CAROB (CERATONIA SILIQUA): SUPER FOOD AND MEDICINE.

LITERATURE UPDATE

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Keywords: Carob; antioxidant; cultivation; cocoa substitute; male fertility; gluten-free; molasses, nutrition.

Carob (Ceratonia siliqua) is one of the important nutritional and medicinal trees of the Middle East and Mediterranean basin, and in recent decades, it has been grown and cultivated in many other regions in the world. Realization and awareness to its unique nutritional and medicinal properties and biological activities are rising rapidly. A great effort of research has been invested and published since our comprehensive review article was published here, in this journal, in 2017. Most recent publications focus on nutrition and efforts to utilize Carob products for numerous food purposes, but medicinal activities of this tree are still drawing major attention, due to their high potential. In this review article, we are presenting a literature update of published research since late 2017. The main objective of this review is to highlight the nutritional applications of Carob products, which many industrial companies in the world, are trying now to convert to commercial food products.

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INTRODUCTION

Carob (Ceratonia siliqua) is a tree that was domesticated in the Middle East in ancient times, but it is currently cultivated in many other regions in the world. Since the publication of our comprehensive review about this tree in 2017, the average number of publication per a year is rising rapidly.

According to our literature study, it is clear that until 2017, most publications focused on studying medicinal activities of various products of this plant, such as extracts and oils. In recent years, nutritional research is gaining larger value and importance, yet, medicinal research is still interesting and exploring new activities. Consequently, in this review we will present in wider view the nutritional aspects of Carob research, and in the discussion section, we will relate to some notable cultivation efforts of this tree.

In the last three years, few important review articles were published about medicinal and nutritional properties of Carob. A. Loullis and E. Pinakoulaki discussed carob pod powder as a potential cocoa substitute. The key importance of this review is the presentation and comparison of active compounds in both foods, mainly phenolics, such as flavonoids and anthocyanins. I. Lakkab and her colleagues published a medium size excellent article about the neuroprotective activities of Carob. They presented the biological relation between oxidative stress and neurodegenerative diseases, medicinal activities of Carob focusing on antioxidant activity, general chemical composition and suggestions for future research. Polysaccharides content of various parts of Carob tree were clearly presented by B-J. Zhu and his colleagues. They showed clear structures and discussed the current and possible applications of Carob nutrients. A small size, yet summarily written review was published by K. Ghedira and P. Goetz. It briefly presented botany, ecology, selected medicinal activities and some active compounds. Finally, a very important review was published by J. I. Lopez-Sanchez and his colleagues, and it discussed the importance of D-pinitol (Figure 1), an active polyhydroxy, cyclic alcohol found in Carob pods. Authors presented the structure, physical properties, natural sources, synthesis and many health promoting effects of this natural product. In addition, many synthetic derivatives are introduced along with their biological activities.

We present here some of these derivatives in Figure 1. Based on their presentation of D-pinitol, authors named this compound “super food”.

Figure 1. D-pinitol and some of its selected derivatives (ref. 7).

MEDICINAL, BIOLOGICAL AND OTHER ACTIVITIES

Research of classical medicinal and related properties is ongoing, but in the last three years, there has been a notable increase in the studies of Carob as food and nutrition source. It is important to emphasize that classical research is still exploring very new activities that were not reported in our previous publication, such as treatment of male infertility. A summary of these properties is presented in Table 1.
### Table 1. Medicinal, nutritional and other properties of *C. siliqua.*

<table>
<thead>
<tr>
<th>Property/Activity</th>
<th>Methods/Results/Reference</th>
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<tbody>
<tr>
<td>Antibacterial, anti fungal and related activities</td>
<td>Hydrodistillation was used to extract essential oil (EO) from dried pods. EO was active against several bacteria species. Leaves and green pods were extracted with ethanol and extract was found active against <em>F. oxysporum</em> and <em>M. fructigena.</em> Leaves were extracted with water and ethanol, and both extracts were active against several species of fish infecting bacteria. Other properties were also reported but not detailed.</td>
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<tr>
<td>Antidiabetic and related activities</td>
<td>Methanolic extract of dry, unripe pods was active against STZ-nicotinamide-induced hyperglycemia in rats. Carob honey was extracted with water and ethyl acetate. Both extracts showed hypoglycemic activity in STZ-induced diabetes in rats.</td>
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<tr>
<td>Antidiarrheal, anti emetic</td>
<td>Dry unripe pods were extracted with 70% aqueous methanol, and extract was fractionized with water and dichloromethane. Both fractions were analyzed for general chemical composition and were found active antidiarrheal and antiemetic in rat model.</td>
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| Antioxidant, and related activities | EO was extracted from dried pods and was tested for antioxidant activity (DPPH). Methanolic extract of dry, unripe pods was active antioxidant (DPPH, FRAP). Carob honey was extracted with water and ethyl acetate and both extracts had antioxidant activity (ABTS). Dry ripe pods were extracted with methanol and extract had activity against lipid peroxidation in rat model. General chemical composition and antioxidant activity (DPPH) were determined for honeys. Aqueous extracts were prepared from the ripe pods of two “Carob” trees: *Ceratonia siliqua* and *Prosopis alba.* Both extracts were analyzed for general chemical composition and their antioxidant activity (DPPH) was compared: *C. siliqua* extract had higher activity. Aqueous extracts of pods in different ripening stages were prepared and analyzed for general chemical composition and tested for antioxidant activity (DPPH). Dry ripe pods were roasted with hot air and microwave radiation, then extracted with water. The extract had high antioxidant activity (DPPH), high sugar and D-pinitol contents, and authors reports that there was 50% energy saving in this roasting process compared with regular roasting. Seeds were extracted with several solvents and analyzed for fatty acids, lipidic compounds, amino acids, phenolics, monosaccharides, all in details. Some activities of galactomannan fractions were tested. Antioxidant activity was determined by DPPH method. Commercial aqueous extracts of pods and seeds, along with Carob fructooligosaccharides were supplemented to mice. This resulted in cardioprotective activity and amelioration of metabolic syndrome in mice. Antioxidant capacity of this mixture was determined (ABTS). This comprehensive research used Carob industry by-products that were extracted with methanol and 50% aqueous methanol. Extracts were analyzed for general chemical composition, total phenolic content, and tested for antioxidant activity (4 methods), ACE inhibition, anti-inflammatory activity (3 tests, triglyceride content (metabolic syndrome). Busulfan-induced infertility in mice was treated with Carob aqueous extract (plant part/s not reported). As a result, sperm quality improved, biochemical parameters related to fertility were also improved, including testosterone. Rabbits were treated with seeds aqueous extract, and there were positive changes in concentrations of sperm, plasma and testosterone. Infertile men were treated with 1500 mg capsules of mature pods aqueous extract, resulting improvement in sperm count and quality. Leaves and fruits were extracted with water and extract was administered to normozoospermic aged men, resulting improvement of sperm and chromatin. Dried pods aqueous extract was injected to mice. The dose of 200 mg kg⁻¹ of body weight, was most efficient in increasing testicular index, sperm parameters and decreased the level of oxidative stress. Infertile men in ages 25-40 years were treated with a combination of Carob syrup and vitamin E. As a result, sperm and sex hormones increased. Infertile men were treated with Carob aqueous extract, resulting improvement of sperm and chromatin. Dried pods were extracted with 96% aqueous ethanol. The extract was supplemented to mice with Pb (lead) reproductive toxicity, resulting improvement of sperm, sex hormones and other biochemical fertility parameters. Carob honey was extracted with water and ethyl acetate. Both extracts showed hepatoprotective activity. Aqueous extracts were prepared from the ripe pods of two “Carob” trees: *Ceratonia siliqua* and *Prosopis alba.* Both extracts were analyzed for general chemical composition and their antioxidant activity (DPPH) was compared: *C. siliqua* extract had higher activity. 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Neuroprotective and brain related activities

Extract of ripe, dry pods was prepared and administered to rats that were exposed daily to tobacco waterpipe smoke. Positive results were recorded in amelioration of lung injuries. Dry unripe pods were extracted with 70% aqueous methanol, and extract was fractionized with water and dichloromethane. Both fractions were antispasmodic in rat model. Aqueous extract of ripe, dry pods was prepared and administered to rats that were exposed daily to tobacco waterpipe smoke. Positive results were recorded in amelioration of brain injuries. Rats were fed with plus-maze resulting emotional disorders and estrogen deficiency. They were treated with pods aqueous extract that altered these diet effects. Parkinson disease model was induced in Zebrafish by neurotoxic 6-hydroxydopamine. After treatment with leaves aqueous extract, antioxidant and anti-AChE activity was recorded, resulting improvement of cognitive function. Aqueous extract of fruits and leaves had notable activity of skin regeneration and wound healing. A follow-up study by the same research group, but with clinical trials. Solid Carob waste from food industry was used for production of bio-hydrogen. The process was studied under various reaction conditions: pH, catalysis, nitrogen environment, water supply; and yield was compared to glucose a control substrate. Pods were extracted with water and bacteria fermented. Several ionic liquids were tested for extraction of dry ethanol instead of ethylene glycol. Microwave assisted extraction of phenolics afforded higher yields than non-radiated extraction. Thin layer pulp drying conditions were studied an optimized. Spray drying tested and proved successful in improving the quality and yields for drying of Carob juice. Various roasting conditions were tested in order to achieve optimal properties, such as sensory, antioxidant, aromas and physiochemical. Pods were extracted for sugar content, using the mathematical Taguchi method to design the extraction. Practically, pods were extracted four times successively. Powder of dry, ripe pods was found successful absorbent of dye pollution (methylene blue). Leaves aqueous extract was used to prepare CeO₂ nanoparticles (NPs) by reaction with Ce(NO₃)₃·6H₂O. These NPs had strong antioxidant and cytotoxic activities. Leaves aqueous extract was reacted with Zn(CH₃COO)₂ to prepare ZnO-NPs. These NPs had strong cytotoxic activity against human breast cancer cells. Proteins, amino acids and sugar contents were determined in pods at different harvesting stages. Leaves were extracted with pressurized hot water and fractionized with various solvents, resulting isolation and characterization of a new natural product, that researchers named siliquapyranone (Figure 2, below table, after note a). Solid-phase microextraction / gas chromatography-mass spectrometry (SPME/GC-MS) analyses of flowers and fruits were performed, and detailed composition of volatile compounds is reported.

Skin Protection

Chemical applications, processing and extraction

Animal food

Human food and nutrition

Chemical composition

Syrup was supplemented to Tilapia Fish (O.mossambicus) in different concentrations. It was found that the optimal was 1.25%, which notably decreased serum glucose, triglyceride, cholesterol levels, it significantly increased the phagocytic activity, phagocytic index, respiratory burst and potential killing activity. No toxicity was recorded. Pods were supplied as food to sheep resulting high nutritional values.

Candies were prepared from pod powder as wheat flour substitute, dates and olive fruits. Physical and chemical parameters (nutritional values, antioxidant capacity, FRAP) were reported. Near Infrared spectroscopy technique was developed to detect Carob flour in cocoa powder. Cakes were prepared from pod powder as cocoa powder partial substitute. Physical and chemical parameters (nutritional values, antioxidant capacity, ABTS, DPPH) were reported. Pod powder was used to replace wheat flour (40 or 60%) in Cupcakes, and physical and chemical properties were improved, especially fat and sugar replacement. Soft drink was prepared from pods pulp and it had high physical and chemical qualities. Analysis of seed peel revealed 90% content of dietary fibers. Rice flour and pods powder were used to prepare gluten-free cakes, and results of various tests are reported. Pod powder was used as wheat flour substitute in muffins and positive physicochemical (weight loss, antioxidant, DPPH, ABTS) results were recorded. Bean molasses was used to produce low-calorie ice cream, with
high nutritional values. Pods molasses were mixed with sesame paste, resulting notable physical changes (color, viscosity) as well as rise in nutritional value (mainly proteins). A follow-up study to the previous one, where pods molasses were analyzed for physicochemical characteristics. Detailed analysis of five samples of molasses to determine the content of 18 nutritionally important phenolic compounds. A comprehensive study that analyzed different parts of Carob tree (excluding roots) for sugars, proteins, enzyme inhibition and other general chemical properties. High quality and pioneering study that analyzed 20 commercial Carob food products used in Cyprus. Detailed analyses of major nutritional parameters are presented. Pastry filling was prepared with pods flour and bean gum, and the rheological (texture, flow) were notably improved. Mathematical model was used for the products design. Adding Carob syrup and/or flour to sponge cakes increased their protein, fiber and carbohydrate contents. Polyphenol-rich pod extract was supplemented to Taekwondo athletes for 6 weeks. As a result, reduction of body weight and improvement of aerobic performance were recorded.

Numerous studies were published about the optimal methods of cultivating this tree. In terms of environmental efficiency, P. J. Corriera and his colleagues recommended growing Carob in mixed orchards with another classical Middle-eastern crops, such as Fig, Almond and Olive trees. A. Gugliuzzo and his colleagues discuss the damage that natural enemies of Carob can do to this tree, especially insects (mainly Carob moth, Ectomyelois collaranae) and fungi. And in order to make the best selection of wild varieties for domestic cultivation, N. Korkmaz and her colleagues discuss the various parameters that affect tree morphology, fruiting yield, quality and composition, in wild-grown trees. Along with these studies, many have investigated the effect of growth stressors such as dry farming, or growth promoters like ultrasonic radiation, and other nutrients and cytokinins.

However, it seems that the hottest medicinal research of Carob in the next few years will be treatment of male infertility. To the best of our knowledge, this activity in regard of Carob tree, is not known in Middle-eastern medicines, contrary to female infertility, which is treated with pollen grains of this tree. Modern review articles did not recognize yet this very important medicinal activity of Carob, but since most studies agree that oxidative stress has major role in inducing male infertility, Carob has great potential of treating this global health concern.

CONCLUSIONS

(1) Medicinal properties of Carob have to be additionally studied, especially treatment of male infertility.

(2) The nutritional and economic potential of Carob are huge. In order to maximize them, more research should be invested in Carob’s health and nutrition benefits.

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Received: 17.05.2020. Accepted: 20.06.2020.