2. Introduction

You herbs born at the birth of time,
Ancient as he gods themselves,
You, who have a thousand powers,
Free this patient from diseases.
When restoring vanished strength,
I hold you herbs within my hand,
And the spirit of disease departs,
Cheated of another death.

2.1. Diabetes mellitus

Diabetes mellitus (DM) is a complex and chronic systemic disease accompanied by metabolic disorders, including hyperglycemia, hyperinsulinemia and hypertriglyceridemia. The incidence of DM has increased considerably and the number of patients has believed to be more than 422 million as of today, which is expected to reach to 592 million by 2035. Currently, the global prevalence has been accounted as 8.5% among adults, which is rising more rapidly in middle- and low-income countries (1).

DM is classified into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T2DM accounts for nearly 95% of individuals. T1DM is characterized by absolute insulin deficiency associated with pancreatic cells destruction while T2DM is mainly due to insulin resistance (IR) and deficiency in insulin secretion. T2DM can cause multiple organ injury and a number of complications. Acute complications, like hypoglycemia, coma, ketoacidosis and lactic acidosis are mostly related to high mortality in diabetics, and chronic complications are the most devastating consequence caused by long-term high level of blood glucose. The microvascular lesions can cause diabetic retinopathy, diabetic nephropathy and diabetic neuropathy. Additionally, the macrovascular complications include cardiovascular and cerebrovascular diseases. Besides, T2DM can also cause the morbidity of depression, sexual dysfunction and dementia. Treatment of T2DM has become very costly for the patients.

2.2. Pharmacological application of plants with antidiabetic activity

The first medicinal plant described with a clear antidiabetic effect was Galega officinalis L. (Fabaceae), which has been prescribed since the Middle Ages to treat DM. From this plant, a guanidine derivative, galegine, was isolated. This compound, whose chemical structure is quite similar to the antidiabetic drug metformin, is responsible for the lowering of blood glucose produced by the plant extract (2,3).

Rutin, a polyphenolic bioflavonoid, decrease the levels of fasting blood glucose and non-fasting blood glucose, decrease serum levels of TG, LDL, VLDL, TC and increase the level of HDL in diabetes models. It also increase the secretion of insulin in isolated rat pancreatic islet, and preserves glucose sensing ability in high glucose condition (4).
With the hydroxylated phenolic structure, the activities of flavonoids are structure dependent. The hyperglycemia amelioration effect of Quercetin is mainly caused by blunting free radical and oxidative stress in T2DM mice. Quercetin can reduce liver inflammation and lipid accumulation under hyperglycemic conditions, and influence the glucose homeostasis via stimulating glucose transporter type 4 (GLUT4) translocation in skeletal muscle and inhibiting glucose-6-phosphatase (G6Pase) in hepatocytes (5).

Curcumin, a bioactive component of Curcuma longa L, showed significant effect on reducing the levels of blood glucose and glycosylated hemoglobin (HbA1C), and improving insulin sensitivity. The possible mechanisms of antidiabetic functions of the compound may be through inhibiting lipid peroxidation, nuclear factor kappa B (NF-kB) activation, and lysosomal enzyme vitality. (6,7). Bisdemethoxy-curcumin is a potent small molecule inhibitor of human pancreatic α-amylase that can be used to treat T2DM (8).

Berberine, a representative isoquinoline alkaloid, can decrease blood glucose level, increase insulin secretion, reduce body weight and lipid levels, and attenuate glucose tolerance and insulin resistance via activating AMPK pathway, elevating glucagon-like peptide-1 (GLP-1) level, attenuating ROS production, reversing mitochondrial dysfunction, reducing endothelial microparticles-mediated oxidative stress, and suppressing inflammation (9). Abscisic acid can stimulate β-pancreatic cells to release insulin, and adjust GLUT4-mediated glucose uptake in vitro (10). Diosgenin, a phytosteroid sapogenin can decrease body weight, blood glucose, insulin resistance and modulate lipid profile in T2DM rats (11), which can also attenuate Endoplasmic reticulum (ER) stress and oxidative stress-mediated damage in pancreas (12).

Rhein can significantly improve glucose-tolerance by preserving β cell mass and inhibiting β cell apoptosis in db/db mice (13). Cinnamaldehyde, a natural flavorant and fragrance agent in kitchen, shows glucolipid lowering effects in diabetic animals by attenuating IR, increasing glycogen synthesis, and ameliorating islets dysfunction (14).

In addition to antidiabetic plant compounds, three examples of microbial origin may be mentioned. Acarbose (from Actinoplanes sp.) is probably the most widely used digestive enzyme inhibitor for the treatment of T2DM, acting on α-glucosidase, α-amylase, sucrase, and maltase, but without insulinotropic properties (2,15,16). Miglitol is a second-generation α-glucosidase inhibitor structurally similar to glucose. Originally obtained from various Bacillus and Streptomyces strains (2). Voglibose is synthesized from valiolamine, which is isolated from a fermentation broth of Streptomyces hygroscopicus subsp. limoneus (17). It is also an α-glucosidase inhibitor, which competitively and reversibly inhibits glucoamylase, sucrase, and
isomaltase but has no activity on α-amylases. It also reduces plasma glucose levels and insulin in a dose-dependent manner (2,18).

The possible mechanisms of natural products with antidiabetic properties are mainly through improving β cell dysfunction, suppressing α-amylase activities, attenuating insulin resistance, balancing glucose tolerance, etc. They can also regulate the expression levels of some key proteins involved in signal pathways. Nevertheless, some natural products effectively targeting the specific proteins, including GLP-1, DPP-4, gastric inhibitory polypeptide (GIP), sodium glucose co-transporter 2 (SGLT2), are needed further investigation.

2.3. Glucosidase inhibitor

Mechanisms by which various medicinal plants achieve antidiabetic effect can be linked to more than one mechanism, such as insulin sensitizing, insulin releasing, α-glucosidase inhibition and so on. Evidences have shown that postprandial hyperglycemia is an important contributing factor to the development of T2DM and complications associated with the disease, especially cardiovascular diseases. Postprandial serum glucose levels may be elevated when fasting serum glucose levels remain normal resulting in the increase of mean haemoglobin A1c concentration which in turn leads to the progressive development of the microvascular and macrovascular complications associated with diabetes. It also causes protein glycation and the formation of advanced glycation end products contributing to a more rapid progression to diabetes.

The elevated blood glucose levels produce their effects through polyol pathway and non-enzymatic glycosylation. Activation of polyol pathway occurs in tissues in which glucose absorption is independent of insulin concentration (e.g. retina, lens, kidneys, glomeruli, peripheral nerves). Increases in blood glucose concentration lead directly to elevated glucose levels in these tissues. This in turn stimulates aldose reductase activity enhance the polyol pathway results in an increase in sorbitol concentration in the tissues, which ultimately produces structural damage. In non-enzymatic glycosylation of proteins, referred as glycation, carbohydrates attach to protein molecules lead to protein polymerization and cleavage. These end products of glycosylation (glycated proteins) have been detected in the glomerular basement membrane, the endothelial cell matrix and lipoproteins, where they impair normal tissue function. Glycated haemoglobin is also an end product of the glycation process.

The mammalian α-glucosidases are glycosyl phosphatidyl anchored enzymes located on the surface membrane of intestinal cells, which catalyze the final step of carbohydrate digestion. These enzymes hydrolyze carbohydrates by acting on the 1,4- α linkages, thereby releasing α-D-glucose from the non-reducing end of the
sugar (19). The α-glucosidase inhibitors act against these enzymes in the gut, slowing down the liberation of D-glucose from dietary complex carbohydrates that lowers available glucose for absorption. Hence, they are useful in reducing post-prandial blood glucose in treating prediabetic conditions and delaying the progression of diabetes. This approach has been called 'delaying absorption as a therapeutic principle in metabolic diseases'. Acarbose, voglibose and miglitol are considered as the first-line treatment for diabetic individuals with post-prandial hyperglycemia. Unfortunately, their continuous use may lead to liver toxicity and adverse gastrointestinal symptoms (20). Hence, there is a dire need in exploiting secondary metabolites from plants, culinary herbs, spices, vegetables and fruits as α-glucosidase inhibitors; i.e. for possibly lesser side effects.

Some well-known species, such as Adathoda vasica Nees (Acanthaceae), have sucrose inhibitory activity, with vasicine and vasicinol as potential active phytochemicals, which may be of interest for the future development of new α-glucosidase inhibitory agents. Fenugreek seeds, Trigonella foenum-graecum L, Fabaceae inhibit intestinal glucosidase in diabetic rats and have a positive effect on glucolytic and gluconeogenic enzymes to restore glucose homeostasis.

α-Glucosidase inhibitors are generally well-tolerated because their gastrointestinal side effects are mainly non-systemic compared to that of other antidiabetic drugs. Since they inhibit digestion of complex carbohydrates in the intestine, the side effects are generally limited to flatulence, abdominal pain and diarrhoea due to bacterial action on undigested carbohydrates (21). One of the greatest advantages of α-glucosidase inhibitors over other anti-diabetic drugs is their localized action accompanied by minimal absorption hence limiting the systemic side effects. Glucosidases are also involved in several important biological processes such as the biosynthesis of glycoproteins and the lysosomal catabolism of glycoconjugates. Glucosidase inhibitors are therefore potentially useful as antiviral, antimetastatic and immunomodulatory agents. They also have a potential to be useful against the HIV-1 infection (22).

The main focus of research into diabetes treatment is moving away from the management of acute metabolic imbalances to the prevention of the long-term complications resulting in patient morbidity and premature death. These chronic complications include nephropathy, neuropathy, retinopathy and macrovascular complications. Macrovascular complications, such as atherosclerosis and coronary heart disease, are mostly associated with Type 2 diabetes and currently make up the major cause of morbidity and mortality in diabetes.
2.3.1. Characteristics of α-glucosidase inhibitors

Forty years have passed since the most renowned glucosidase inhibitor, nojirimycin, was first reported in 1966. Since then, an enormous number of glucosidase inhibitors have been discovered, synthesized, and have had their inhibitory activities investigated. The reported α-glucosidase inhibitors have some of the following characteristics: (1) sugar (substrate)-mimic structures, (2) the ability to form ionic bonds with nucleophilically catalyzing residues, (3) transition-state-like structures, (4) the ability to form hydrogen bonds with catalytic acid residues, (5) the ability to make ionic and hydrophobic interactions at sites other than the active site, and (6) the ability to form covalent bonds with enzymes through an epoxy or aziridine group. These model structures are reported to be potent inhibitors that combine the above-mentioned (1)-(6) features. The target of these glucosidase inhibitors is the glycon binding subsite (subsite -1) of α-glucosidases. However, non-sugar-mimicking α-glucosidase inhibitors have recently been reported and these inhibitors might bind to the aglycon binding site (subsite +1) or elsewhere (23). The structure modulation of glucosidase inhibitors are shown in figure 1.

![Figure 1. Classification of α-glucosidase inhibitors](image)

Natural products isolated from medicinal plants that showed α-glucosidase inhibitory activity are structurally incorporate into terpene, alkaloid, quinine, flavonoid, phenol, phenylpropanoid and steride frameworks. They are rich in organic acid, ester, alcohol

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and allyl functional groups. A majority of the compounds reported contain flavonoid, terpene and phenylpropanoid ring structures.

**Flavonoids**

Seven triprenylated flavonols, isolated from the roots of *Dorstenia psilurus*, exhibited high inhibitory activity against α-glucosidase. Compound with three unmodified prenyl groups, was the most active, while compound with only one unmodified prenyl group, was the least active. Thus, α-glucosidase inhibitory activity increases with the increase in the number of prenyl groups in the structure (24). Three known compounds, quercetin-3,6,7-trimethyl ether (A), isovitexin-4µ-methyl ether (B), isovitexin (C) and a new compound acaetin-6-C-(6µ-acetyl-β-D-glucopyranoside)-8-C-α-L-arabinopyranoside (D) were obtained from *Achillea fragrantissima*. Among them, compound (D), exhibited the highest α-glucosidase inhibitory activity in a concentration-dependent manner followed by compound A. The potent inhibitory activity of compound D may be attributed to the presence of two sugar residues; a di-glycoside is supposed to exert stronger competitive inhibitory action against the target enzyme (25).

**Phenols**

Gallic acid, an important constituent of many plants species, has shown strong inhibitory activity against glucosidase. Moreover, methyl gallate obtained from the dried stem and bark extracts of *Terminalia superb* (26) and propyl gallate isolated from green tea extracts (27) showed strong α-glucosidase inhibitory activity.

**Phenylpropanoids**

Three curcuminoids isolated from *Curcuma longa* have shown strong inhibitory activity on α-glucosidase (IC$_{50}$ = 37.2, 42.7, and 23.0 µmol/L, respectively). In addition, the synthesized analogs of such compounds have been made and were regarded as good α-glucosidase inhibitors (28). Two structurally similar phenylpropanoids were isolated from the Devil tree (*A. scholaris*), and in spite of structural similarity, their inhibitory activity toward small intestinal sucrase and maltase activity profiles were significantly different. Therefore, the configuration of a compound could significantly influence its inhibitory activity (29).

Seven stilbenoids isolated from the ethanol extracts of *Syagrus romanzoffiana* seeds possess potent inhibitory activity against α-glucosidase type IV. From SAR studies, it was determined that by keeping the basic pharmacophore constant, the glucosidase inhibitory activity could be increased by increasing the number of OH substitutions in the aromatic ring (30). The phenylpropanoids, stewartiside, lunariifolioside, phlomispentanol, tiliroside were isolated from the entire plant body of *P. Stewartii*, have shown better α-glucosidase inhibitory potential than acarbose. Tiliroside was
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the most active, whereas its methoxy derivative phlomispentanol showed the least activity. This indicated that the presence of hydroxyl group in ring C had an important role in enzyme inhibition. The activity of stewartiiside was comparable with that of lunariifolioside. This means that the glycone part did not play an important role in enzyme inhibition (31). Ten honokiol oligomers, including four trimers and four dimers obtained from *Momordica charantia* had good inhibitory effects on α-glucosidase enzyme. The potency of honokiol trimers were found to be higher than those of dimers, and among the sixdimers, the potency of the carbon oxygen linkage dimers appeared to be much stronger than those of the carbon acarbon linkage ones. Among all the tested compounds, the honokioltrimer (IC$_{50}$ = 1.38 µmol/L), exhibited the most significant inhibitory activity toward α-glucosidase in a concentration-dependent manner and was 128-fold more potent than that of honokiol (IC$_{50}$ = 177.03 µmol/L) (32).

Other compound types

Numerous organic compounds of plant origin containing functional groups such as organic acid, ester, alcohol, allyl, and others have shown strong α-glucosidase inhibitory activity. For example, vanillic acid isolated from the bark of Rutaceae *F. tessmannii* (33), 4-hydroxybenzoic acid from the seeds of *S. romanzoffiana* (Cham.) (30) and 4-hydroxyphenylacetic acid isolated from *C. Plicata* have shown inhibitory activity against yeast α-glucosidase (34).

2.4. Bioactivity guided fractionation

Development of a new drug is a complex, time-consuming and expensive process. The time taken from discovery of a new drug to its reaching the clinic is approximately 12 years, involving more than 1 billion US$ of investment in today’s context. Essentially, new drug discovery involves the identification of new chemical entities (NCEs), having the required characteristic of druggability and medicinal chemistry. These NCEs can be sourced either through chemical synthesis or through isolation from natural products. Before the advent of high throughput screening and the post genomic era, more than 80% of drug substances were purely natural products or were inspired by the molecules derived from natural sources (including semi-synthetic analogs). There are various examples of development of new drugs from the plant sources. Morphine was isolated from opium produced from cut seed pods of the poppy plant (*Papaver somniferum*) approximately 2000 years ago. Pharmaceutical research expanded after the Second World War to include massive screening of microorganisms for new antibiotics, inspired by the discovery of penicillin. Few drugs developed from natural sources have undoubtedly revolutionized medicine, like antibiotics (e.g. penicillin, tetracycline, erythromycin), antiparasitics (e.g. avermectin), antimalarials (e.g. quinine, artemisinin), lipid control agents (e.g. lovastatin and analogs), immunosuppressants for organ transplants.
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(e.g. cyclosporine, rapamycins), and anticancer drugs (e.g. paclitaxel, irinotecan) (35). The botanical sources are known to provide the following classes of NCEs for drug discovery processes.

- Bioactive compounds for direct use as drug, e.g. digoxin.
- Bioactive compounds with structures which themselves may act as lead compounds for more potent compounds, e.g. paclitaxel from Taxus species.
- The novel chemophore which may be converted into druggable compounds with/without chemical analoging.
- Pure phytochemicals for use as marker compounds for standardization of crude plant material or extract.
- Pure phytochemicals which can be used as pharmacological tools.
- Herbal extracts as botanical drugs, e.g. green tea extract.

Usage of botanical sources as starting point in the drug development program is associated with few specific advantages.

- Mostly, the selection of a candidate species for investigations can be done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active compounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use. At certain time point afterward, one may attempt upon synthesis of active molecule and reduce pressure on the resource. Drug development from Rauwolfia serpentina, Digitalis purpurea, etc. in the past fall under this category of approach.

- Sometimes, such approaches lead to development of novel molecules derived from the source due to inherent limitations of the original molecule. For instance, podophyllin derived from Podophyllum hexandrum was faced with dose-limiting toxicities. Such limitations could be overcome to a great extent by semi-synthesis of etoposide, which continues to be used in cancer therapy today. Similar was the case with camptothecin (originally isolated from Camptotheca sp. and subsequently from Mappia sp.), which led to development of novel anticancer molecules like topotecan and irinotecan.

- Natural resources as starting point has a bilateral promise of delivering the original isolate as a candidate or a semi-synthetic molecule development to overcome any inherent limitations of original molecule.

2.4.1. Druggability of isolated phytochemical compounds

Challenges in the new drug development are mainly encountered from two categories: the prevailing paradigm for drug discovery in large pharmaceutical...
industries and technical limitations in identifying new compounds with desirable activity. Koehn and Carter (36) have enumerated the following unique features of the compounds isolated from natural products:

- Greater number of chiral centers
- Increased steric complexity
- Higher number of oxygen atoms
- Lower ratio of aromatic ring atoms to total heavy atoms
- Higher number of solvated hydrogen bond donors and acceptors
- Greater molecular rigidity
- Broader distribution of molecular properties such as molecular mass, octanol water partition coefficient, and diversity of ring systems

These unique features of chemical entities of natural origin pose a string of challenges for medicinal chemists as they start working upon development of analogs, either to improve the absorption or to reduce the toxicity and improve upon efficacy which is often achieved by addition or deletion of selected functional groups. According to Ehrman et al., different bioactive plant compounds like alkaloid, steroid, triterpene, limonoid, diterpene, sesquiterpene, monoterpane, tanin, isoflavonoid, flavonoid, polycyclic aromatic, lignan, coumarin, simple phenolic, aliphatic, etc have been isolated. Alkaloid may be distributed as 20%, flavonoids as 15%, triterpenes and simple phenolics around 10%, and remaining others below that, with limonoid being the least (37).

It can be safely presumed that large number of natural products, despite being biologically active and having favorable ADMET profile (absorption, distribution, metabolism, excretion, and toxicity), do not satisfy the criteria “drug likeness.” The challenge is of building a physio-chemical tuned natural products library in line with the lead generation to promote natural products to their full potential. Lipinski propagated simple set of calculated property called “rule of five” based on the drug candidates reaching Phase II clinical trials (38). This rule is an algorithm consisting of four rules in which many of the cutoff numbers are five or multiples of five, thus originating the rule’s name. To be drug-like, a candidate should have:

- less than five hydrogen bond donors;
- less than 10 hydrogen bond acceptors;
- molecular weight of less than 500 Da; and
- partition coefficient log P of less than 5.

The aim of the “rule of five” is to highlight possible bioavailability problems if two or more properties are violated. Had Lipinski’s rule been applied, paclitaxel would never have become a drug. Since it does not comply with “rule of five,” a biggest challenge is to find alternative druggability criteria for the compounds of natural origin.
2.4.2. Selection of candidate plant species for screening

To available estimates, the total number of higher plants species (comprising angiosperms and gymnosperms) is approximately 250,000 species. Of them, only 6% have been reportedly screened for biological activity and about 15% have been screened for phytochemical activity. Initial listing of the candidate species for screening of biological activity is a major task of specific importance in itself. As following two approaches have been followed for screening of the plants selected randomly for the purpose of new drug discovery (39); i) Screening for selected class of compounds like alkaloids, flavonoids, etc.: While this route is simple to perform, however, it is flawed in the sense that it provides no idea of the biological efficacy. However, chances of getting novel structures cannot be denied following this approach. ii) Screening of randomly selected plants for selected bioassays: National Cancer Institute (NCI) of National Institute of Health, USA, has studied about 35,000 plant species for anticancer activity, over two decades from 1960 to 1980. It resulted in two success stories, which were those of paclitaxel and camptothecin. This route, therefore, has been applied for both focused screening as well as general screening, showing some success in focused screening. If target-based bioassays are used, e.g. screening against α-glucosidase chances of success would probably be more.

2.4.3. Ethnopharmacology approach

The approach of ethnopharmacology essentially depends on empirical experiences related to the use of botanical drugs for the discovery of biologically active NCEs. This process involves the observation, description, and experimental investigation of indigenous drugs, and is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines like anthropology, archaeology, history, and linguistics (40). This approach based on ethnomedicinal usage history, has seen some success, e.g. *Andrographis paniculata* was used for dysentery in ethnomedicine and the compounds responsible for the activity were isolated as andrographolide. Morphine from *Papaver somniferum*, Berberine from *Berberis aristata*, and Picroside from *Picrorrhiza kurroa* are some examples of this approach. Some of the plants which are not selected on the basis of ethnomedical use also had some success stories, like L-Dopa from *Mucuna prurita* and paclitaxel from *Taxus brevifolia*.

2.4.4. Traditional system of medicine approach

Countries like India and China have a rich heritage of well-documented traditional system of medicine in vogue. Though these codified systems of medicine use largely botanical sources as medicines, however, these stand apart from ethnomedicine specifically on three accounts:

- The ethnomedicinal practice is based on empirical experiences. On the other hand, these codified systems built up the empirical practices on strong
conceputal foundations of human physiology as well as of pharmacology (though the tools of their investigations in those times were far different from the existing ones).

The pharmaceutical processes have been more advanced as against the use of crudely extracted juices and decoctions in ethnomedicinal practices. Due to this phenomenon, the concept of standardization was known to the system.

They are well documented and widely institutionalized.

On the other hand, the ethnomedicinal practices are localized and may be largely controlled by few families in each of the community. However, in terms of historicity, ethnomedicinal practices might be older than codified systems of medicine. Discovery of artemisinin from *Artemisia alba* for malaria, guggulsterones from *Commiphora mukul* (for hyperlipidemia), boswellic acids from *Boswellia serrata* (antiinflammatory), and bacosides from *Bacopa monnieri* (nootropic and memory enhancement) was based on the leads from these codified systems of medicine prevailing in China and India. However, it can be stated that such approach for selecting candidates in drug discovery programs has not been adopted much so far. Nonetheless, the approach has a distinct promise in terms of hit rates. But the distinct example for this approach has been the discovery of reserpine from *Rauwolfia serpentine*, which was based on the practices of Unani medicine.

2.4.5. Zoo-pharmacognosy approach

Observation of the behavior of the animals with a view to identify the candidate plants for new drug discovery is not a distant phenomenon. Observation of straight tails linked to cattle grazing habits in certain regions of South America led to identification of a plant *Cestrum diurnum* and three other plant members of family Solanaceae, which probably are the only known plant sources of the derivatives of Vitamin D₃. This approach, however, needs close observation and monitoring of the behavior of animals.

The key objective of this project is to emphasize on the usage of traditional wisdom in selection of candidate species on the basis of ethnomedicinal records. A few published studies and classical Indian traditional medicinal system for antidiabetic drug plants as the major source for drug discovery is reviewed below. Based on traditional and ethnobotanical wisdom, it is possible to apply the traditional knowledge on herbs to identify the better leads for research and development to find out good antidiabetic drugs.

2.4.6. Bioactivity guided fractionation for compound isolation

Bioactivity guided fractionation has been the process deployed to identify the lead druggable candidate from any given phytochemical matrix. Two approaches might be
followed as the design of extraction for bioactive guided fractionation leading to compound isolation to act as a lead compound: i) Parallel approach, this approach may be applied when the biological activity of the plant is known by its traditional use. The objective of this approach is to isolate compounds responsible for the activity based on their biologic activity. As explained in Figure 2, in parallel extraction approach, three types of extracts are obtained, viz. 100% methanolic extract, 50% methanolic extract, and 100% aqueous extract from a crude plant. The most active fraction based on the primary screening for bioactivity is chosen for further extraction and evaluation. ii) Sequential approach, this approach may be useful when the biological activity of the subject plant is not known and random selection strategy is adopted for plants. The extraction is mainly done based on the polarity of the solvents and fractions are obtained in a sequential process using hexane, chloroform, ethyl acetate, and butanol as solvents. Further extraction involves purification stage where structural elucidation is done for different compounds.

Since the bioactivity is assessed at two stages, two distinct models should be chosen keeping in view the end points. The screening model for stage I should be designed to elicit the efficacy. On the other hand, the screening model for secondary screening should be designed with an orientation toward mechanism of action. For example, for discovering potential antidiabetic molecules from a natural source, glucose uptake assay can be employed as the primary screening model. At stage II, it would be desirable to choose a secondary assay model like Glut 4, PI3 K, and IRTK, enzyme inhibition assay which may provide some clue for the mechanism of action. It is also desirable to include an assay for cytotoxicity so as to elicit the safety profile during secondary screening level.

Bioactivity guided fractionation of any crude extract from natural source in any case would lead to a wide array of possible outcomes at different stages. Also, these outcomes might provide unforeseen opportunities for modulating the discovery design during subsequent stages. Figure 3, depicts the possible outcomes of a typical bioactivity guided fractionation.

2.4.7. Way Forward and Conclusions

There is a pertinent need to renew scientific enthusiasm toward natural products for inclusion in drug discovery program. One of the important concerns related to natural products has been the predictability of hit rate during various phases of drug development. Such predictability is expected to be lower in case of random selection of candidate species considering the overall complexity of botanical sources for NCEs. In order to enhance the predictability, strategic selection and shortlisting of candidate species is necessary. Documented clinical experience with botanical medicines as codified in traditional systems of medicine might simplify the issues associated with poor predictability. New functional leads picked up from the
traditional knowledge and experiential database may help to reduce time, money, and toxicity, which are the three specific hurdles in the drug development (41).

An integrative approach by combining the various discovery tools and the new discipline of integrative biology will provide the key for success in natural product drug discovery and development. Since plant selection is the major step involved, it needs a well-designed strategy. The following scheme may be followed for appropriate plant selection:

- Identification of plants: Through a tactical application of traditional wisdom, especially in the context of usage frequency.
- Listing of all the formulations and their herbal ingredients.
- Frequency analysis of the ingredients.
- Arriving at hypothesis of the principal of traditional medicine system.
- Mapping of the ingredients identified against traditional medicine system attributes.
- Shortlisting of those plant species which match both frequency analysis as well as the traditional medicine system attributes.

Once the task of enumerating potential candidates for screening is over, the extraction procedure can go by a parallel approach instead of the sequential approach as followed for randomly selected species. Rest of the investigational course shall follow the following steps:

- Screening of biological activity on selective assays.
- Bioassay guided fractionation of the identified plant.
- Isolation and structure elucidation of the active compound.
- Evaluation of chemical do-ability, druggability, and patentability.
- Go or no go decisions based on safety, biological activity screening.

It is time for large-scale pharmaceutical organizations to open up the developmental strategies. In view of the increasing cost of development of new drugs, alternative approaches like development of herbal extracts hitting multiple targets as new drugs need serious consideration. Obviously, the cost of development shall be substantially lower in case of herbal extracts. Such strategy would not only enhance the chances of success in terms of providing effective and safe drugs, but also is considered to minimize the risk of post-marketing withdrawals. Such a complementary scenario shall go a long way in safeguarding the interests of both pharmaceutical industry and common man.
Figure 2. Parallel approach for bioactivity-guided fractionation

Figure 3. Array of outcome – A schematic representation
2.5. Molecular docking and ADMET studies

Docking is most commonly used in the field of drug design. It is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecules. A binding interaction between a small molecule (Ligand) and an enzyme protein may result in activation or inhibition of the enzyme. If the protein is a receptor, then ligand binding may result in agonism or antagonism. Most drugs are small organic molecules, and docking may be applied to: (i) Hit Identification: Docking combine with scoring function can be used to quickly screen large database of potential drugs \textit{in silico} to identify molecules that are likely to bind protein target interest. (ii) Lead Optimization: Docking can be used to predict in where and in which relative orientation a ligand binds to proteins (also referred to as the binding mode or pose). This information may in turn be used to design more potent and selective analogues. (iii) Bioremediation: Protein ligand docking can be used to predict pollutants that can be degraded by enzymes. To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been determined using a biophysical technique such as X-ray crystallography, or less often, NMR spectroscopy. This protein's structure and a database of the potential ligands serve as inputs to a docking program.

2.5.1. Maltase-glucosylamylase (MGAM)

MGAM is an enzyme with duplicated catalytic centers that anchor on the small-intestinal brush-border membrane via an O-glycosylated link stemming from their N-termini. MGAM can be divided into an N-terminal subunit (MGAM-N) and a C-terminal subunit (MGAM-C). All two subunits exhibit similar exoglucosidase activities against linear α-1,4-linked maltose substrates but different preferences for oligosaccharides substrates with various lengths. MGAM-N has maximal activity against substrates with two glucoses, while MGAM-C prefer oligosaccharides with up to four glucose residues (42). MGAM-C has much higher activity than MGAM-N. Consequently, inhibition of the activity of MGAM-C has proven to be an efficient treatment for some diseases, such as type 2 diabetes or obesity (43). Acarbose, an oral anti-diabetic medicine currently in use, shows a stronger level of inhibition against MGAM-C than MGAM-N (44).

2.5.2. The X-ray crystal structure of MGAM

The MGAM-C structure can be divided into five major domains: a trefoil Type-P domain (residues 955–1001); an N terminal domain (residues 1002–1220) composed of a series of anti-parallel β-barrels; a catalytic domain (residues 1221–1632) consisting of a (β/α)8-barrel with two loop inserts (Insert 1, residues 1317–1386 and Insert 2, residues 1424–1477) a proximal C-terminal domain (residues 1633–1711)
and a distal C-terminal domain (residues 1712–1857). The last four residues in the distal C-terminal domain are disordered in both crystals and were not included in the final models (Figure 4).

Figure 4. Human MGAM domains with amino acid boundaries

Figure 5 represents the ribbon diagram of the MGAM-C/acyclovir complex. Individual domains are coloured as follows: trefoil Type-P domain (blue), N-terminal domain (yellow), catalytic (β/α)8 domain (red), catalytic domain Insert 1 (orange), catalytic domain Insert 2 (pink), proximal C-terminal domain (ProxC) (green), and distal C terminal domain (DistC) (purple). The bound inhibitor acarbose is shown as a stick model and coloured cyan. N, N terminal; C, C terminal.
2.5.3. Acarbose and MGAM-C

Acarbose, a pseudo-tetrasaccharide that is composed of an acarviosine group α-(1-4) linked to a maltose, is a competitive inhibitor of MGAM-C. In the complex structure, acarbose was found in the active site of MGAM-C (Figure 5). Acarbose spans subsites from −1 to +3 of MGAM-C, with its non-hydrolyzable N-linked bond occupying the catalytic center. Numerous hydrogen bonds and hydrophobic interactions are involved in the interactions between MGAM-C and acarbose (Figure 6 and 7). At subsite−1, atoms NE2 of His1584 and OD2 of Asp1279 form hydrogen bonds with chemical groups C3-OH and C4-OH of the unsaturated cyclitol unit of acarbose. Additional stabilization of the first sugar ring may result from hydrophobic interactions with bulk side chains of residues Tyr1251, Trp1523 and Trp1418. At subsite +1, the side chains of Asp1157 form two hydrogen bonds with the C2-OH and C3-OH groups of 4,6-dideoxy-4-amino-D-glucose of acarbose. Additionally, atom NH1 of Arg1510 makes a hydrogen bond with the C3-OH group of the second ring. Residues Trp1355 and Phe1559 stack with the first and second rings of acarbose, further stabilizing the acarbose molecule. Most importantly, the residue Asp1526 forms one hydrogen bond with atom N4B of acarbose, which is a candidate for an acid/base catalytic residue. At subsite +2, the side chain of residue Trp1369 stacks with the third ring of acarbose. At subsite +3, the two residues Phe1560 and Pro1159 stabilize the fourth ring through hydrophobic interactions.

Figure 6. Interaction of MGAM-C with acarbose

Figure 6. represents the stereo view of the 2Fo-Fc electron density map in the active site of the MGAM-C/acarbose structure contoured at the 2.0σ level and shown in blue. Acarbose is represented as thick cyan sticks and the active-site residues are represented as thin green sticks.
2.5.4. Prediction of ADMET properties for the isolated compounds

Computed physicochemical properties associated with compounds that have good oral bioavailability, less or no toxicity and optimum values of physicochemical properties are key parameters for the drug discovery. The physicochemical properties such as lipophilicity calculated partition coefficient (log P), molecular weight (MW), topological polar surface area (TPSA), number of hydrogen bond donors, hydrogen bond acceptor, number of rotatable bonds (nRot) and predicted aqueous solubility are the key parameters in drug design and development. Early prediction of the safety endpoints through in silico techniques screening have become regular practice for both designing new molecule and screening of the isolated compounds. Most frequently measured end points to evaluate potential safety issues include inhibition of cytochrome P450 (CYPs) mono oxygenase enzymes to determine potential for drug-drug interactions, inhibition of hERG potassium ion channel effects, lethal rat acute toxicity (LD50) and other crucial toxicity (AMES toxicity, skin sensitization, and hepatotoxicity). Drugs are often withdrawn at the different phases of the clinical trials due to poor ADMET properties and adverse effects probably associated with their molecular structures. Therefore, it is important to predict ADMET properties during the lead optimizations. The current study utilizes the pharmacodynamics prediction to elucidate the drug-likeness, Lipinski’s rule, and absorption, distribution, metabolism, excretion, and toxicity properties of isolated compounds as MGAM inhibitor.