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Original Research

Evaluation of Biological Activity of Natural Compounds: Antihyperglycemic, Anti-Inflammatory, Analgesic and Anticoagulant Activity

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Abstract:
All forms of life contain natural compounds, each of which has a unique structure and can be found in a variety of forms. Tannins, anthocyanins, and alkaloids are only some of the metabolites that function as a defence mechanism in living organisms. These metabolites are obviously molecules that are of interest to the food, cosmetic, and pharmaceutical sectors. Plants, microbes, and insects are all examples of suppliers of biomolecules that have a wide range of actions, yet in many cases, these biomolecules have not been thoroughly investigated. In order to make use of these molecules for a variety of purposes, it is necessary to have a solid understanding of their structure, concentrations, and the potential for biological activity. Since the 1950s, in vitro techniques have been developed that measure the biological activity of the molecules of interest without the presence of the molecules themselves. As of right now, a variety of strategies have arisen in order to overcome some of the restrictions that these old procedures have, primarily through reductions in the amount of time and money required. The purpose of this study is to provide an overview of the interactions between herbal drugs that have been used for the treatment of blood coagulation, as well as the effect of herbal pharmaceuticals on anticoagulant therapy in secondary metabolites composed of medicinal plants. This review is comprised of multiple papers. It has been demonstrated that tridax procumbent possesses a multitude of active constitutions that have therapeutic value and are a significant source of anticoagulant treatment. The urgent requirement to expand the capacity for analysis of an increasing number of biomolecules that have been reported is the driving force behind the continued appearance of these developing technologies. Inflammation is a pathologic condition that encompasses a wide variety of diseases, including rheumatic and immune-mediated ailments, diabetes, cardiovascular accidents, and a variety of other conditions.

Keywords: Natural product; bioactive compounds; antimicrobial; antioxidant

Introduction:

In order to alleviate or treat ailments, eighty percent of the world's population employs medicinal plants-based medicine, as stated by the World Health Organisation (WHO) [1]. Furthermore, although figures differ depending on what constitutes a drug that is derived from natural goods, it is reliable to assert that natural products are the source of origin for as much as fifty percent of the drugs that are currently on the market [2]. Every day, new compounds derived from natural resources that have the potential to exhibit bioactivity are reported; however, only a small number of these molecules are investigated to determine whether or not they are suitable for use as pharmaceuticals [3]. The first phase in the process of drug development is the identification of bioactive molecules, sometimes known as hits or leads. Therefore, it is essential to choose appropriate bioassays in order to evaluate not only the effectiveness against the disease but also the potency of the treatment. Target-based screening is mostly utilised for the goal of identifying chemicals that affect the activity of a target that is involved in a disease. This screening makes use of a variety of in vitro biological assays that are intended to quantify primary activities, selectivity, cellular toxicity, and activity that is physiologically relevant. The earliest stages of a target-based screening cascade often involve the utilisation of a variety of in vitro assays, particularly high-throughput screening (HTS). Despite this, the research is costlier and more time-consuming [4,5]. However, this is not always possible, particularly when working with natural compounds or natural extracts. In the first instance, the assays might be chosen by taking into consideration the fact that molecules that are structurally related have similar biological activity.

Traditional medicine frequently makes use of extracts from a wide variety of plants, either on their own or in combination with other substances. However, in many instances, only a small number of these extracts are tested for their biological activity. On the other hand, it is not always simple to identify the molecules that are responsible for the activity of these extracts. This inability can be attributed to insufficient fractionation processes or the breakdown of active compounds that occurs during separation. Due to the fact that getting an isolated compound frequently necessitates the presence of infrastructure and personnel with specialised knowledge, as well as the fact that it is costly, it is difficult to provide pharmacological options of this kind to individuals who have low incomes. Natural extracts continue to be an alternative for treating certain diseases in people that have little resources or who live in remote places [6]. This is due to the fact that medicinal herbs can be grown locally at a cost that is fair. Using crude extracts rather than isolated molecules has a number of advantages, one of which is the existence of molecules within the extract that have the potential to interact synergistically with the bioactive component, hence amplifying the therapeutic effect of the substance [7].

However, despite the fact that natural extracts have the potential to be accessible treatment choices and sources of bioactive molecules for drug development, it is important to note that, just like other pharmacological alternatives, natural extracts can also exhibit undesirable effects that need to be taken into consideration and examined [8]. When looking for bioactive chemicals, it is essential to have a large number of assays available to either dismiss or confirm activities. This is because the objective of the search for therapeutic agents is to locate a method that is acceptable and can screen the source material for bioactivity. In this context, the current review presents some of the most common in vitro assays that are currently used for the identification of the pharmacological activity of bioactive compounds [9] or natural extracts. These assays allow for the preliminary identification of the biological potential and possible targets of the compounds in question. In addition, each section provides an overview of the benefits and drawbacks associated with different procedures, so assisting the reader in selecting the most appropriate assays according to the kind of sample and the resources that are at their disposal. Each section concludes with a

discussion of potential alternatives or developments in the future that are related to these techniques.

Antihyperglycemic:

Exercice The disease known as diabetes is a global health concern that affects 422 million people all over the world. In the absence of treatment, this condition is characterised by increased blood glucose levels, which, if left untreated, can result in severe failure of several organs and 1.5 million fatalities annually. Antihyperglycemic drugs are a category of molecules that can be generated through chemical synthesis or by isolating them from natural sources. These agents have the ability to either reduce the amount of glucose that is present in the blood or to prevent itself from increasing. The methods that are used to evaluate the in vitro antidiabetic properties of natural compounds are summarised in Table 2. These methods can be classified into two major groups: (i) assays that are based on the inhibition of isolated enzymes that are involved in the regulation of blood glucose levels, and (ii) assays that are used to measure major cellular processes that directly alter glucose levels, primarily glucose uptake and insulin secretion [10].

The enzymes that are part of the first group are those that are responsible for the breakdown of poly- and oligosaccharides. These enzymes include α-amylase and α-glucosidase, respectively. An antidiabetic molecule is one that inhibits the enzymes that have been discussed, as well as other enzymes that have a similar role in the digestion of carbohydrates. The decrease in the concentration of glucose that is available to be absorbed in the gut has the effect of preventing an additional rise in blood glucose levels. α-amylase and α-glucosidase inhibition tests are reactions that involve commercially available enzymes and substrates. These reactions take place under ideal conditions, which include buffer, pH, and cofactors. These conditions enable for the identification of the reaction product(s) [11]. When measuring α-amylase, starch is the substrate that is utilised the most frequently. It is based on the interaction of starch with dinitrosalicylic acid (DNS), which combines with reducing sugars to produce 3-amino-5-nitrosalicylic acid, which is detected spectrophotometrically at 540 nm. The method is based on this reaction. The spectrophotometric detection of p-nitrophenol, which is produced after the hydrolysis of p-nitrophenyl-α-D-glucopyranoside (pNPG), is utilised in the majority of α-glucosidase tests. This p-nitrophenol can be detected at a wavelength of 400 nodes. Both dipeptidyl peptidase IV (DPP4) and tyrosine phosphatase 1B (TP1B) have a role in the indirect regulation of glucose levels. DPP4 is responsible for modifying insulin secretion, whereas TP1B is responsible for signalling.

The serine exopeptidase known as DPP4 is responsible for the cleavage of a variety of peptides, one of which is GLP-1, which is a significant regulator of insulin production in response to glucose [75]. Therefore, blocking DPP-4 in vivo results in an increase in the availability of GLP-1 and insulin production, which ultimately leads to a reduction in blood glucose]12. Through the process of dephosphorylating the insulin receptor (IR) and its downstream signalling components, TP1B is able to exert a detrimental influence on the insulin and leptin signalling pathways. Insulin signalling is released from TP1B-mediated dephosphorylation when TP1B is inhibited, which also makes it possible for insulin to signal downstream. Assays that are designed to detect the possible inhibitory effects of various compounds on relevant cellular processes that govern blood glucose levels, such as glucose absorption and insulin production, are included in the second set of assays. The internalisation of a labelled glucose analogue is the foundation of the glucose uptake assay. However, due to the alteration of the glucose analogue, it is not possible to have its full utilisation. As a result of its accumulation within the cells, its detection becomes easier. The output that is produced by the accumulation of labelled analogues is proportional to the glucose uptake, and it is able to be detected and measured with the help of common equipment such as fluorescence

or bioluminescence readers or fluorescence-activated cell sorting (FACS) [13].

It is possible to carry out these tests on mammalian cell lines, which is a significant advantage when it comes to determining the physiologically significant effects. There have been reports of yeast cells being used as an alternative to mammalian cell lines in some experiments. A recent work, for instance, provided a label-free way for measuring glucose uptake in yeast cells by utilising pHluorin, which is a pH-sensitive green fluorescent protein that is genetically encoded. In most cases, ß-cells that have been isolated from pancreatic or islet cell cultures are utilised in the process of insulin secretion determination. For the purpose of determining the effect of the drug or plant extract on insulin secretion modulation, the cells are first stimulated by glucose and then incubated with the compound.

ELISA and radioimmunoassay are two methods that can be utilised to test insulin after it has been released from cells. Assays for α-amylase and α-glucosidase inhibition involve reactions of enzymes and substrates that are readily available in the market. These reactions take place under ideal conditions, including buffer, pH, and cofactors, which enable the detection of the reaction product(s). When measuring α-amylase, starch is the substrate that is utilised the most frequently. It is based on the interaction of starch with dinitrosalicylic acid (DNS), which combines with reducing sugars to produce 3-amino-5-nitrosalicylic acid, which is detected spectrophotometrically at 540 nm. The method is based on this reaction. The spectrophotometric detection of p-nitrophenol, which is produced after the hydrolysis of p-nitrophenyl-α-D-glucopyranoside (pNPG), is utilised in the majority of α-glucosidase tests. This p-nitrophenol can be detected at a wavelength of 400 nodes. Both dipeptidyl peptidase IV (DPP4) and tyrosine phosphatase 1B (TP1B) have a role in the indirect regulation of glucose levels. DPP4 is responsible for modifying insulin secretion, whereas TP1B is responsible for signalling. The serine exopeptidase known as DPP4 is responsible for the cleavage of a variety of peptides, one of which being GLP-1, which is a significant regulator of insulin production in response to glucose.

The availability of GLP-1 and insulin production is increased as a result of suppressing DPP-4 in vivo, which results in a decrease in blood glucose levels. Assays that are designed to detect the possible inhibitory effects of various compounds on relevant cellular processes that govern blood glucose levels, such as glucose absorption and insulin production, are included in the second set of assays. The internalisation of a labelled glucose analogue is the foundation of the glucose uptake assay. However, due to the alteration of the glucose analogue, it is not possible to have its full utilisation. As a result of its accumulation within the cells, its detection becomes easier. The output that is produced by the accumulation of labelled analogues is proportionate to the glucose uptake, and it is able to be detected and measured with the help of standard equipment such as fluorescence or bioluminescence readers [79,80] or fluorescence-activated cell sorting (FACS). It is possible to carry out these tests on mammalian cell lines, which is a significant advantage when it comes to determining the physiologically significant effects. There have been reports of yeast cells being used as an alternative to mammalian cell lines in some experiments.

Enzymes belonging to the class of amylases are responsible for hydrolyzing starch, which results in the production of low molecular weight dextrins and sugars. Amylases also play a significant part in the digestion of carbohydrates. A significant reduction in the post-prandial increase of blood glucose can be achieved through the inhibition of α-amylase in conjunction with α-glucosidase. This treatment approach has the potential to be an essential technique for controlling blood glucose levels in patients with type 2 diabetes. Therefore, the pancreatic α-amylase and stomach glucoamylases present themselves as the primary therapeutic targets for the treatment of type-2 diabetes mellitus. Amylase inhibitors are already available for use in the treatment of diabetes in their current market form. It is also possible to utilise amylase inhibitors for

the purpose of managing obesity. The use of amylase inhibitors is not limited to medical applications; they also play a role in the management of pests. Because of this, amylase inhibitors are an essential component in the treatment of pests.

Organising the inhibitors of alpha amylase into categories:

Alpha amylase inhibitors can be broken down into a few different categories. Each class is comprised of inhibitors that have their own unique structural components. These are some of the classes that are described in more detail:

Alpha amylase inhibitors that are similar to lectin:

There are 250 amino acid residues that make up the alpha amylase inhibitors of the lectin type. These residues are responsible for the production of five disulfide bonds. Higher plants, cereals, and legumes are the places where you can find them. They have a considerable impact on the suppression of the activity of salivary and pancreatic amylase in both in vitro and in vivo experimental settings. Amylase inhibitors have the potential to be used in a variety of disciplines, including crop protection, because plants use them as a source of defensive strategy. There are three isoforms of alpha-amylase inhibitors that are found in pulses, the most common of which are isoforms 1, 2, and 3. Isoform 1 of the alpha-amylase inhibitor is also referred to as (Alpha-AI1), and similarly, isoforms 2 and 3 of the alpha-amylase inhibitor are referred to as (Alpha-A12) and (Alpha-AIL) respectively. The alpha amylase 1 is further subdivided into two types, which are referred to as the alpha amylase 1 P1 and the alpha amylase 1 Pa2 respectively. When it comes to the suppression of the many alpha amylase enzymes that are present in the case of storage pests, each person plays a unique role. However, neither of these two isoforms may be considered inhibitory factors for the alpha amylases found in mammalian cells [14].

Kunitz like alpha amylase inhibitors:

A chain of 180 amino acids is contained within the kunitz-like alpha amylase inhibitor, which is characterised by the inclusion of four cysteines. Cereals tend to have a significant amount of them. A beta-trefoil protein is the building block of the subtilisin inhibitor, which is also referred to as the barley alpha amylase inhibitor due to its presence in barley protein. The isozyme 2 of the alpha amylase classification is inhibited by the soyabean trypsin inhibitor while the barley seed is germinating. This occurs during the process of seed germination. About 21 kilodaltons is the molecular weight of the alpha amylase that is found in rice [15]. It has also been discovered that legumes, such as flame tree, also known as Delonix regia, have an alpha amylase inhibitor similar to Kunitz. A significant possibility exists that the mortality rate of Callosobruchus maculatus could be increased by an alpha amylase inhibitor found in legumes. In addition to this, it damages the larval stage of the insects, which has an effect on the development of the insects. In contrast to the rice alpha amylase inhibitor, this inhibitor has the ability to inhibit the alpha enzymes of both mammalian organisms and insect organisms. The molecular weights of their extended family range from 6 kDa to 24 kDa, and they are unique from one another along the way. Pigeon pea and wheat are two other sources from which a Kunitz-like inhibitor can be isolated [16]. Additionally, they operate on a distinct mechanism, which contributes to the excellent thermal stability of their protein. The formation of a dozen hydrogen bonds and salt bridges by these molecules leads to the formation of significant electrostatic forces in the active regions of the catalytic site, which in turn prevents the substrate from entering the site.

Knot type alpha amylase inhibitors:

Proteinaceous alpha amylase inhibitors are among the smallest in size, and they are among the most effective. There are only 32 amino acid residues in their structure, and there are only three disulfide connections included. The inhibitory activity that it possesses is accomplished through the utilisation of a complicated process that is founded on the water-mediated hydrogen bonds. Through this particular method, the alpha amylase inhibitory attaches to the space that exists between

the domains of the catalytic site, which ultimately results in the formation of a salt bridge. A number of different species, including Tenebrio molitor and Tribolium castaneum, are inhibited by the knot type alpha amylase inhibitor [17]. Amaranth is the primary source of inspiration for this inhibitor. In 1994, Chagolla and his colleagues were the ones who made the initial discovery. There are only four cysteine knot alpha amylases that have been shown to include two to four prolines, according to the aforementioned investigations.

Recent studies on medicinal plants have been carried out because of their potential to treat diabetes.

There are a number of medicinal plants that have been found to have alpha-amylase inhibitory activity, and it has been reported that over 500 different plant species have qualities that are beneficial in the treatment of diabetes. Studies have already been conducted, and it has been noted that when rats were given an aqueous extract derived from the leaves of the P.S. cuminigua plant, there was a reduction in the amount of glucose that was found in their blood. It was also observed that the seeds of Amaranthus caudatus had an inhibition rate of roughly 80 percent. According to reports, numerous portions of a variety of plant species were collected, and investigations have already been carried out, with the purpose of determining the alpha amylase inhibitory activity in plants. According to the findings, the highest percentage of inhibition of the enzyme alpha amylase was recorded in the case of the flowering plant Hibiscus sabdariffa, while the percentage of inhibition in the case of the perennial legume Cajanus cajan was almost one hundred percent. The inhibition percentage was 93% in the case of Bergenia ciliate rhizome [18]. This was the result of the previous step. In the case of extracts of bark and leaves of Balanitesa egyptiaca L and Murraya koenigii L, the percentage of alpha amylase inhibition was 57 and 56, respectively. Similar to the previous example, the proportion of alpha amylase inhibition in the case of annual herbaceous plants, specifically Andrographis paniculate, is 52%.

Anti-Inflammatory Activity:

A given organism's immune system generates inflammation as a defensive response to dangerous external agents, such as pathogens, poisons, or irritants. Inflammation is a protective response. The healing process is facilitated by this system, which helps the body recover from infections, diseases, and injury to tissues [19]. Inflammatory responses involve the activation of macrophages by pro-inflammatory mediators like lipopolysaccharide (LPS), interleukin-1β (IL-1β), interferon-γ (IFN-γ), and the nuclear factor kappa B (NF-κB). These pro-inflammatory mediators trigger the pathways of cyclooxygenase (COX) and lipoxygenase (LOX), as well as the production of nitric oxide (NO), tumour necrosis factor-α (TNF-α), and interleukin-6 (IL-6) most prominently [20]. Through in vitro research, each of these pro-inflammatory mediators is being investigated in order to determine the anti-inflammatory capabilities of natural compounds. Non-steroidal anti-inflammatory medicines have been proven to suppress the formation of arachidonic acid and to lower prostaglandin levels, which contribute to a reduction in both pain and inflammation [21]. Furthermore, these drugs have been shown to be effective in treating inflammation. The usage of these products, on the other hand, has been linked to a number of adverse consequences, such as stomach ulcers, indigestion, headaches, allergic responses, and an increase in cardiovascular problems. The incredible variety of phytoconstituents that are found in plants, such as flavonoids, alkaloids, saponins, coumarins, anthraquinones, saccharides, glucosinolates, tannins, phenolic acids, and nitrile glycosides, are, fortunately, excellent sources for the development of drugs due to the vast biological activities that they possess. In particular, flavonoids [22], anthocyanins, and some polyphenols have been demonstrated to possess anti-inflammatory effects. In a similar manner, secondary metabolites, such as atranorin derived from lichens and sulphated polysaccharides derived from the brown alga Sargassum cristaefolium, are beneficial in that they decrease the inflammatory process [23]. The

great potential of natural extracts for the development of medications that offer anti-inflammatory properties is highlighted by these few examples. This article provides a comprehensive analysis of natural active compounds that have the ability to reduce inflammation. Overproduction of nitric oxide (NO), which is related with tissue toxicity, many inflammatory diseases, and carcinomas [24, 25], is a straightforward method for evaluating inflammation. This method is also a simple technique to measure inflammation.

Analgesic Activity:

Pain is a sign of a wide variety of disorders that call for the administration of analgesics. An analgesic is a medication that is used to alleviate pain without causing the patient to lose consciousness. The central neural system or the peripheral nervous system can be affected by analgesics [26]. In order to alleviate pain, there are two primary categories of medications: (i) non-steroidal anti-inflammatory analgesics, which alleviate pain by reducing local inflammatory responses; (ii) opiate analgesics, which are highly effective because they reduce but do not block the perception of the central nervous system and even induce sedation, transforming pain into a non-bothersome sensation [27]. Among the several types of opioid receptors are the following: There are three types of receptors: μ-receptors, which are responsible for producing the typical effects of narcotic analgesics when they are stimulated; κ-receptors, which are responsible for spinal analgesia; and δ-receptors, which are responsible for cardiovascular manifestations. When the drug acts on these receptors, it does not have addictive effects but brings about unpleasant hallucinations. In order to evaluate the potential analgesic action of novel drugs, radioligand binding experiments are utilised to determine the affinity and selectivity of the new molecules for particular receptors. There are three different types of radioligand binding assays that can be conducted experimentally: saturation, competitive, and kinetic assays [29]. The most common type of experiment is the competitive assay, which is used to investigate equilibrium binding at a constant dose of radioligand and with varying quantities of an unlabeled competition. There is a growing problem of pain all throughout the world. It has been estimated that twenty percent of adults around the world experience pain, and ten percent of those adults receive a new diagnosis of chronic pain every year [30]. More than eighty percent of patients seek medical attention because they are experiencing pain, and the majority of these patients experience pain that is for a brief period of time and is rapidly forgotten. But unfortunately, for some people, the pain does not go away; rather, it becomes a constant burden and a source of agony that is interrupted.

There are various compounds that are released by the injured tissues when they sustain an injury, regardless of whether the injury was caused by germs, trauma, chemicals, heat, or any other phenomena. These substances trigger severe secondary alterations in the tissues that are around the injured tissues. The term "inflammation" refers to the full complex of changes that occur in the tissue [31]. Restoring tissue homeostasis and starting the healing process in the body are both accomplished through this mechanism, which is of the utmost importance. Pain, discomfort, avoidance of motor reflexes, and changes in autonomic output are all symptoms that are linked with it. It is primarily the product of a complex interaction between sensory, emotional, and behavioural elements, and it manifests itself in the form of suffering. It is inherently unpleasant. As a result of the fact that perception of pain is always subjective, it varies from person to person and even from time to time within the same individual. It has been found that there is a strong connection between the pharmacological potency of opiate agonists and antagonists in vivo and their capacity to displace radiolabeled molecules like naloxone, which is a chemical that binds to opiate receptors [32]. Through the utilisation of the correlation that was discussed earlier, new substances can be evaluated. These tests are carried out on animal brain membranes, which have a high density of the receptors of interest; in the assay, varied doses of the test chemical are tested against a set concentration of specific radioligands [33, 34].

Figure 3 illustrates the results of these experiments. Using these assays, it is possible to identify molecules that recognise the same binding site as radiolabeled ligands that are already known.

Conclusion:

These plants were found to have hypoglycemic effects, and they have the potential to be utilised in the treatment of a variety of secondary problems that are associated with diabetes mellitus. Despite the fact that plants have emerged as a valuable source of medicine for the treatment of a wide range of diseases, a significant number of plants and the active chemicals that can be extracted from plants have not been adequately characterised. It is necessary to do additional research in order to determine the precise mechanism of action of medicinal plants that possess antidiabetic and insulino mimetic activities. It is a common misconception that plants are safe to consume; nevertheless, there are numerous plant components that are not suitable for human consumption. Because of this, it is imperative that toxicity studies of these plants be provided prior to the consumption of these plant materials.

Among the many components that make up alternative medicine, herbal medicine is among the most significant. Many research have demonstrated that certain herbs play a function in the reduction of inflammation, and these findings have been published. Clinical data is, of course, more reliable than other types of data; among the research data that we have, the Curcuma longa had the most clinical evidence about various inflammatory disorders such as rheumatoid arthritis, uveitis, and inflammatory bowel disease (IBD). We introduce some herbs whose anti-inflammatory effects have been evaluated in clinical and experimental studies.

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