REVIEW

Medicinal Plants: dual source of enzymes and enzyme modulators

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ABSTRACT

Medicinal plants are recognized for their magical medicinal properties and they act as an imperative reservoir for drug discovery. Plant enzymes offer a complete spectrum of useful activities, such as amylase for digesting starches, cellulase to catalyze cellulose, and protease for the proteolysis of proteins. Enzymes are considered as the worker bees that make things happen. The presence of appropriate levels of an enzyme is critical for a healthy body system. Over or under-expression of enzymes may lead to an abnormal cascade of biological events, which eventually results in various disorders. From ancient times, humanity has been treating various ailments with medicinal plants which could find basis in their being a source of enzymes or enzyme modulators. Therefore, medicinal plants are a rich source of enzymes or bioactive natural products which could be used as enzyme modulators for the management of various disorders. For example, Gaucher's disease is a progressive lysosomal storage disorder caused by the lack of glucocerebrosidase, which leads to dysfunctions in multiple organ systems. Enzyme replacement therapy (ERT) with two therapeutic enzymes, commercialized as Replagal (Agalsidase alfa) and Fabrazyme (agalsidase beta), is currently used as a therapy for Gaucher's disease. Celiac disease is a digestive malabsorption disorder associated with an allergic response to foods containing gluten. Papaya-derived protease commercialized as Gluten-Ade is efficient in some cases of gluten intolerance.

KEYWORDS: Medicinal plants, Enzymes, and Enzyme modulators.

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INTRODUCTION

Medicinal plants are used by 80% of people in the world for their simple health requirements. The link between medicinal plants, humans and drugs derived from the medicinal plants describes the past of men. Medicinal plants are the generous basis of natural drug molecules (Rauf *et al.* 2017). Medicinal plants could be used as a source of enzymes. Plant-derived enzymes play a diversified role in many sides of everyday life, including treatment and management of various conditions such as dyspepsia and digestive disorders, the production of food, and find several industrial uses (Roxas 2008). Enzymes are nature's catalysts. Therefore, people have used them for several years to carry out important chemical reactions for making products such as beer, cheese and wine. Yogurt and bread also owe their texture and flavor to a variety of enzyme producing organisms that were domesticated many years ago (Gurung *et al* 2013).

Medicinal plants have been utilized to treat several disorders. However, pure compounds were not isolated from plants until the 1800s, paving the way for modern pharmaceuticals. In 1805, morphine was isolated the opium poppy (*Papaver somniferum*) by the German pharmacist Friedrich Serturner. Felix Hoffmann synthesized aspirin in 1897, after the isolation of salicylic acid from the bark of the willow tree (*Salix alba*). The artemisinin (antimalarial drug) was discovered in 1972 from the Chinese herb qinghao (sweet wormwood, *Artemisia annua* L.). These examples explain the rich history of plant-derived medications. In traditional Chinese medicine (TCM), numerous plants are used for the treatment of angiogenic ailments such as chronic wounds and rheumatoid arthritis. Thus, it is rationale to consider these medicinal plants as a source for new angio-modulators (Fan *et al.* 2006). In this article, we review the plant-based angio-therapy to prove that medicinal plants could be used also to modulate enzymes' activities.

Classification of enzymes

Different kinds of enzymes are believed to exist in the human body, each with a specific role. There are three general classes of enzymes: digestive enzymes, metabolic enzymes, and food or plant enzymes. The digestive enzymes category involves the enzymes produced within your own body to help break down food into its basic components for digestion. Metabolic enzymes are found throughout our entire body – in our organs, blood, bones, and even within the cells that produce them. They function in support of our lungs, kidneys, heart, and brain. Food and plant enzymes naturally exist in raw food. They generally assist the same role as digestive enzymes, but these are the enzymes that we may consume through our diets, as opposed to the ones that our bodies produce. We can obtain these enzymes by eating fresh, raw and uncooked foods like fruits, vegetables, unpasteurized dairy, eggs, meat and fish (Howell 1995).

The modern diet generally revolves around processed and cooked food, but these processes destroy the naturally occurring enzymes contained in the food. This places a substantial burden on our bodies to promote the enzyme requirement for breaking down that food. Raw food comprises the necessary amount and types of enzymes required to digest itself. This remains one of the biggest benefits of a diet focused on raw food (Shaffer 2009). The major components of the food (sugar, protein, starch, and fat) and their respective caloric amounts determine what type and quantity of enzymes also exist. For example, the enzyme amylase is found in high carbohydrate fruits like apples and peaches. Fruits that are rich in fat, such as avocados, contain the enzyme lipase. Below, we will focus on enzymes we obtain from food sources (animal, plant and fungal) and their corresponding usefulness.

Plant-derived enzymes

Fruits and vegetables are commonly consumed in their raw and natural form. This alleviates the main issue with animal-based enzymes by conserving the integrity of the enzymes themselves. Additionally, plant-derived digestive enzymes are effective over a broad range of pH levels. This range is generally supposed to be between 3.0 and 9.0, which is highly well-matched with the human gastrointestinal environment. Therefore, plant-derived enzymes are compatible with supporting comprehensive digestive health (Rachman 1997).

Four vital enzymes often found in plants are amylase, lipase, protease, and cellulase. Amylase helps our body with the breakdown and consequent absorption of carbohydrates. Lipase facilitates the digestion of fat. When our diet includes lipase-rich foods, it eases the production burden on the gall bladder, liver and pancreas. Protease breaks down the protein that can be present in meat, fish, poultry, cheese, and nuts. Cellulase is present in many fruits and vegetables, and it breaks down food fibers, which increases their nutritional value to our bodies. The presence of cellulase in plant-derived sources is important because it does not naturally exist in the human body.

Vegetables and fruits are a perfect source of enzymes. They are enzyme-rich and easily eaten without being cooked or processed, ultimately preserving the full functionality of the enzymes (Rachman 1997).

Fungal-derived enzymes

Fungal-derived enzymes have several uses. They are critical in the preparation of many food products, like beer, soy sauce, miso, baked goods, dairy, and processed fruit. One of the oldest known applications is the role of yeast in alcohol fermentation. Fungal enzymes are commonly produced from a fungal source called Aspergillus. For example, *Aspergillus oryzae* is used in the preparation of sake and soy sauce, while *Aspergillus sojae* is also used in soy sauce preparation as well as in miso soup (Park *et al.* 2017). One of the most popular and well known culinary fungi is the mushroom. Some mushroom species produce enzymes, including hydrolases, esterases, and phenol oxidases. Fungi and their enzymes can also be found in yeast spreads and certain types of cheeses, such as Camembert and blue cheeses.

Fungi also contain a variety of enzymes, such as protease, amylase, lipase, cellulase, and tilactase (aids lactose absorption). Like plant enzymes, fungal enzymes are acid-stable and can withstand the pH range of the stomach. They are also suitable for a vegetarian diet, unlike animal-sourced enzymes (Østergaard *et al.* 2011).

Plant versus animal-derived enzymes: Which should you use?

Plant-derived enzymes have very similar functions as their pancreatic counterparts. Trypsin and chymotrypsin are pancreatic proteases. Proteases from plant and fungal sources have trypsin-like functions; they all cleave proteins. But the effectiveness of the enzymes can vary based on their source. Pancreatic enzymes cannot function in acidic conditions. The gut goes to great lengths to reduce the acid content in the food mass once it enters the small intestine. Sodium bicarbonate is produced along with the pancreatic enzymes and is released into the gut at the same time. The bicarbonate raises the pH of the food mass and the pancreatic enzymes go to work. Plant enzymes, however, have no pH limitations. They can perform the same job as the pancreatic enzymes, either in acid or alkaline conditions. Plant enzymes are happy to go to work as soon as they dissolve in the stomach fluid and can begin the business of food breakdown much quicker. In fact, by the time the plant enzyme-enhanced food mass enters the small intestine, much of the food will have already been degraded. For the person with food intolerance - the time difference can be

crucial. Proteins and peptides are not absorbed from the stomach. Using an acid-stable enzyme blend can degrade gluten, casein, soy, and other food proteins to the extent that those foods are tolerated once they enter the gut and absorption occurs (Roxas 2008). In summary, if you're interested in increasing your enzyme intake efficiently, the usefulness of plant-sourced and fungal-sourced enzymes outweighs that of animal-sourced enzymes.

Table 1: List of medicinal plants contain enzymes with their applications.

Plant-derived enzymes			
Plant	Enzyme	Role	Ref.
Ananas comosus (Pineapple)	Bromelain, sulfhydryl-containing, proteolytic enzymes	Bromelain can be purchased in powdered form to help tenderize tough meats. It's also widely available as a health supplement to help people who struggle to digest proteins. It can be used for grain protein solubilization, beer clarification, etc. It acts as an enzymatic browning inhibitor in fresh apple juices. It also helps to treat acne, wrinkles, and dry skin. Anti-inflammatory and anti-cancer agent	(Sivakumar *et al.* 2016), (Arshad *et al.* 2014)
Carica papaya (Papaya)	Papain *Make sure to eat papayas ripe and raw, as high heat will destroy their digestive enzymes. Also, unripe or semi-ripe papayas can be dangerous for pregnant women, as it may stimulate contractions.	Proteases that help digest proteins (papaya-based formula) may help ease digestive symptoms of IBS, such as constipation and bloating. Detergent, healing burn wound, textiles, cosmetics industry Papain act as a debris-removing agent, with no negative effect as it acts at a specific tissue, which particularly lacks in alpha-1 antitrypsin plasmatic anti-protease which has the property to inhibit proteolysis in healthy tissue. Papain shows feature similar to cysteine protease and also the folding pattern around the active site that has its use for drug manufacturing.	(Meara *et al.* 1996), (Chatterjee *et al.* 2018)
Mangifera indica (Mango)	Amylases	They help break down carbs so that they are easily absorbed by the body.	(Stanley *et al.* 2005)
Musa acuminata and *Musa balbisiana*	Amylases and Glucosidases	Two groups of enzymes that break down complex carbs like starch into smaller and more easily absorbed sugars.	(Stanley *et al.* 2005)

(Banana)			
Persea Americana (Avocados)	Lipase	This enzyme helps digest fat molecules into smaller molecules, such as fatty acids and glycerol, which are easier for the body to absorb.	(Chase 1921)
Zingiber officinale (Ginger)	Zingibain	It digests proteins into their building blocks It helps to increase the body's production of digestive enzymes like amylases and lipases. Anti-proliferative agent	(Thompson *et al.* 1973)
Actinidia deliciosa (Kiwifruit)	Actinidain	protease that helps digest proteins and is commercially used to tenderize tough meats	(Boland 2013)
Legumes (beans and peas) and potato tubers.	Lipoxygenase From Soybeans	It acts as an ingredient for the production of bread. It also acts as an aroma enhancer. It affects color, off-flavor, and anti-oxidant properties of foods in a negative way.	(Baysal *et al.* 2007)
Ficus carica (fig latex)	Ficin	Protease that helps digest proteins	(Chatterjee *et al.* 2018)

Plants enzymes: applications for enzyme replacement therapy (ERT)

α-Galactosidases (EC 3.2.1.22) are glycosidases that break down the terminal α-linked galactose residues from glycol-conjugate substrates. α-Galactosidases contribute to the turnover of cell wall-associated galactomannans in plants and the lysosomal degradation of glycosphingolipids in animals. Insufficiency of human α-galactosidase A (α-Gal A) results in Fabry disease (FD), a genetic, X-linked lysosomal storage disorder, characterized by the accumulation of globotriaosylsphingosine (lysoGb3) and globotriaosylceramide (Gb3) (Germain 2002). FD current management comprises enzyme replacement therapy (ERT). To study the α-galactosidases for their use in FD therapy, an activity-based probe (ABP) which covalently labeling the catalytic nucleophile of α-Gal A was designed. Here, we report that these ABP labels proteins in *Nicotiana benthamiana* leaf extracts, aiding the identification and biochemical characterization of the *N. benthamiana* α-galactosidase, we name here A1.1 (gene accession GJZM-1660). The transiently refined over-expressed enzyme was a monomer lacking N-glycans and was active toward the 4-methylumbelliferyl-α-D-galactopyranoside substrate (Km = 0.17 mM) over a wide pH range. Structural analysis of A1.1 by X-ray crystallography exposed marked similarities with human α-Gal A, even including A1.1's capability to hydrolyze Gb3 and lysoGb3 that are not endogenous in plants. Of note, A1.1 uptake into FD fibroblasts decreased the elevated lysoGb3 levels in these cells, consistent with A1.1 delivery to lysosomes as revealed by confocal microscopy. The simplicity of production and the features of A1.1, such as stability over a wide pH range, and its capacity to degrade glycosphingolipid substrates, need further analysis for its value as a potential therapeutic agent for ERT–based FD management (Kytidou *et al.* 2018).

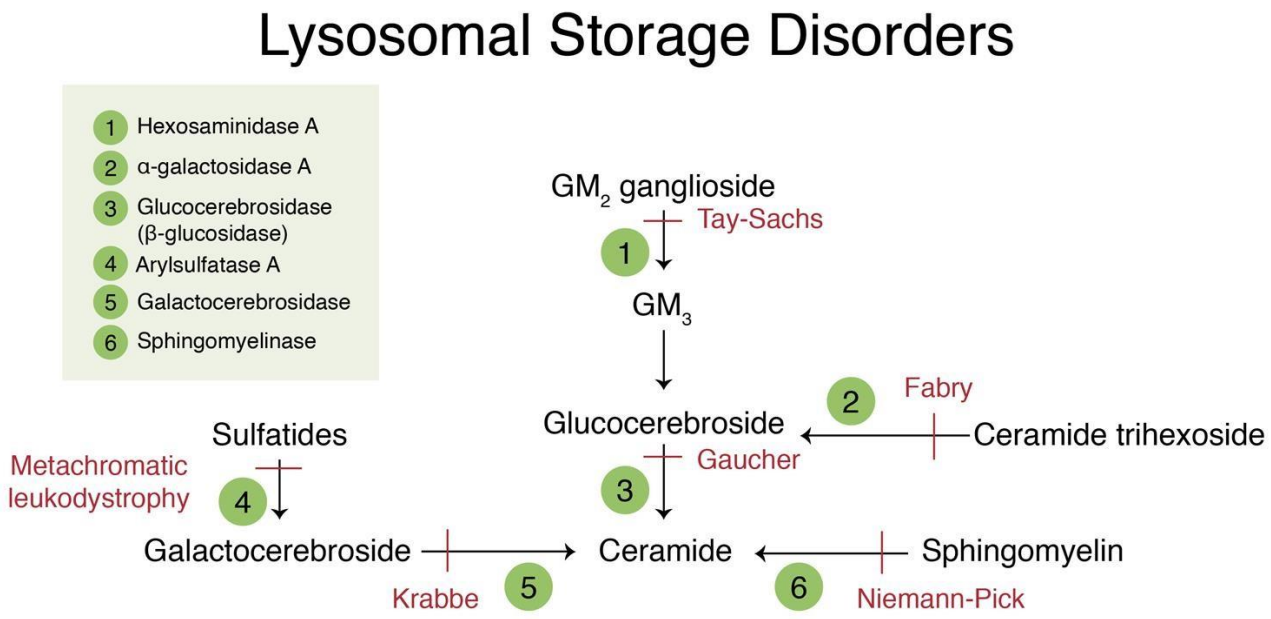

Figure 1: Enzymes associated with lysosomal storage disorders (https://step1.medbullets.com/).

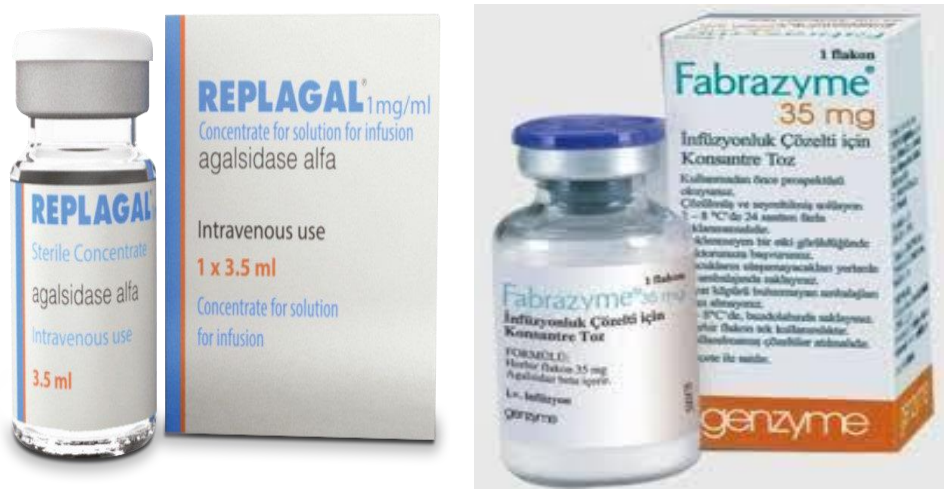

Figure 2: Two recombinant protein therapeutics, Replagal (agalsidase alfa, Pharm`net-dz.com) and Fabrazyme (agalsidase beta, http://www.3scorporation.com/), have been accepted in Europe as enzyme replacement therapies for Fabry disease. Both contain the same human enzyme, α-galactosidase A, but they are produced via different protein expression systems and have been permitted for administration at different doses.

Weaknesses of plant enzymes for ERT

The effectiveness of the present ERT interventions is considered to be poor: ERTs can fail to reach all organs, particularly the kidney and heart, which often make the complications in Fabry patients. According to the researchers, this possibly occurs

as a result of the insufficient usage of the therapeutic enzyme to enter these organs.

Opportunities in ERT

After isolating the plant enzyme, the team saw that its structure revealed obvious similarities with the human alpha-galactosidase A. Additionally, A1.1 was able to break down both types of fat that accumulate in Fabry's disease – globotriaosylceramide and globotriaosylsphingosine – with an effectiveness similar to that of Fabrazyme (agalsidase beta).

Researchers then tested A1.1's effectiveness in a specific type of cells, called fibroblasts, from Fabry disease patients. Cells incubated overnight with A1.1 had lower levels of fat accumulation, reaching those commonly found in healthy fibroblasts.

Production of a plant-derived alpha-galactosidase A, enzyme carries considerably lower costs compared to those of current human recombinant enzymes, permitting the use of higher doses. Also, A1.1 showed no cross-reactivity with neutralizing antibodies directed against the human form of the enzyme (Ferraz *et al.* 2014).

Overall, these results reinforce "further research on the optimization of plant alpha-galactosidases like A1.1 to decrease the toxic lysoGb3 [globotriaosylsphingosine] in Fabry disease should be considered to encounter the need for an affordable treatment of this devastating disorder" researchers wrote.

Preclinical and phase I Investigation of plant-derived recombinant human gluco-cerebrosidase enzyme

Gaucher's Disease

Gaucher's disease is a progressive lysosomal storage disorder caused by the lack of glucocerebrosidase, which leads to the dysfunction in multiple organ systems (Lee 1982). Intravenous enzyme replacement is the recognized standard treatment. Safety and pharmacokinetics of a new human recombinant glucocerebrosidase enzyme expressed in transformed plant cells (prGCD) has been assessed by administering to primates and human subjects. Short term (28 days) and long term (9 months) recurrent injections with a regular dose of sixty units/kg and a high dose of 300 units/kg were administered to monkeys (n = 4/sex/dose). Neither clinical drug-related side effects nor neutralizing antibodies were found in the animals. In a phase I clinical trial, six healthy volunteers were treated by intravenous infusions with rising single doses of prGCD. Doses of up to 60 Units/kg were administered at weekly intervals. The prGCD infusions were very well tolerated. Anti-prGCD antibodies were not detected. The pharmacokinetic profile of the prGCD showed a prolonged half-life compared to the commercial enzyme (imiglucerase) that is manufactured in a costly mammalian cell system. These studies reveal the safety and absence of the immunogenicity of prGCD (Aviezer *et al.* 2009).

Enzyme replacement therapy (ERT) with two therapeutic enzymes, commercialized as Replagal (Agalsidase alfa) and Fabrazyme (agalsidase beta), is currently used as a therapeutic strategy. ERT provides an external source of alpha-galactosidase A, where the enzyme is produced in mammalian cells.

Celiac Disease

Celiac disease is a digestive malabsorption disorder associated with an allergic response to foods containing gluten, a protein found in some grains, including wheat, rye, and barley (Barker and Liu 2008). Gluten-Ade is a gluten digestive papaya-derived formula that is efficient in some cases of gluten intolerance (Krishnareddy et al. 2017). In one case study, oral papain was administered to an adult male celiac patient suffering from intestinal malabsorption who had partial atrophy of the intestinal wall. Before treatment, he was placed on a gluten-free diet, resulting in weight gain and symptom improvement; however, steatorrhea persisted. The

patient administered 1,800 mg of an enteric-coated papain enzyme tablet with each meal and was capable of tolerating some gluten. After one month on this protocol, the patient no longer experienced loose stools and absorption normalized (Messer *et al.* 1976).

Research applications of proteolytic enzymes in molecular biology

Proteolytic enzymes' action is essential in several physiological processes, e.g., in the digestion of food proteins, signal transduction, cell division, apoptosis, processing of polypeptide hormones, and the life-cycle of many disease-causing organisms comprising the replication of retroviruses (Neurath *et al.* 1976, Devlin 2006). They have great medical, pharmaceutical, and academic importance because of their crucial role in the life-cycle of many pathogens and hosts.

Proteases are extensively applied enzymes in several areas of industry and biotechnology. A lot of research applications require their use, including the Klenow fragments production, peptide synthesis, unwanted proteins digestion during nucleic acid purification, cell culture experiments and tissue dissociation, preparation of recombinant antibody fragments, diagnostics and therapy, exploration of the structure-function relationships by structural studies, affinity tags removal from fusion proteins in recombinant protein methods, peptide sequencing, and proteolytic digestion of proteins in proteomics (Mótyán, Tóth et al. 2013).

Klenow Fragment Production

The large fragment of the *E. coli* DNA polymerase I enzyme is termed as the Klenow fragment. While the holoenzyme has 5'→3' polymerase, 3'→5' and 5'→3' exo-nuclease activities, the Klenow fragment has only the polymerase and the 3'→5' exo-nuclease activities. The Klenow fragment has several uses in the recombinant DNA technology, such as labeling, sequencing, and site-specific mutagenesis of DNA.

The enzymatic method to produce the large protein fragment by proteolysis from the DNA polymerase I holoenzyme was published in 1970 (Klenow and Henningsen 1970). Subtilisin-catalyzed proteolytic cleavage was used to yield Klenow fragment leading to retaining of the polymerase and the 3'→5' exonuclease activities and the loss of 5'→3' exonuclease activity of the intact polymerase.

Enzymatic Peptide Synthesis

The enzymatic method of peptide synthesis has been recurrently used for pharmaceutical and nutritional purposes. This method has numerous benefits compared to chemical methods, such as stereo-specificity with side-chain protection, the non-toxic nature of solvents and the possibility of recovering the reagents used for synthesis. Enzymes have been chosen according to their specificity for amino acid residues (Table 3), but this is limited by the possibility of the peptide bond hydrolysis (Morihara 1987). Enzymatic peptide synthesis can be prepared by equilibrium- or kinetically-controlled methods.

Cell isolation and tissue dissociation

Cell biology studies frequently require the primary tissues dissociation and viable cells isolation for tissue culturing. The enzymatic digestion is the most preferable to isolate the junctions connecting the cells and the surrounding extracellular matrix components, by which the cells can be produced from a wide variety of tissues. Several enzymes are available in the market for cultured cells detachment, cell dissociation and cell component or membrane-associated protein isolation (Mótyán *et al.* 2013). Besides the polysaccharidases, nucleases, and lipases, the proteases are the most important enzymes used broadly to dissociate cells from tissues.

Antibody fragment production

The monoclonal antibodies fragments are widely used in diagnostics, therapeutics and biopharmaceutical research having valuable properties over the whole immunoglobulin molecules due to their smaller size and lower immunogenicity (Rader 2009). Fragments of whole immunoglobulin molecules can be produced using recombinant DNA technology or by enzymatic digestion. Here we discuss the proteolytic antibody fragmentation method. Commonly, the papain and ficin proteases are used for the specific digestion of IgG molecules. Digestion of an antibody by papain (cysteine protease), produces three fragments due to the cleavage of peptide bonds in the hinge region between C_{H1} and C_{H2} domains: one Fc (crystallizable) and two identical Fab (antigen binding) fragments are released (Figure 3B). While both released Fab fragments carry one antigen-binding site, the Fc fragment does not have the antigen-binding ability. The ficin, which is also a cysteine protease, can release both $F(ab')_2$ or Fab fragments (Figure 3C), based on the cysteine concentration. The digestion of monoclonal antibodies by papain or pepsin is still used to produce Fab or $F(ab')_2$ fragments. In some cases, it could be better to obtain the Fab fragments in high quality by recombinant expression in cell lines and produce the fragment in sufficient quantity. Therefore, these types were found to be more suitable for crystallization experiments (Zhao *et al.* 2009).

Table 2: Some proteolytic enzymes Substrate specificity which are used in molecular biology research.

Enzyme	Main source	Cleavage site
A. Endopeptidases		
i. Serine proteases		
Proteinase K	fungal	-aromatic, aliphatic or hydrophobic ↓nonspecific-
ii. Cysteine proteases		
Bromelain	plant	-nonspecific↓nonspecific-
Papain	plant	-Arg (or Lys)↓nonspecific-
Ficin (ficain)	plant	-nonspecific↓nonspecific-
B. Exopeptidases		
- Serine proteases:		
Carboxypeptidase Y	yeast	-nonspecific↓nonspecific

* Proteases are classified according to their catalytic mechanisms; moreover, the main sources and enzyme specificities are listed. The arrows refer to the cleavage sites.

Table 3: Examples of peptides synthesized by proteases.

Peptide	Sequence	Enzyme(s)	Reference
Nutritional peptide	Tyr-Trp-Val	papain	(Kimura *et al.* 1990)
Oxytocin	Cys-Tyr Tyr-Ile Pro-Leu Leu-Gly	Papain	(Rizo *et.al.* 1992)
Mouse EGF (21–31)	His-Ile-Glu-Ser-Leu-Asp-SerTyr-Thr-Cys	Papain	(Widmer *et al.* 1984)

Table 4: Enzymes used for cell isolation and tissue dissociation.		
Enzyme	Source	Action
Papain is a cysteine peptidase	*Carica papaya* latex	Papain has amidase and esterase activities similarly to elastase and has a wide specificity. Papain has a lower harmful impact on tissues and therefore, it is characteristically used for cell dissociation of neuronal tissues. papain is also broadly used for the solubilization of integral membrane protein and proteoglycans digestion.

Antibody fragments have many useful properties for *in vivo* applications compared to whole antibody molecules. Their smaller size gives them higher mobility, tissue penetration, and cell membranes permeability. As antigen-binding fragments lack the Fc fragments, they have lower immunogenicity, as they contain only their antigen-binding domain sites (Fab, Fab' or $F(ab')_2$ fragments) and do not have the regions responsible for antibody effector functions.

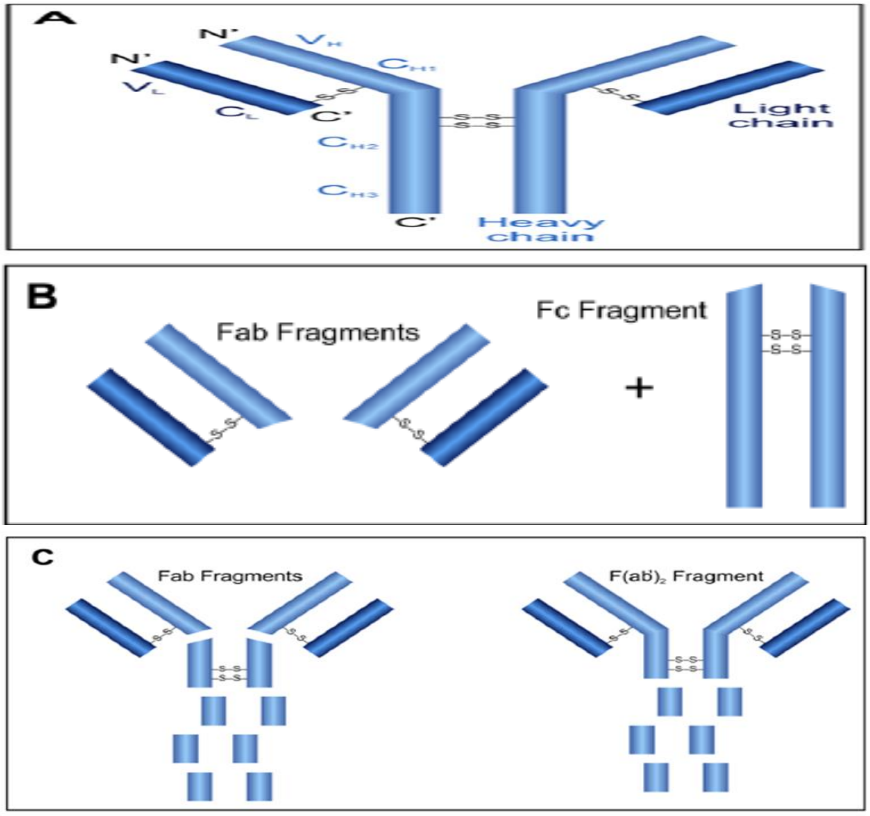

Figure 3: Structure of (A) IgG antibody molecules, (B) and (C) fragments released after proteolytic digestion using papain and ficin, respectively (Mótyán 2013).

Those fragments have several clinical and therapeutic applications. They could be used to prevent the development of a disease (e.g., restenosis), for the management of some diseases (e.g., macular degeneration), applied during the diagnosis (e.g., metastatic breast and colon

cancer), or to detect toxins or neutralize snake venoms (Flanagan and Jones 2004). The current number of antibody-based therapeutics accepted by the FDA is 35, while many other antibodies are in clinical trials (Mótyán *et al.* 2013)

Proteomic applications

Proteomic studies aim to identify, characterize, and quantify the required samples and typically include mass spectrometric (MS) analysis. Chemical properties, post-translational modifications, and structural properties of proteins could also be revealed by MS, as well as the determination of the composition of protein complexes.

Generally, the samples to be analyzed have a mixture of various proteins and/or polypeptides that have to be separated and digested into smaller fragments before the MS analysis. Protein separation can be performed efficiently by polyacrylamide gel electrophoresis, then the separated proteins can be digested by chemical cleavage or by enzyme-catalyzed digestion of peptide bonds. The process in which the bands or spots are cut out from the gel followed by the addition of protease(s) to the gel containing the protein(s) of interest is called in-gel digestion (Steen and Mann 2004). Whole protein proteolysis leads to the release of smaller peptides with different molecular masses which are appropriate for MS analysis (Figure 4).

Papain, subtilisin, and some other proteases are also appropriate enzymes for fragmentation in MS analysis (Granvogl, Plöscher *et al.* 2007).

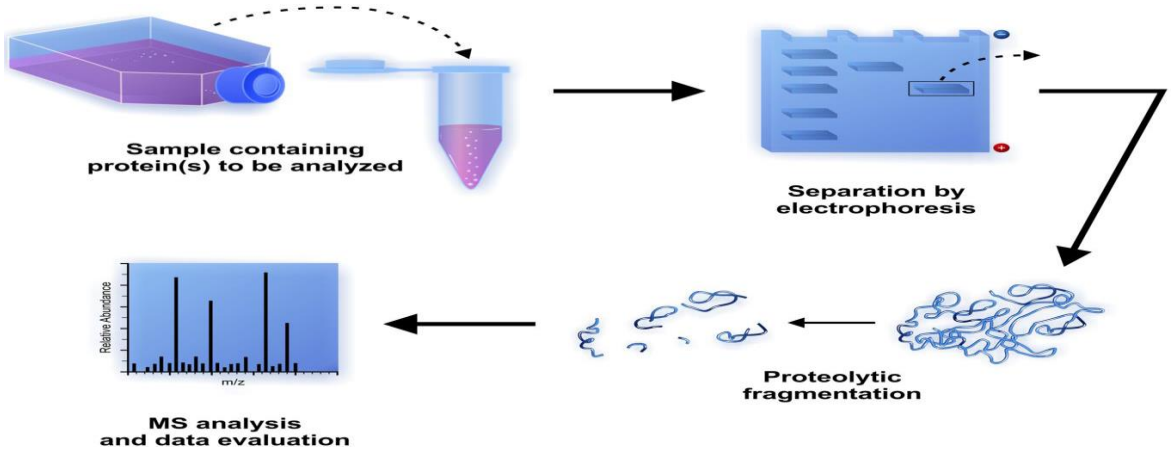

Figure 4. Steps of proteomic analysis using mass-spectrometry after separation and in-gel digestion of proteins of interest (Mótyán 2013).

Applications developed for *in silico* protein fragmentation are beneficial to predict the proteolytic fragments and to choose the most proper enzyme for the most effective digestion depending on the enzyme specificities (http://prospector.ucsf.edu).

Generally, highly sequence-specific proteases are favored for protein fragmentation rather than less specific enzymes, as the latter ones produce a very complex fragments mixture. In the case of the efficient peptide fragmentation, the fragments have a suitable length and are released in high yield, and the complete sequence of the whole protein can be covered by the analysis of the proteolytic fragments.

Medicinal plants as a source of enzyme modulators (inhibition/activation)

Impairment of enzyme activity (high or low) produces several health disorders. Therefore, enzyme modulatory agents are an attractive area in drug discovery because of their application in

treating several conditions. Medicinal plants are rich in secondary metabolites that showed wide-spectrum enzyme modulatory potential. Bioactive secondary metabolites can deliver excellent pharmacophore patterns for medications associated with numerous illnesses (Rauf *et al.* 2017). The second part in this article is planned to document the enzyme modulatory potential of plant extracts, and their isolated pure compounds as angiogenic modulators.

Angiogenesis is a common denominator of many diseases

In 1994, The Angiogenesis Foundation (http://www.angio.org) announced angiogenesis a 'common denominator' in the most serious diseases of society. In many ailments states, the body loses angiogenesis control.

Angiogenesis is a physiological process by which new blood vessels are formed from pre-existing vasculature. It is tightly controlled by a balance of angiogenic factors and inhibitors and occurs only in wound healing, embryonic development, and the female reproductive cycle. Angiogenic diseases result from new blood vessels growing either insufficiently (e.g. ischaemic heart disease and chronic wounds) or excessively (e.g. diabetic retinopathy, psoriasis, and cancer).

Excessive angiogenesis

Excessive angiogenesis occurs in diseases like (cancer, age-related macular degeneration, psoriasis, and endometriosis), when diseased cells produce large amounts of angiogenesis factors abnormally (e.g. vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2 and hepatocyte growth factor), diminishing the effects of natural angiogenesis inhibitors (e.g. endostatin, angiostatin, and thrombospondin). In these conditions, new blood vessels act as a supply for the diseased tissues and destroy the normal ones. In cancer, tumor cells utilize the new vessels to escape into the circulation and lodge in other organs (tumor metastasis). Anti-angiogenic therapies, aiming to suppress the growth of new blood vessels, are being established to treat these chronic diseases (Malecic and Young 2017).

Insufficient angiogenesis

In chronic wound, stroke, coronary artery disease, and non-union fracture, inadequate (in size and/or number) blood vessels grow and circulation is not well restored, leading to the risk of tissue death and, in the case of alopecia, hair loss. Inadequate production of angiogenesis growth factors and/or extreme amounts of angiogenesis inhibitors leads to insufficient angiogenesis. Therapeutic angiogenesis, aiming to stimulate neovascularization with growth factors, is being discovered to inverse these conditions (Bisht *et al.* 2010).

Angiogenesis stimulators and inhibitors target one or more of these steps:

I. Synthesis and release of angiogenic factors; in response to hypoxia, injured or diseased tissues.
II. Angiogenic factors are binding to their receptors on endothelial cells (ECs).
III. ECs activation.
IV. Proteases are released to dissolve the basement membrane.
V. ECs migration and proliferation.
VI. Adhesion molecules (e.g. integrin avb3 and avb5) help to pull the sprouting blood vessel forward.
VII. Matrix metalloproteinases (MMPs) are formed to dissolve the extracellular matrix (ECM) and to initiate remodeling.
VIII. Angiopoietin–Tie-2 interaction modulates tubule formation.
IX. Regulation of loop formation by the EphB–ephrin-B system.
X. Pericytes are combined to stabilize the newly formed blood vessel.

Plants as a source of angiogenesis-modulating compounds

Recent genomics-concentrated drug-discovery efforts have failed to obtain the expected large number of compounds aimed at 'novel' targets (Szymkowski 2003). The chronic diseases multifactorial nature is probably the most critical problem concerning target discovery. By contrast, the identification of the efficacious drugs from medicinal plants has a long, albeit difficult, precedent.

Therefore, there is always a value in supplementing rational design and high-throughput screening in drug discovery by paying attention to traditional medicines.

Medicinal plants are complex chemical cocktails that contain numerous active ingredients with properties that modern pharmaceuticals cannot mimic. A wide range of plants contains pure compounds with angiogenesis modulating properties as shown in Table 6.

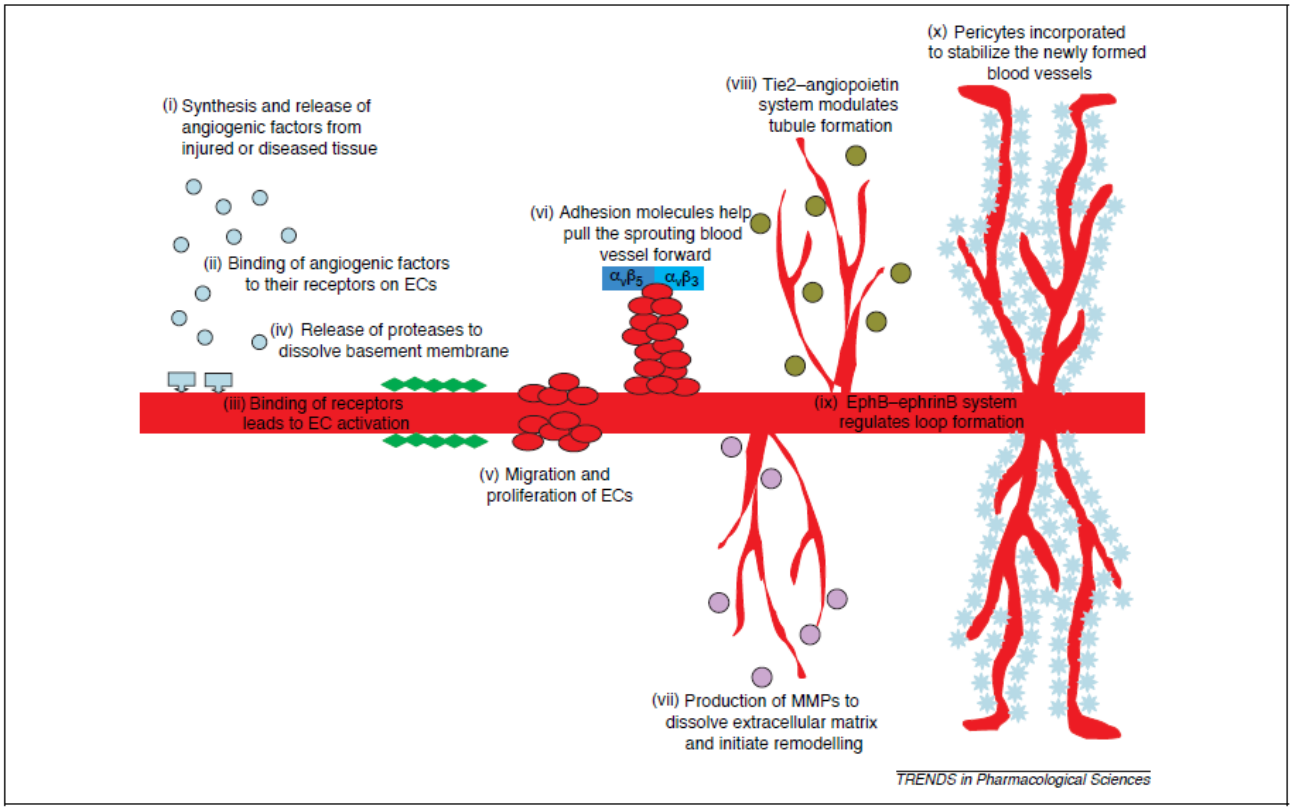

Figure 5. The ten consecutive steps of angiogenesis. Only the key cellular and molecular events are represented. Based on the microenvironment (e.g. leucocyte infiltration, oxygen tension, and release of antiangiogenic factors such as transforming growth factor-b and platelet factor 4), the newly formed vasculature either undergoes maturation into a functional network or retreats to keep the original vascular density (Fan 2006).

Table 5: Conditions that need angiogenic modulation either by stimulation or suppression.	
Angio-stimulation	Chronic wound
	Myocardial infarction
	Non-union fracture
	Alopecia
Angio-suppression	Tumor, arthritis, psoriasis
	Atherosclerosis, endometriosis
	Diabetic retinopathy
	Obesity

Table 6: Plant-derived angiogenic and antiangiogenic compounds and their mechanisms.		
Plant species	Compound	Mechanism
Angiogenic compounds		
Aloe vera	Beta-Sitosterol	Stimulation of the HUVEC motility; enhances von Willebrand factor, VEGF, VEGF receptor Flk-1 and laminin expression.
Vitis spp. (grape)	Resveratrol	VEGF and Flk-1 upregulation.
Panax ginseng	Ginsenoside Rg1	Upregulates eNOS expression, which leads to the PI3K–Akt pathway activation.
P. ginseng	Ginsenoside Re	Activation of the EC proliferation, migration and tube formation.
Astragalus membranaceus (huangqi)	Unknown	Unknown
Angelica sinensis (danggui)	Unknown	Unknown
Salvia miltiorrhiza (danshen)	Salvianolic acid B	Upregulation of the genes encoding MMP-2, VEGF, VEGF receptor 2 and Tie-1
Antiangiogenic compounds		
Glycine max (soybean)	Genistein	Suppresses the expression of VEGF and FGF-2. Inhibition of receptor tyrosine kinase. inhibition of NF-$_k$B and Akt signaling pathways
Camellia sinensis (green tea)	EGCG	Inhibition of the VEGF signaling by interfering VEGF receptor 2 complex formation.
Vitis spp. (grape)	Resveratrol	Disrupts Src-dependent VE cadherin tyrosine phosphorylation
Taxus brevifolia (Pacific yew tree)	Taxol	Disrupts microtubule cytoskeleton; inhibits VEGF production; inhibits HIF-1alpha protein
Vinca rosea (periwinkle)	Vincristine	Disrupts microtubule cytoskeleton. Inhibits

		production of VEGF.
Camptotheca acuminata	Camptothecin	Blockage of topoisomerase I. Inhibits EC proliferation and tube formation; Decreases the expression of HIF-1a and VEGF.
Combretum caffrum (African bush willow tree)	Combretastatin	Tubulin assembly inhibition.
Glycyrrhiza uralensis (liquorice)	Isoliquiritin	Tube formation inhibition.
P. ginseng	Ginsenosides Rb1, Rb2, and Rg3	Inhibition of the VEGF production by tumor cells
S.miltiorrhiza (danshen)	TIIA Cryptotanshinone	G1–G0 arrest of ECs Apoptosis of ECs
Sinomenium acutum	Sinomenine	G1–G0 arrest of ECs
Tripterygium wilfordii Hook.f.	Triptolide	Inhibits VEGF expression and secretion from ECs; inhibits COX-1, COX-2, and 5-lipoxygenase; decreases transcription of the gene encoding inducible nitric oxide synthase
Cordyceps militaris	Unknown	Inhibits FGF-2 expression in ECs and MMP-2 expression in tumor cells
Ganoderma lucidum	Polysaccharide peptide	Causes EC apoptosis by reducing Bcl-2 expression and increasing Bax expression; decreases VEGF secretion from tumor cells

CONCLUSION

This review revealed that medicinal plants are important in drug discovery. Medicinal plants are rich in enzymes that provide safe treatment for digestive mal-absorption disorders, such as exocrine pancreatic insufficiency. These enzymes also have several applications in molecular biology, proteomics applications, and treatment of lysosomal storage disorders such as Fabry and Gaucher's diseases.

On the other hand, medicinal plants are rich in natural compounds that could be used as natural enzyme modulators either by inhibition or activation. Those enzyme modulators have several applications in the treatment and management of various disorders such as impaired angiogenesis. Angiogenic activators could be used for chronic wound, myocardial infarction, and alopecia, while the angiogenic inhibitors are useful for tumor, arthritis, psoriasis, atherosclerosis, endometriosis, and diabetic retinopathy. Therefore, medicinal plants are a double-edged sword as a source for enzymes and a source for enzyme modulators.

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Conflict of interest statement

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Authors' contributions

Farid A. Badria: Suggested the article subject its design, and submitted the manuscript. Rowida M. Omar: collected, analyzed data and prepared the manuscript. Amal A. Galala: Supervising Rowida during the preparation of the manuscript.

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