**UNIT-V (BOP-474)**

**Biological Fingerprinting of Herbal Samples by Means of Liquid Chromatography:**

Biological chromatographic fingerprinting is a relatively new concept in the quality control of herbal samples. Originally it has been developed with the application of HPLC, and recently herbal samples' biological profiles have been obtained by means of thin-layer chromatography (TLC).

 **Introduction**

* Fingerprint construction has become an important quality control tool of herbal samples in the light of constantly growing interest in natural origin medicines. Fingerprint analysis has been accepted by WHO as a methodology for the quality control of herbal samples .
* It is applied to identify closely related plant species, to detect adulterations, to control the extraction process or to study the quality of a finished product. Herbal sample fingerprint can be defined as a set of characteristic chromatographic or spectroscopic signals, whose comparison leads to an unambiguous sample recognition.
	+ - Several chromatographic methods have been applied for fingerprint construction, namely, high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), gas chromatography (GC), or high-speed counter current chromatography (HSCCC). However, it is difficult to indicate with 100% certainty which signals (peaks, bands, etc.) should be present in the obtained fingerprint to confirm sample identity.
* For that purpose the analyzed sample can be compared with a defined Botanical Reference Material (BRM) or a set of standard compounds. Defining and obtaining BRM for every plant species is a difficult task, therefore, new solutions are sought for fingerprint comparison.
* More recently a concept of multiple fingerprints construction and multidimensional fingerprinting have gained much attention, as large amount of chromatographic and/or spectroscopic signals enable more comprehensive data analysis.Multiple chromatographic fingerprint consists of more than one chromatographic profile.
* As far as herbal medicines are considered their biological activity is an important issue. However, traditional chromatographic fingerprint analysis provides the researchers only with qualitative and quantitative information. A critical issue is the fact, that compounds present in low concentration may exert more potent biological activity than those present in greater amounts. It is, therefore, important to introduce screening of the biological activity into the chromatographic fingerprint analysis.

**HPTLC**

The current scenario appears to demand for plant drugs throughout the world because of their safety and efficacy. Now a days folklore medicine is being reevaluated by extensive research on different plant species and their therapeutic principles. Chromatographic and spectral fingerprint analysis plays an important role in the quality control of complex herbal medicines. Thin layer chromatography (TLC) is the preliminary step to identify the phytochemical constituents in a sample. High performance thin layer chromatography (HPTLC) can provide an electronic image of the chromatographic fingerprint and a densitogram to detect the presence of marker compounds in a plant sample. Both the methods are efficient, faster, reliable and reproducible.

It involves the same theoretical **principle** of thin layer chromatography. **principle** **hptlc** have similar approach and employ the same physical **principles** of TLC (adsorption chromatography) i.e. the principle of separation is adsorption.

 **Instrumentation of HPTLC**

1. **Solvent Resorvoir** : Mobile phase contents are contained in a glass resorvoir. The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample.
2. **Pump** : A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system’s column and detecter. Depending on a number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated.
3. **Sample Injector** : The injector can be a single injection or an automated injection system. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).
4. **Columns** : Columns are usually made of polished stainless steel, are between 50 and 300 mm long and have an internal diameter of between 2 and 5 mm. They are commonly filled with a stationary phase with a particle size of 3–10 µm. Columns with internal diameters of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the mobile phase and the column should be kept constant during an analysis.
5. **Detector** : The HPLC detector, located at the end of the column detect the analytes as they elute from the chromatographic column. Commonly used detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical detectors.
6. **Data Collection Devices** : Signals from the detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The computer integrates the response of the detector to each component and places it into a chromatograph that is easy to read and interpret.

# *High performance thin layer chromatography fingerprinting, phytochemical and physico-chemical studies of anti-diabetic herbal extracts.*

## Introduction

* Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine.
* According to World Health Organization (WHO), about three-quarter of the world population relies upon traditional remedies for the health care of its people. In fact, plants are the oldest friends of mankind. They not only provided food and shelter, but also served the humanity to cure different ailments.
* The plants and their extracts are a common elements in Indian systems of medicine. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. The plant-based, traditional medicine systems continue to play an essential role in health care.
* In the world, diabetes is a serious disease due to irrational food habits. Most of the hypoglycemic agents used in allopathic practice to treat diabetes mellitus are reported to have side effects in long term use.
* Hence, there is a need to search for effective and safe drugs for these ailments. Pharmaceutical research across the world shows that natural products are potential sources of novel molecules for drug development.

*The recent global resurgence in herbal medicines has led to an increase in the demand for them. Commercialization of these medicines to meet this increasing demand has resulted in a decline in their quality, primarily due to a lack of adequate regulations pertaining to this sector of medicine.*

* Various methods of phytochemical standardization, such as preliminary phytochemical screening, fingerprint profiling and quantification of the marker compound with reference to plant extracts and polyherbal formulations are used.
* Standardization is necessary to make sure the availability of an uniform product in all parts of the world. It assures a consistently stronger product with guaranteed constituents.

### Plant material and market extracts

The five herbal anti-diabetic drugs and their extracts, chosen for this study were

Gymnema sylvestre  - Asclepiadaceae (Madhunashini - leaves),

Pterocarpus marsupium  - Fabaceae (Vijaysara - heart-wood),

Enicostema littorale . - Gentianaceae (Mamejaka - whole plant),

Syzygium cumini (L.) - Myrtaceae (Jambu - seeds) and

Emblica officinalis - Euphorbiaceae (Amalaki - whole fruits).

***HPTLC vs. TLC***

HPTLC arises from the need for major separation capacity, has it is obtained by the use of precoated plates with smaller particles (5 *vs*. 15 µm), in order to obtain the efficacy needed for plant mixtures, but problems for TLC have also other origins.

Planar chromatography is mainly based on critical manual steps: it is an open system, dependent on environmental factors (temperature, light, fumes, humidity), that can influence the resulting data; also using the best rigor, despites all the extracautions of the same operator, results can not be fully controlled and analyses are not totally reproducible. In other words, data are scientifically not totally reliable and confident. TLC still remains a craftsman's performance. HPTLC, High Performance Thin Layer Chromatography - the TLC of the 21th Century - is the last evolution of planar chromatography, whose mission is change the weakness into strengths. In modern HPTLC, the plate is the central tool of a complex automatic instrumentation system developed to control analysis conditions, to optimize reproducible results and to allow a complete comparison between different laboratories. The operator is the director of a tentative to reproduce Nature's symphony tricking with sophisticated machines. In this regard, HPTLC is much more complicated than TLC, but complex problems need complex solutions. Being a multistep process, HPTLC performance requires separated devices for each step of the sequence: sample application, chromatogram development, derivatisation, visualization and documentation. The full power of HPTLC comes from the proper use, compatible and complementary, of each device in an integrated system (Reich & Schibli, 2007).

Frequent goal of HPTLC is the fingerprint, as authentic maker of the biological complexity. A fingerprint is the individual chromatographic track representing, as near as possible, a mixture of organic substances. By the fingerprint approach, it is possible to obtain a proper identification of the plant material, but also determine and accept the limits of the biological changes. Variations in HPTLC tracks of the same species are mainly quantitative, not qualitative.

**The successful use of HPTLC performed in our laboratory is here reported in cases concerning:**

a) adulteration, the use of other constituents besides those reported in label composition

b) substitution, the utilization of botanical species not reported in pharmacopoeias and other regulatory references instead of the official ones

c) comparison, between extracts of the same species differently obtained.

HPTLC is a potent tool for easy comparison of similar products. Lots of the same product can be compared in the same plate and quantities assessed by densitometric inspection. In particular, we were able to examine the composition of several products marketed by the name of neem cake. Neem tree, *Azadirachta indica* A. Juss, features a long traditional utilization in agriculture, for the body care (cosmetics) and medicine but currently, there is a huge amount of products obtained from this multipurpose tree, due to the anti-fungal, anti-bacterial and insecticide properties. The most commercially important product is the neem oil extracted from the seeds, also known as margosa oil.

 Neem, identified by WHO/UNEP1989 as an environmentally powerful natural pesticide, is considered to be one of the most promising trees of the 21stcentury for its great potential in pest management, environment protection and medicine.

**Conclusion**

The utilization of HPTLC, owing to the high automatization recently obtained, can be considered as an useful tool in the analysis of complex mixtures of natural products, such as those introduced nowadays in the market. Still some aspects remain to be improved, like the cost of instruments, and the sensibility, still not comparable with HPLC. Also in this field, like in the other analytic approaches, the use of hyphenated techniques, as already obtained in GC/MS. Hyphenated HPTLC/MS instruments are already available, and HPTLC/NMR are under study, to solve in one step the problem of separation and identification.

**THE INTERACTIONS OF HERBS AND DRUGS**

Until about 150 years ago, all medicines were derived from natural materials .  Most of those early medicines are described under the broad heading “herbs,” although that term may prove misleading. Even though people often think of herbs as plants or plant-derived materials, several commonly used items were obtained from animals and minerals.  Further, although the term “herbs” suggests something that is beneficial and has little potential for harm, numerous toxic materials were used, such as foxglove, deadly nightshade, and jimson weed (datura).

Herbalists sometimes processed the herbs to change them from their original form , and even isolated some active constituents, so that the end products were not as nature presented them.  For example, aconite was processed extensively in China to reduce its toxicity so that it could more readily be used, and borneol, the active constituent found in a few tropical plants, was isolated centuries ago in relatively pure form, a translucent crystal, for both internal and external use.

 The use of potent and toxic substances and the intentional alteration of natural substances are characteristics of production of modern drugs.  Thus, some issues that arise today about interactions of herbs and drugs may have already been encountered in earlier times when herbs were combined with each other.

In the formative period of traditional Chinese medicine, some concerns were raised about the intermixing of herbs .  This could be considered the genesis of cautions about drug interactions (using the term “drug” here for medicines in general.

**INTRODUCTION:**

Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. Synergistic therapeutic effects may complicate the dosing of long-term medications. Use of herbal and dietary supplements is extremely common: in one US survey of adults who regularly take prescription medication, 18·4% reported the concurrent use of at least one herbal product or high-dose vitamin (and 61·5% of those who used unconventional therapies did not disclose such use to their physicians) .

A basic problem is that the phrase "herb-drug interaction" routinely appears in the media, without definition and an assumption that everyone knows what is meant. Herbal medicines are ubiquitous: the dearth of reports of adverse events and interactions probably reflects a combination of underreporting and the benign nature of most herbs used. Experimental data in the field of herb drug interactions are limited, case reports scarce, and case series rare.

This lack of data is also true of drug drug interactions: published clinical studies are mainly case reports (controlled trials are scarce, since the random assignment of patients to trials that examine unintended effects is not ethical). The true prevalence of drug interactions is substantial but unknown. One study of 1000 elderly people admitted to a hospital from the emergency department found that 538 patients were exposed to 1087 drug-drug interactions; 30 patients experienced adverse effects as a consequence of these interactions 2.

In clinical practice, poly pharmacy is common, and to the mixture physicians prescribe, patients add various over-the-counter medications, vitamins, herbs, and foods. All ingested substances have the potential to interact. Herbs are coming under increasing attack for being potentially dangerous to patients who are already taking prescription medications. The concerns are multiplied for those patients currently taking multiple medications, often prescribed by multiple physicians who may or may not be in communication with each other regarding their medical reasoning.

**Mechanism of herbal-drug interactions:**When there are any interactions between herbals and drugs occur that can be caused by either pharmaco-kinetic or pharmacodynamic mechanisms.

**Pharmacokinetic interactions:**When an herbal changes the absorption, distribution, metabolism, protein binding, or excretion of a drug that results in altered levels of the drug or its metabolites that is called pharmacokinetic interactions. Most of the current evidence of pharmacokinetic drug interactions involves metabolizing enzymes and drug transporters . Although drug interactions can involve enzymes such as glutathione S-transferases and uridine diphosphoglucuronyl transfereases (UGTs), most herbal drug interactions are related to oxidative metabolism by the cytochrome P-450 system (CYP) or by the effect of an herbal on the efflux drug transporter P-glycoprotein.

**Interference with Absorption:**Oral bioavailability of medications can be decreased by this latter mechanism when they are combined with soluble and insoluble fibers; for example, with psyllium (*Plantago psyllium*) or tannins like those found in tea (*Camellia sinensis*), pomegranate (*Punica granatum*), cinnamon (*Cinnamomum*spp), and rhubarb (*Rheum*spp) 3.This was demonstrated in a clinical trial in which patients taking lovastatin with pectin or oat bran experienced an increase in low-density-lipoprotein (LDL) levels, which returned to normal after fiber supplementation was discontinued .

The most likely cause of this interaction was a decrease in lovastatin absorption due to binding of lovastatin by pectins or bran fibers in the intestinal lumen .

**Drug Metabolism:**The liver, intestines, kidneys, and lungs are the sites of drug metabolism and pharmacokinetic interactions .Of those, the liver is the major site of drug metabolism, with the intestines playing a secondary yet potentially important role .Drug metabolic pathways historically have been classified into 2 groups-phase I and phase II. These 2 phases work in sequence, with most metabolites of phase I passing through phase II. Phase I enzymes are a super family of hemoproteins called cytochrome P450s (CYP). They oxidize relatively non-polar molecules, increasing their polarity and allowing them to be excreted in the urine. The main CYP isoforms are 1A2, 2D6, 2C9, 2C19, and 3A4 .

An estimated 60% of drugs are metabolized and excreted via the CYP3A4-dependant pathway .Phase II enzymes follow a different chemical process, which is referred to as conjugation. Phase I and phase II activities must be coordinated or the induction of phase I could cause production of too many intermediate metabolites for phase II to process. Similarly, excessive phase II substrates can lead to an increase in reactant concentrations, sometimes with fatal results.

**Pharmacodynamic Interactions:**Pharmaco-dynamic interactions are related to the pharmacologic activity of the interacting agents and can affect organ systems, receptor sites, or enzymes. A Pharmacodynamic interaction may occur when herbals that possess antiplatelet activity are administered with antiplatelet/anticoagulant drugs, thus increasing the risk for bleeding. Other examples are when herbals that depress the central nervous system (CNS), such as kava, are administered with CNS depressant drugs or when herbals that may lower blood glucose are given with antidiabetic drugs.

An example of an antagonistic interaction is when an herbal with high caffeine content, such as guarana, is administered with a sedative-hypnotic. In addition, herbals with the potential to cause organ toxicity may cause further risk of toxicity when drugs with similar toxicity are administered concurrently, such as when the hepatotoxic herbal comfrey is given with large and prolonged doses of acetaminophen.

Some herbs were reported to interact with others in a beneficial way, such as by reducing the toxicity or other adverse effects that might be experienced (see Table 1)

**Table 1: Chinese Herbal Concept of Interaction of Drugs.** The common term used for interaction is *xiang*, literally, mutual or reciprocal action (that is, the drugs act upon one another).

|  |  |
| --- | --- |
| **Type of Interaction** | **Examples** |
| *Xiangfan* (incompatibility): yields toxic reaction or side effects. | Raw aconite is incompatible with raw pinellia; licorice is incompatible with sargassum; veratrum is incompatible with scrophularia. |
| *Xiangwu* (antagonism): reducing the desired effect of one drug by combining with another drug. | Raphanus inhibits the action of ginseng; ginseng inhibits the action  of pteropus; clove inhibits the action of curcuma. |
| *Xiangsha* (detoxification) or *Xiangwei* (inhibition): one drug reduces the toxicity or side effects of another. | Fresh ginger and alum each reduce the toxicity of pinellia and arisaema. |
| *Xiangshi* (enhancement): one drug enhances the effect of the other. | An herb that tonifies the spleen, when combined with an herb that is diuretic will enhance the diuretic action. |
| *Xiangxu* (synergism): drugs of similar nature reinforce each other’s effects. | Rhubarb and mirabilitum are each purgatives that produce a more reliable and stronger purgative action when used together. |

The move from natural source to synthetic drugs was not necessarily any reflection of anti-herb bias by the medical profession or pharmaceutical manufacturers.  Even the relatively new drug Taxol was originally extracted from yew trees.  It was found in very minute quantities in the bark.  The demand for Taxol (a drug widely used for treating breast and ovarian cancers, and increasingly tried for other cancers) grew so rapidly that the yew forests would have been decimated by now if it weren’t for the development of a synthetic version, which was a very difficult process due to the complexity of the molecule.  Had Taxol been derived from an easily cultivated and renewable natural resource, the natural product would have been used instead.

Many whole herbs have remained in the Pharmacopoeias of nations that now rely on modern medicine, but the difficulties in procuring and handling the high-quality herbal materials has led to replacements by isolates and synthetics.  Within the context of using intensively regulated, pure chemical drugs, medical doctors have correctly raised questions about the identity and variability of herbs that have been provided as alternatives without adequate clinical testing and without monitoring of adverse effects once on the market.  On the other hand, herbs (as well as other natural healing approaches) were sometimes dismissed under the incorrect premise that modern medicine had already provided a satisfactory replacement for all the earlier health care practices that had been relied upon for centuries.

For the most part, the possibility of herb-drug interactions had been largely ignored during this revival of medicinal herb use.  A strong impetus to look at the problem more closely came from the finding that grapefruit juice could impair drug metabolism and result in significant changes in the expected drug activity as a result.  This observation, first published in 1989, did not come to public attention until several years later, when the use of herbs had become even more widespread.  The question then immediately arises: if an ordinary food like grapefruit could cause this response, why not herbs?   Among the other issues of herb-food interactions were these:

       it was recognized that certain foods interacted with a broad class of antidepressant drugs, making people wonder if it was safe to eat pizza while using the drugs (so, how safe could combining with herbs be?);

       it was noted that green vegetables could antagonize the effect of warfarin, the most commonly used blood thinner (many herbs appear no different than green vegetables);

       tetracycline absorption was markedly impaired by ingestion of milk or milk-based food products (perhaps herbs could impair the absorption of drugs as well).

During the past five years, a few herbs that had been widely popularized (partly because new labeling laws allowed medical use claims to accompany their sale), such as ginkgo, ginseng, and St. John’s wort, were specifically cited as causing, or suspected of causing, interactions with drugs.  The drugs of most concern for interactions with herbs were those that people took continuously, such as blood thinners prescribed after a heart attack or stroke, and antidepressants.  However, interactions with anti-infection drugs, which might fail to work if their blood levels fell too low, could also present a serious problem.

**Table 2: Commonly Used Chinese Herbs with Furanocoumarins.**

|  |  |
| --- | --- |
| **Herbs** |  **Furanocoumarins** |
| citrus varieties, including citrus, blue citrus, chih-shih, chih-ko, citrus seed | Bergapten |
| *Angelica*species; including angelica, chiang-huo, tu-huo, tang-kuei | psoralen, xanthotoxin, imperatorin, bergapten, angelin, marmesin, oxypeucedanin, isopimpinellin, phellopterin, byakangelicin |
| cnidium fruit | bergapten, isopimpinellin, columbianetin, cnidiadin |
| psoralea | Psoralens |

Some herbs can increase drug metabolism rather than decrease it, resulting in lower drug availability.  This effect was recently discovered with the use of St. John’s wort by patients with HIV infection .  It was found that the use of this herb resulted in much lower levels of the protease inhibitor drug Indinavir, with the possible consequence that the drug combination would not work and that resistant strains of HIV would emerge.  A warning was issued by several agencies involved with AIDS treatment.  St. John’s wort was also blamed for a sharp drop in cyclosporin availability ; since this drug is used to prevent transplant rejection, a lowered blood level could lead to initiation of the rejection reaction, which may be difficult to reverse.  On the other hand, the herb has also been reported to inhibit CYP3A4 , which could result in some drugs being made available to the blood at elevated levels.

**Fermented foods and mao inhibitors:**

A broad class of antidepressant, anti-anxiety drugs are the monoamine oxidase inhibitors (MAOIs).  These drugs reduce the activity of the enzyme (MAO) that breaks down neurotransmitters that are monoamines (have one amino group), such as serotonin, dopamine, and norepinephrine.  Monoamine oxidase is found in the outer membranes of mitochondria.  The monoamines are constantly produced, released, and then inactivated, and changes in the rates of any of these actions affects the levels of the compounds and their effects on the nervous system.

Two well-known effects of having low levels of monoamines are depression and hypertension.  If MAO activity can be inhibited, then the levels of the monoamines will rise and the symptoms of deficiency are likely to be alleviated.  MAO inhibitor drugs, such as phenelzine (Nardil), are used to treat certain types of depression, mainly “situational depression.”  However, such drugs are often reserved for use when others fail because of the potential side effects, including reduced sexual function, hypotension, fluid retention, and nervous agitation.  These MAO inhibitor drugs are powerful, and they often interact with other drugs, especially others that have as a secondary effect, inhibition of MAO, thus increasing the level of serotonin and other monoamines to harmful levels.  Not only can the side effects increase with such drug interactions, but the patient can experience episodes of mania that may not have occurred prior to drug use.  High levels of serotonin can have immediate effects and, if prolonged, might have permanent damaging effects on the heart and kidney.

**Tetracycline and milk:**

Tetracycline is the most commonly used of a group of related antibiotic drugs that are labeled -cyclines.  The significant inhibition (50–90%) of absorption of tetracycline by consuming milk was reported in 1976 (25).  Patients were advised to avoid drinking milk within 3 hours of tetracycline ingestion.   Because this drug is so widely used, the interaction with milk became one of the most widely cited cases of food-drug interactions.

However, few people outside the pharmaceutical field realize that the absorption problem of these cycline drugs with milk is actually a reflection of the drug’s binding with polyvalent metals (that is, those that normally lose more than one electron to form an ion).  The interaction with milk, in fact, is an interaction with calcium (Ca++) in the milk.  The same type of interaction occurs with iron (Fe++), magnesium (Mg++), zinc (Zn++), and aluminum (Al++).  The cycline antibiotic complexes with the mineral and becomes an insoluble salt that is not absorbed (26).  The mycin antibiotics (such as kanamycin and neomycin) also form salts with polyvalent metals.  Antacid preparations contain these metals, and are contraindicated when taking the antibiotics.

Inhibition of the drug’s absorption can also occur when taking nutritional supplements that provide minerals (iron has a somewhat stronger effect than calcium) and, potentially, with any mineral rich food or herb.  Chinese herbal formulas may be made with calcium-rich materials such as oyster shell, dragon bone, gypsum, haliotis, and mother of pearl.  Oysters (the meaty portion) are rich in zinc; dried figs are rich in calcium; and raisins are rich in iron, and iron is a dominant component of the Chinese mineral compounds lapis, hematite, and magnetite.  Laxative and mass-resolving Chinese herb formulas containing mirabilitum will provide considerable amounts of magnesium.

Ginger, used in Chinese and Western medicine to treat nausea, is postulated to have, as part of its action, the binding and inactivation of substances in the stomach that cause nausea.  By this same mechanism, ginger might bind drugs (reducing their nauseant effect but, potentially, reducing their absorption as well).  Recently, phytic acid, a significant component of corn that is also present in other grains and in some legumes, was promoted as a cancer inhibitor (under the name IP-6; see: *Questionable cancer therapies*).  This substances is well-known as a chelator of minerals, and might also bind up drugs; phytic acid is one of the major binding agents in fiber.

**Blood thinners and green vegetables :**

During the past decade, it has become standard procedure to place patients who have survived a heart attack or stroke on a regimen of daily use of “blood thinning” drugs (e.g., warfarin).  These drugs are also used in other circumstances where it is feared that a clot may form, such as after leg surgery (a clot forming as a result of the surgical damage could release and migrate to the brain).  The drugs don’t actually make blood “thinner,” but reduce the ability of its platelet component to clot (the clumped platelets represent a “thickening” of the blood).  The drugs are administered with the intent of preventing a harmful or catastrophic clot.  The dose of the anticoagulant must be adjusted carefully so that there is adequate clotting at the site of an injury, otherwise, the person could seriously bleed (even to the point of fatality) from a relatively minor cut or from a bleeding ulcer.  Yet, there must be enough impairment of  unnecessary clotting that a heart attack or stroke is unlikely to occur.  One of the fears associated with use of blood thinning drugs is the possibility that the patient will suffer from a cerebral hemorrhage.  This is a rare event, but unlike bleeding from a cut that might simply stop a little more slowly under the influence of the drug compared to normal, even the small amount of blood flow in a cerebral hemorrhage can be fatal.  Usually, platelet aggregation rates of the patients on blood thinning regimens are measured weekly, so as to assure that the rate is within desired bounds; if it is not, the drug dosage can be changed or factors that are influencing it can be removed.

Green vegetables (especially broccoli, spinach, peas, cabbage, and cucumbers) were found to have a measurable impact on anticoagulant therapy.  The usual advice given to deal with substances that interact with drugs—to avoid them—didn’t seem an appropriate way out when the health benefits of green vegetables were so well established and so strongly promoted.  Therefore, the solution offered was to avoid changing the amounts of green vegetables being consumed, so as to provide a stable environment for the drugs to work in.

The main active ingredient in the green vegetables is vitamin K.  It has coagulation promoting qualities that overcome the effects of the anticoagulant drug, thus making the therapy less effective (the drug dosage has to be increased). According to one recent literature review, it was suggested that patients try to limit their vitamin K intake from their diet to 65–80 micrograms (15).  The current recommended daily requirement for adults not undergoing warfarin therapy, is 65 micrograms for women and 80 micrograms for men; thus, the recommendation is to avoid exceeding the levels suggested for normal nutritional needs.  Vitamin K is produced by intestinal bacteria; use of antibiotics that inhibit intestinal bacteria can also change the vitamin K content of the patient’s blood.

**Steroids and chinese herb:**

Many Chinese herbs have been reported to be useful in the treatment of autoimmune disorders.  One mechanism of action is to enhance the circulating levels of corticosteroids by slowing their metabolism. This action will not only increase the amount of natural corticosteroids (hormones produced by the adrenal cortex), but also any steroids administered as drugs.  When the steroid levels administered for treating inflammation are high enough to cause some side effects, elevation of the drug level by herb action could increase the side effects.  The sex hormones are metabolized by both the same and by similar enzymes as the corticosteroids.  Enhanced levels of sex hormones in aged individuals may be one of the mechanisms by which antiaging Chinese herbs are able to improve patient conditions.  The enhancement may arise at the level of hormone production or by inhibition of hormone degradation.  If hormone degradation is slowed, administered hormones may be present in amounts higher than expected.  This situation is similar to that which arises with inhibition of the CYP enzyme system.

**General concerns:**

Some doctors have raised general concerns about using herbs, without being highly specific about the interaction that might occur.  An anesthesiologist has raised the concern for his colleagues that herbal therapies might interact with the anesthesia, and has suggested, perhaps excessively, that herbal therapies be discontinued at least two to three weeks prior to surgeries that require anesthesia . Some medical doctors and pharmacists have advised that, until more is known, herbs not be mixed with drugs at all , citing as examples several potential cases of interactions.  A Chinese physician has expressed theoretical concerns about herb-drug interactions, especially with regard to antibiotics .  For example, he suggested that antibiotics that are somewhat toxic to the liver, such as chloromycetin, erythromycin, tetracycline, riampicini, and rimifon, might become more toxic in the presence of hydrated tannins, as found in pomegranate rind, sanguisorba, gall (*webeizi*), and terminallia.  The general concern is that the herbs have a mild liver toxicity that reinforces that of certain drugs.  This might also apply to other herbs (without tannins) that are safe in normal usage but which become liver toxic in high dosage, such as xanthium.  He also expressed concern over combining antibiotics with herbs rich in organic acids or alkaline substances (may change absorption characteristics of antibiotics), with herbs containing cyanosides (such as apricot and peach seeds; may cause respiratory inhibition), enzymes (as found in gallus, which may break down the drugs), licorice (decrease absorption), and blackened herbs (as used to stop bleeding, may decrease absorption of drugs).   Table 3 provides a summary of some potential interactions for herbs used in the Chinese tradition; St. John’s wort, though rarely used in Chinese medicine, is increasingly used in the West and the main herb for which drug interactions are reported.

 **Table 3: Some Herbs Recently Mentioned as Having Potential for Drug Interactions.**  According to modern journal reports, these “should not be used” with the drugs indicated.  The herbs listed in this table are used in the Chinese tradition.  Warfarin is the drug most often suggested to have interactions.

| **Herb** | **Source** | **Interactions Reported or Suspected** |
| --- | --- | --- |
| St. John’s wort*tianjihuang* | *Hypericum perforatum*(tops) | warfarin (to cause bleeding); serotonin-uptake inhibitors (to cause mild serotonin syndrome); indinavir (decreased bioavailability); digitoxin, theophylline, cyclosporin, phenprocoumon, and oral contraceptives (all with reduced bioavailability) |
| Ginseng*Renshen* | *Panax ginseng*(root) | antidepressants such as phenelzine sulfate (to cause manic episodes, headaches); warfarin (to cause bleeding or to decrease effectiveness); corticosteroids (potentiation); estrogens (potentiation) |
| Ginkgo*Yinxingye* | *Ginkgo biloba*(leaf) | warfarin (to cause bleeding) |
| Ginger*Jiang* | *Zingiber officinale*(rhizome) | sulfaguanidine (enhance absorption) |
| Garlic*Dasuan* | *Allium sativum*(bulb) | warfarin (to cause bleeding) |
| Tang-kuei*Danggui* | *Angelica sinensis*(root) | warfarin (to cause bleeding) |
| Salvia*Danshen* | *Salvia miltiorrhiza*(root) | warfarin (to cause bleeding) |
| Rhubarb*Dahuang* | *Rheum officinale*(root) | cardiac glycosides and antiarrhythmic agents (potentiating by reducing potassium via laxative effect) |
| Aloe*Luhui* | *Aloe ferox*(leaf sap) | cardiac glycosides and antiarrhythmic agents (potentiating by reducing potassium via laxative effect) |
| Ma-huang*Mahuang* | *Ephedra sinica*(leaf) | MAO inhibitors (to cause hypertension); cardiac glycosides or halothane (to produce cardiac arrhythmia); caffeine (to intensify cardiovascular side effects) |
| Astragalus*Huangqi* | *Astragalus membranaceus*(root) | cyclosporine, azathioprine, methotrexate (to impair intended immuno-suppressive effects). |
| Bupleurum*Chaihu* | *Bupleurum falcatum*(root) | sedatives (potentiation) |
| Licorice*Gancao* | *Glycyrrhiza uralensis*(root) | corticosteroids and thiazide diuretics (potentiation); digitalis or other cardiac glycosides (increased sensitivity) |

**ADVICE**

Practitioners who prescribe herbal therapies must ask their patients about drug use and keep an updated record of the drugs.  Drug interactions may be avoided entirely by not prescribing herbs, and may be minimized by having the patients take the herbs and drugs at different times (about one hour apart to avoid any direct interactions in the digestive tract; about 1.5 hours apart to avoid having maximum blood levels of the drug and herb occurring at the same time).  The dosage of herbs that are aimed therapeutically at the same function as the drugs (e.g., both are sedatives; both are hypoglycemics; both are anticoagulants), should be reduced to alleviate concerns about additive or synergistic effects that are too great.

Patients that are taking multiple drug therapies are at greater risk of interactions between each of the drugs, between drugs and foods, and between drugs and herbs.  Therefore, greater caution must be exercised in considering use of herbs for these patients and, especially, herbs that may have a similar therapeutic action. Patients who are in fragile health and using multiple drug therapies should avoid most herbs.  In order to more skillfully combine drug and herb therapies, it behooves the practitioner to learn as much as possible about the mechanisms of action of both the drugs and the herbs (pharmacological action, not the action in ancient terminology).  Increasingly, this is the language used to describe potential interactions that might be avoided.  When patients report that they have had an experience that they believe might be the result of an interaction between one or more drugs that they are taking and the herbs that have been prescribed, it is very helpful to be able to know the possibilities that are present for such interactions.  It is reassuring to the patient if they know that the potential for any herb-drug interactions has been carefully considered in selecting or designing the herb therapy (see Table 4).

**Table 4: Information for Practitioners to Relay to Patients about Interactions with Drugs.**The interactions depicted here may involve foods, herbs, or nutritional and non-nutritional supplements; the information stated in terms of herbs should be applied to any substance with a potential for drug interactions.

| **Type of Interaction** | **Examples** | **Patient Information** |
| --- | --- | --- |
| Drug absorption inhibited by binding, resulting in low drug levels. | Tetracycline with minerals; alkaloids with tannins; pectins, resins, and fibers may bind several drugs. | Take herbs at least one hour apart, preferably 1.5 hours apart from taking drugs. |
| Drug absorption inhibited by rapid transit time, resulting in low drug levels. | Diarrhea or frequent bowel movements due to colitis or laxative intake speeds transit of all materials through the intestinal tract. | Treat diarrhea and avoid excessive use of laxatives.  Induction of diarrhea is an intended treatment strategy in Chinese medicine for nephritis. |
| Drug absorption and/or elimination modified. | Saponins may improve absorption and elimination of drugs, altering the blood levels and rate of change of drug levels; strongly acid or alkaline herbs may alter absorption of drugs. | Take herbs at least 1.5 hours apart from drugs; avoid herbal preparations that have high saponin content. |
| Drugs metabolized too slowly resulting in elevated drug levels. | Grapefruit juice and herbs that inhibit CYP enzyme system can result in much higher levels of drugs in the bloodstream, and longer persistence of the drugs. | Take herbs at least 1.5 hours apart from drugs, preferably taking the drugs first (so that drug metabolism is already under way by the time the herbs can inhibit enzyme systems). |
| Potassium decreased when using cardiac drugs, resulting in adverse cardiac conditions. | Laxative and diuretic herbs may reduce potassium; these types of herbs are often given together for weight loss. | Avoid any strong laxative or diuretic action while using cardiac drugs.  To compensate for mild diuretic or laxative treatments, consume high-potassium foods. |
| Drug action is intensified by similar effect of herbs. | Blood vitalizing herbs and blood thinning drugs may prevent adequate clotting; hypoglycemic herbs and hypoglycemic drugs may lower blood sugar too far; caffeine or ephedrine containing herbs and CNS stimulants disturb nerve functions. | When the drug therapy is already addressing a particular therapeutic goal, avoid adding an herbal therapy with the same goal. Intensify monitoring of blood conditions affected by the drugs. |
| Drugs cause adverse reaction to occur when certain substances are ingested. | MAO inhibitors can cause hypertension when an ordinary food component, tyramine, is ingested; some drugs can cause severe nausea when alcohol is ingested. | Learn the known reactions and take reasonable steps to avoid problematic herbs. It may be unnecessary to have total abstinence from an herb that reacts with a drug. |
| Miscellaneous: reported drug interactions. | St. John’s wort decreases bioavailability of indinavir. | Learn the known reactions and avoid using the combination. |
| Desired drug effect is counteracted by herb effect. | Immune-enhancing herbs may counteract intended immunosuppressive action of drugs in autoimmune disorders, including transplant rejection reactions. | If herbs with known immune-enhancing actions are to be used, limit the dosage to avoid counteracting the drugs. |

**Definition and history of bioavailability enhancers**

‘Bioavailability enhancers’ are drug facilitators, they are the molecules which by themselves do not show typical drug activity but when used in combination they enhance the activity of drug molecule in several ways including increasing bioavailability of the drug across the membrane, potentiating the drug molecule by conformational interaction, acting as receptors for drug molecule and making target cells more receptive to drugs. A ‘bioenhancer’ is an agent capable of enhancing bioavailability and bioefficacy of a particular drug with which it is combined, without any typical pharmacological activity of its own at the dose used.

These are also termed as ‘absorption enhancers’ which are functional excipients included in formulations to improve the absorption of a pharmacologically active drug.

The term ‘bioavailability enhancer’ was first coined by Indian scientists at the Regional Research Laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine, Jammu), who discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979.

## Mechanism of action of bioenhancers of herbal origin

There are several mechanisms of action by which herbal bioenhancers act. Different herbal bioenhancers may have same or different mechanisms of action. Nutritional bioenhancers enhance absorption by acting on gastrointestinal tract. Antimicrobial bioenhancers mostly act on drug metabolism processes.

Among the various mechanisms of action postulated for herbal bioenhancers some are (a) reduction in hydrochloric acid secretion and increase in gastrointestinal blood supply , (b) inhibition of gastrointestinal transit, gastric emptying time and intestinal motility , (c) modifications in GIT epithelial cell membrane permeability, (d) cholagogous effect , (e) bioenergetics and thermogenic properties and (f) suppression of first pass metabolism and inhibition of drug metabolizing enzymes and stimulation of gamma glutamyl transpeptidase (GGT) activity which enhances uptake of amino acids.

### Mechanism of action of piperine:

Mechanism of Action of Bioenhancers The following are the chief mechanisms via which the various bioenhancers exert their bioavailability enhancing properties on the drug molecules:

**1**. By enhancing the absorption of orally administered drugs from gastrointestinal tract by increase in blood supply.

**2**. By modulating the active transporters located in various locations eg. P-glycoprotein (P-gp) is an efflux pump which pumps out drugs and prevent it from reaching the target site. Bioenhancers in such case act by inhibiting the P-gp.

**3.** Decreasing the elimination process thereby extending the sojourn of drug in the body.

Different mechanisms for the bioenhancer activity of piperine have been proposed including DNA receptor binding, modulation of cell signal transduction and inhibition of drug efflux pump.

 In general, it inhibits drug metabolizing enzymes, stimulates absorption by stimulating gut amino acid transporters, inhibits the cell pump responsible for drug elimination from cells and inhibits intestinal production of glucuronic acid.

It may increase the absorption of drug in the GIT, or inhibit enzymes responsible for drug metabolism, especially in the liver when the drug passes through the liver after absorption from GIT. Oral administration of piperine in rats strongly inhibited the hepatic arylhydrocarbon hydroxylase and UDP-glucuronyltransferase activities.

Another study demonstrates that piperine modifies the rate of glucuronidation by lowering the endogenous UDP-glucuronic acid content and also by inhibiting the transferase activity.

Piperine inhibits human P-glycoprotein and cytochrome P450 3A4 (CYP3A4)Both the proteins contribute to a major extent to first-pass elimination of many drugs.

Some of the metabolizing enzymes inhibited or induced by piperine include CYP1A1, CYP1B1, CYP1B2, CYP2E1, CYP3A4 etc. Most of the drugs metabolized by these enzymes will therefore be influenced by bioenhancers.

Some other suggested mechanisms include making target receptors more responsive to drugs, acting as receptors for drug molecules, increasing GIT vasculature by vasodilation to increase absorption of drugs, modulation of the cell membrane dynamics to increase transport of drugs across cell membranes.

Piperine Piperine (1-piperoyl piperidine) is an amide alkaloid found in plants of Piperaceae family like Piper longum (long pepper), Piper nigrum (blackpepper). The bioenhancing property of piperine was first utilized in the treatment of tuberculosis in human. Piperine was found to increase the bioavailability of rifampicin by about 60% and hence reduce the dose from 450 to 200mg . In human medicine piperine is approved to be combined with antitubercular drugs. Piperine also showed enhanced bioavailability when combined with Nevirapine, a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase which is used in combination with other antiretroviral agents for the treatment of HIV-1 infection.Piperine also increases the bioavailability of curcumin, the active principle of Curcuma longa (turmeric). A 20 mg dose of piperine can increase the bioavailability of curcumin by 20 fold in humans.Several animal studies on piperine have shown promising results in bioenhancing capacity of piperine for various drugs

## Need for bioavailability enhancers:

Lipid solubility and molecular size are the major limiting factors for molecules to pass the biological membrane and to be absorbed systematically following oral or topical administration.

Several plant extracts and phytoconstituents, despite having excellent bioactivity in vitro demonstrate less or no in vivo actions due to their poor lipid solubility or improper molecular size or both, resulting poor absorption and poor bioavailability. It is often found that, when individual constituents are isolated from the plant extract there is loss of specific bio-activity. Sometimes some constituents of the multi-constituent plant extract are destroyed in gastric environment when taken orally. They reduce the dose, shorten the treatment period and thus reduce drug resistance problems. Due to dose economy, they make treatment cost-effective, minimize drug toxicity and adverse reactions.

## Problems/disadvantages/with bioenhancers:

##  Although bio-enhancers in drug delivery has been successful, not all approaches have met with the same success. New bio-enhancers being developed come with challenges which have to be solved. However some of the challenges encountered have been and are still being tackled by modifying the physicochemical characteristics of the nanomaterials to improve properties such as long circulation in the blood, increased functional surface area, protection of incorporated drug from degradation, crossing of biological barriers and site-specific targeting.

Another challenge of research and development of herbal bio-enhancers is large scale production. There is always a need to scale up laboratory or pilot technologies for eventual commercialization. The challenges of scaling up include low concentration of nanomaterials, agglomeration and the chemistry process-it is easier to modify nanomaterials at laboratory scale for improved performance than at large scale. Maintaining the size and composition of nanomaterials that enhances bioavailability at large scale is also a challenge.

Advances in herbal bio-enhancers also provide new challenges for regulatory control. There is an increasing need to have regulations that would account for physicochemical and pharmacokinetic properties of nano drug products, which are different from conventional drug products. The United States' Food and Drug Administration and the European Medicines Evaluation Agency have taken the initiative to identify some possible scientific and regulatory challenges.

## Future prospects:

With bioenhancers the dosage is reduced and dangers of drug resistance are minimized. Toxicity of drug is minimized because of reduced dosage; This is especially true of anticancr drugs like taxol.

There are ecological benefits too. Taxol used to treat ovarian cancer or breast cancer is derived from bark of pacific yew tree, one of the slowest growing tree in the world. At present to treat one patient, six trees of 25-100 years old needed to be axed. With bioenhancers fewer will be destroyed.