oil is oil extracted from mustard seeds, which depending on the mustard variety, contain between 29% and 36% fixed oil. Residues from the extracted seed kernels are known as "press cake". Well refined mustard oil has pleasurable flavor with a brownish yellow color.

3. Definition of Bioactive Compounds in Plants

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages (e.g. vitamins and minerals), nutrients in plants are generally not included in the term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals.

3.1. Synthesis and Purpose

Secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes of compounds aimed at plant growth and development, such as carbohydrates, amino acids, proteins and lipids. They can be regarded as products of biochemical "side tracks" in the plant cells and not needed for daily functioning of the plant. Phylogenetically, the secondary bioactive compounds in plants appear to be randomly synthesized – but they are not useless junk. Several of them are found to hold important functions in the living plants. For example, flavonoids can protect against free radicals generated during photosynthesis. Terpenoids may attract pollinators or seed dispersers, or inhibit competing plants. Alkaloids usually ward off herbivore animals or insect attacks (phytoalexins). Other secondary metabolites function as cellular signaling molecules or have other functions in the plants.

3.2. Classification of Bioactive Compounds in Plant

Bioactive compounds in plants are classified according to different criteria. A presentation based on clinical function – their pharmacological or toxicological effects – is relevant for the clinician, pharmacist or toxicologist. An approach based on biological effects is complicated by the fact that the clinical outcome is not exclusively connected to chemically closely related compounds; even chemically very different molecules might produce similar clinical effects. A botanical categorization based on families and genera of the plants producing the bioactive compounds might also be relevant, as closely related plant species most often produce the same or chemically similar bioactive compounds. However, there are also ranges of examples that species even genetically less related produce similar secondary compounds.

4. Phytochemical Constituents of Mustard Seeds

Mustard seeds are rich sources of energy, having on average 23-30% fixed oil, 29-36% protein and 12-18% carbohydrate content. Depending on the variety of mustard, area of cultivation and condition of growth, the chemical composition of the crop varies considerably. Usually, Oriental and brown varieties of mustard seeds contain higher amounts of fixed oil, whereas, yellow varieties have lower concentrations of oil and higher protein and mucilage.

4.1. Phenolics

The *Brassica* genus contains phenolic compounds, one of the bioactive compounds that have positive effects on human health. These compounds show antioxidant activity by inhibiting the biological activation of carcinogens and by increasing the detoxification of Reactive Oxygen Species (ROS). Phenolic compounds are a large group of phytochemicals that occur naturally in a wide variety of plants and contain at least one aromatic ring and different numbers of hydroxyl substituents. Phenolics are classified as simple, having low molecular weight, single aromatic cyclic compounds, large and complex tannins and derivatives of polyphenols.

They are categorized on the basis of the number and arrangement of carbon atoms in flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones and others) and nonflavonoids (phenolic acids, hydroxycinnamates, stilbenes and others). Flavonoids and hydroxycinnamic acids are the most common and heterogeneous group of polyphenols in *Brassica* species. In addition, *Brassica* vegetables also contain anthocyanins which cause pigmentation in red cabbage and broccoli sprouts.

They are produced from phenylalanine as secondary metabolite derivatives through the shikimate pathway with phenylalanine ammonia-lyase as the first key enzyme. Their structure can vary from a single-ringed phenolic compound to a very complicated polyphenol with high degree of polymerization. Phenolic compounds play various physiological and ecological roles in the growth and reproduction of plants such as controlling growth hormones, facilitating pollination by attracting insects, and providing protection against herbivores, insects, and pathogens such as fungi, bacteria and viruses. In epidemiological studies phenolic compounds were associated with many health benefits such as anti-inflammatory, anti-bacterial, anti-allergic, cardio-protective, and anti-carcinogenic activity. One of the key actions of dietary phenolics is their antioxidant activities which are attributed to their redox properties, free radical scavenging, chain breaking, metal chelating, and other biological activities. Flavonoids, lignans, tannins, and phenolic acids are the four dominant phenolic compounds in the plant kingdom. Phenolic acids are divided into two groups: hydroxycinnamic acids and hydroxybenzoic acids. Hydroxycinnamates are phenylpropanoid compounds characterized by the C6-C3 structure. The major derivatives of cinnamic acid which occur widely in various crops including fruits, vegetables, cereals, legumes and oilseeds are caffeic, ferulic, *p*-coumaric, and 15 sinapic acids, which mainly exist as conjugates of sugar or other hydroxycinnamic acids.

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Biography sketch

Odangowei Inetiminebi Ogidi is a native of Okoloba/Sabagriea in Kolokuma/Opokuma Local Government Area of Bayelsa State, Nigeria. He attended Niger Delta University, Wilberforce Island,

Bayelsa State and Federal University of Technology, Owerri, Imo State, where he graduated with Bachelor of Science (B.Sc.) honors Degree in Biochemistry in 2009, Master of Science (M.Sc.) Degree in Biotechnology in 2016 and Doctor of Philosophy (Ph.D.) Degree in Biotechnology respectively. He is a Lecturer in the Department of Biochemistry in Federal Polytechnic, Ekowe, Bayelsa State, He is a fast growing researcher who has over thirty (30) Academic Journal Publications and over fifteen (15) Conference Papers and Presentations to his name. His research has focused on novel areas in Environmental Biotechnology, using indigenous agro-wastes for the purification of industrial effluents which is economically feasible and eco-friendly aand Ethnopharmacology for complementary and alternative medicine as a panacea for health care and various therapeutic applications, among other research interests. He has made a lasting impression and leadership achievements at Federal Polytechnic, Ekowe, Bayelsa State as Ag. Head, Department of Science Laboratory Technology from 2017-2018. Ag. Head Department of Biochemistry from 2018 till date. He is a member of various professional bodies which include: International Society of Tissue Engineering and Regenerative Medicine, Foundation for African Development through International Biotechnology and Nigerian Environmental Society.