5. INTRODUCTION

5. a. Herbal Medicine

5. a.i. Introduction to Herbal Medicine

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. As examples, we have Western medicine with origins in Mesopotamia and Egypt, The Unani (Islamic) and Ayurvedic (Hindu) systems centered in Western Asia and the Indian subcontinent and those of the orient (China, Japan, Tibet, etc.) How and when such medicinal plants were first used is, in many cases; lost in prehistory, indeed animals other than man, appear to have their own materia medica. Following the oral transmission of medical information came the use of writing (e.g. the Egyptian Papyrus Ebers c.1600BC), Baked clay tablets (some 660 cuneiform tablets c. 650 BC from Ashurbanipal’s library at Nineveh, now in the British museum, refer to drugs well-known today)1.

Again illustrating the same trend, the editor of Journal of Natural Products, 1999, writes that in response to the increasing prominence of herbal remedies, additional contributions describing scientific investigations of a rigorous nature are welcomed. Undoubtedly the plant kingdom still holds many species of plants containing substances of medicinal value, which have yet to be discovered; large numbers of plants are constantly being screened for their possible pharmacological value (particularly for their Anti-inflammatory, Hypotensive, Hypoglycemic, Amoebicidal, Anti-fertility, Cytotoxic, Antibiotic and Anti-parkinsonism properties). Phytopharmacists with a multidisciplinary background are able to make valuable contributions to these rapidly developing fields of study2.

5. a.ii. History of herbal medicine

Plants have always been a source of medicines for humans and other animals. Even the word drug comes to us from the Old Dutch term ‘droogen’ meaning dried roots. The World Health Organization estimates that 80% of the world’s population relies totally on herbal medicines. But even so 40-50% of their medicines are either direct extracts of plants or are synthetic copies of plant ingredients, from the height of Tibet, across the arid grass lands of Africa, to the dense rain forests of South America, plants remain our most important and most used medicine3.
INTRODUCTION

The plants for research back in comfortable laboratories are selected by studying the plant medicines used by the jungle peoples. The most advanced medical technologies ever known can develop new medicines only by learning from the most primitive societies living on earth. Such is the power of plant medicine. Unlike all other technologies, new developments in medicine do not mean all previous knowledge is obsolete, superseded or consigned to the bin if not the museum.

Herbs been used to transform, diagnose, and treat spiritual, emotional and physical ills in every tradition from the shamanic cultures of Africa, Mexico and Tibet to the highly regulated medical herbalists of today. Twenty-five hundred years ago, Hippocrates (the father of medical literature), stated as part of his oath: “I will give no deadly medicine to anyone.” Hippocrates used only food and herbs and is best known for the sayings: “Let your food be your medicine and let medicine be your food,” “Sickness is caused by the body’s inability to digest its environment.”

5. a. iii. The truth of Herbal Medicine

The Herbal Medicine is defined as the oldest form of healthcare system known to humans. The first drug towards treatment of any disease is from the Mother Nature, i.e., from the plants. Many modern drugs, commonly used today, are of herbal origin. Approximately 25% of all prescription drugs are derived from trees, shrubs or herbs. Digitalis is extracted from the leaves of foxglove; morphine and codeine are derived from poppy; quinine from cinchona bark etc. Some are made from plant extracts; others are synthesized to mimic natural plant constituents. The essential difference between herbalism and conventional medicine is that, while in conventional medicine the most active constituent is extracted from the plant and then synthesized in the laboratory, in herbal medicine extracts from the whole plant are used. Herbal medicinal products can offer an alternative to conventional medicines in non-life-threatening conditions, provided they are of adequate quality and safety and are used in an appropriate manner by suitable individuals.

5. a. iv. Indian Traditional Medicine and Tribal Medicine

India is rich in its ethnic diversity of which many aboriginal cultures have retained traditional knowledge concerning the medicinal utility of the native flora. Southeast Indians have been known to put a great emphasis on traditional knowledge systems and practices, which is supported by their vast intra-ethnic diversity. India has over 537 different aboriginal and other ethnic groups constituting approximately eight percent of the country’s population. Traditional knowledge systems including various medicinal
plant utilities appear to vary according to local population domain. Documentation of these local knowledge systems concerning medicinal plants may have high impacts from a bio-economic point of view\(^6\).

Tribal communities living in biodiversity rich areas possess a wealth of knowledge on the local utilization and conservation of food and medicinal plants. This traditional knowledge, which developed over years of observation, trial and error, inference and inheritance, has largely remained with the aboriginal people. However, these cultures and their associated botanical knowledge may be in peril and may even become extinct. Migration from one area to another in search of improved livelihoods is a key feature of human history. Many aboriginals in India migrate to access emerging opportunities and industrialization. This widens the gap between Traditional Aboriginal Knowledge (TAK) and modern knowledge associated with workplace and social skills of the developed mainstream populations. It is a fact that as traditional healers who value Traditional Aboriginal Knowledge (TAK) are becoming very old; younger generations exhibit a lack of interest in Traditional Aboriginal Knowledge (TAK) with a trend toward migration to cities for lucrative jobs. Traditional Aboriginal Knowledge (TAK) in India is declining\(^7\).

The study of ethno botanical research is deeply rooted within India. There are many examples of medicinal ethno botanical surveys conducted in India in the past that have recorded many botanical remedies among many aboriginal groups: Malasars; Malamalasars; Malayalis; Irulas; Gonds; Koysd, Konda reddis, Valmikis, Koyas, Chenchus, Lambadis, Jatapus, Savaras, Bagatas, Kammaras, Khondas, Nukadoras, Porjas, Jatapus; Paliyar; Kanikar ; Todas, Kotas; Kattunayakas; Apatani ; Chellipale . Although there are many descriptive qualitative surveys of Traditional Aboriginal Knowledge (TAK), to our knowledge, there are no ethnobotanical studies within India that consider variation in Traditional Aboriginal Knowledge (TAK) among informants using a quantitative consensus analysis\(^8\).

5. b. Natural Products
5. b.i. Natural products as drug candidates

The first written records on medicinal applications of plants date back to 2600 BC and report the existence of a sophisticated medicinal system in Mesopotamia, comprising about 1000 plant-derived medicines. Egyptian medicine dates back to about 2900 BC, but its most useful preserved record is the “Ebers Papyrus” from about 1550 BC, containing more than 700 drugs, mainly of plant origin\(^{10-11}\).
The knowledge on the medicinal application of plants in the Western world is mainly based on the Greek and Roman culture. Of particular importance are the compendia written by the Greek physician Dioscorides (1st century AD), and by the Romans Pliny the Elder (1st century AD) and Galen (2nd century AD). The Arabs preserved a large amount of the Greco-Roman knowledge during the Dark and Middle ages (i.e., 5th to 12th centuries), and complemented it with their own medicinal expertise, and with herbs from Chinese and Indian traditional medicines. The invention of letterpress by Johannes Gutenberg led to a resurrection of the Greco-Roman knowledge in the 15th and 16th century, and to the compilation of several very influential herbal books that were widely distributed in Europe, like The Mainz Herbal and The German Herbal (1485), both edited by Gutenberg’s partner Peter Schöffer, the Herbarium Vivae Icones (Otto Brunfels; 1530), the Kreütter Buch by Hieronymus Bock (1546) that was written in German, and De Historia Stirpium by Leonhart Fuchs that was published in Latin in 1542 and also in German in the following year. During all that time, medicinal plants were only applied on an empirical basis, without mechanistic knowledge on their pharmacological activities or active constituents. It was only in the 18th century that Anton von Störck, who investigated poisonous herbs such as aconite and colchicum, and William Withering, who studied foxglove for the treatment of edema, laid the basis for the rational clinical investigation of medicinal herbs.

5.b.ii. Drug Discovery from plants

Rational drug discovery from plants started at the beginning of the 19th century, when the German apothecary assistant Friedrich Sertürner succeeded in isolating the analgesic and sleep-inducing agent from opium which he named morphium (morphine) after the Greek god of dreams, Morpheus. He published a comprehensive paper on its isolation, crystallization, crystal structure, and pharmacological properties, which he studied first in stray dogs and then in self-experiments (Sertürner, 1817).

This triggered the examination of other medicinal herbs, and during the following decades of the 19th century, many bioactive natural products, primarily alkaloids (e.g., quinine, caffeine, nicotine, codeine, atropine, colchicine, cocaine, capsaicin) could be isolated from their natural sources.

Apothecaries who specialized in the purification of these compounds were the progenitors of pharmaceutical companies. The first one was H.E. Merck in Darmstadt (Germany) who started extracting morphine and other alkaloids in 1826 (Kaiser, 2008). Subsequently, efforts were undertaken to produce natural products by chemical
synthesis in order to facilitate production at higher quality and lower costs. Salicylic acid was the first natural compound produced by chemical synthesis in 1853\textsuperscript{14}.

After the discovery of penicillin (1928), an era of drug discovery from microbial sources was initiated in the 1930s, that laid the scientific and financial foundation of the modern pharmaceutical industry after World War II. At that time, the therapeutic use of extracts and partly purified natural products was increasingly replaced by the use of pure compounds. Despite the advent of combinatorial chemistry and HTS campaigns during the last decades, the impact of natural products for drug discovery is still very high. Of the 1073 new chemical entities belonging to the group of small molecules that had been approved between 1981 and 2010, only 36\% were purely synthetic, while more than the half were derived or inspired from nature. A substantial number of these compounds have been discovered in higher plants. Particularly prominent examples of plant-derived natural compounds that have become indispensable for modern pharmacotherapy can be found in the field of anti-cancer agents, e.g., paclitaxel and its derivatives from yew (Taxus) species, vincristine and vinblastine from Madagascar periwinkle (Catharanthus roseus (L.) G. Don), and camptothecin and its analogs initially discovered in the Chinese tree \textit{Camptotheca acuminata} Decne. Further notable examples include the cholinesterase inhibitor galanthamine that has been approved for the treatment of Alzheimer’s disease and was initially discovered in \textit{Galanthus nivalis} L.\textsuperscript{15}, and the important antimalarial and potential anti-cancer agent artemisinin originally derived from the traditional Chinese herb \textit{Artemisia annua} L.\textsuperscript{15}.

5. b.iii. Plants: perfect sources for successful leads

Medicinal plants have historically been a rich source for successful drugs, and still represent an important pool for the identification of new pharmacological leads today. Renewed scientific interest in plant-derived natural product-based drug discovery is evident from the analysis of PubMed publications trends (Fig. 1). Plants are producing numerous chemically highly diverse secondary metabolites which are optimized for exerting biological functions and are still far from being exhaustively investigated. Resulting from the revived scientific interest in natural product-based drug discovery, new approaches for the identification, characterization, and resupply of natural products are being developed, that may address some of the challenges related with the development of plant-based therapeutics. One major asset of medicinal plant-based drug discovery is the existence of ethnopharmacological information providing hints for compounds therapeutically effective in humans. In order to harvest its full potential, of
particular importance is the adoption of a broad interdisciplinary approach involving ethnopharmacological knowledge, botany, phytochemistry, and more relevant pharmacological testing strategies (e.g., early in vivo efficacy studies and compound identification strategies including metabolism and synergistic action of the plant constituents). Resupply from the original plant species is very often unfeasible to meet market demands upon commercialization of a natural product, and alternative resupply approaches are being developed that rely on biotechnological production or chemical synthesis. Total chemical synthesis is an effective resupply strategy in case of natural products or natural product derivatives with simple structures such as acetylsalicylic acid and ephedrine. For complex structures with multiple chiral centers, however, total synthesis is, at present, both difficult and economically unfeasible in most cases, requiring significant technological advances to be successfully applied. For the resupply of complex natural products usually harvesting from plant sources and semi-synthesis from naturally occurring precursors still remain the most economically-viable approaches. While natural product-based drug discovery and development represents a complex endeavor demanding a highly integrated interdisciplinary approach, the presented recent scientific developments, technologic advances, and research trends clearly indicate that natural products will be among the most important sources of new drugs also in the future.\(^{16}\)

![Figure 1. PubMed publication trend analysis, demonstrating increased scientific interest in plant-derived natural product pharmacology, chemistry, and drug discovery.](image-url)
The data were retrieved with MEDSUM (http://webtools.mf.uni-lj.si/public/medsum.html) on 15th of June 2015, and cover the time period 1982–2012 (newer data are not included because of the lack of coverage). As indicated, the used search keywords were plant chemistry, plant pharmacology, plant natural product, plant compound, plant drug discovery, plant bioactivity, and the total number of PubMed publications per year was retrieved by search with the symbol *. The trend analysis reveals that the increase of PubMed citations in the target areas is faster than the increase in the total number of annual PubMed citations (indicated by the steeper slopes of the respective trend lines).

5. c. Weeds

5. c.i. Definition of weeds

A weed may be defined as any plant or vegetation that interferes with the objectives of farming or forestry, such as growing crops, grazing animals or cultivating forest plantations. A weed may also be defined as any plant growing where it is not wanted. For example, a plant may be valuable or useful in a garden or on a farm or plantation – but if the same plant is growing where it reduces the value of agricultural produce or spoils. an aesthetic or environmental value, then it is considered a weed.

5. c.ii. Benefits of weeds

Despite the negative impacts of weeds, some plants usually thought of as weeds may actually provide some benefits, such as: Stabilizing and adding organic matter to soils, providing habitat and food for wildlife, providing nectar for bees, offering aesthetic qualities, serving as a genetic reservoir for improved crops, providing products for human consumption and medicinal use, creating employment opportunities. Even though weeds may be considered as unwanted for a number of reasons, the most important one is that they interfere with food and fiber production in agriculture, but there are many weeds having ethnomedicinal and pharmacological value, like the phrases in the poem wrote by Gerard Manley Hopkins’ "What would the world be, once bereft, of wet and wilderness? Let them be left. O let them be left; wilderness and wet; Long live the weeds and the wilderness yet." A number of weeds, such as the dandelion (Taraxacum officinale F.H.Wigg.) are edible, and their leaves and roots may be used for food or herbal medicine. Greater Burdock (Arctium lappa L.) common weed over much of the world, and is sometimes used to make soup and other medicine in East Asia. These so-called "beneficial weeds" may have other beneficial effects, such as drawing away the attacks of crop-destroying insects, but often are breeding grounds for insects and pathogens that attack other plants. Dandelions are one of several species which
break up hardpan in overly cultivated fields, helping crops grow deeper root systems. Some modern species of domesticated flower actually originated as weeds in cultivated fields and have been bred by people into garden plants for their flowers or foliage.

An example of a crop weed that is grown in gardens is the corncockle (*Agrostemma githago* L.) which was a common field weed exported from Europe along with wheat, but now sometimes grown as a garden plant. White clover (*Trifolium repens* L.) is considered by some to be a weed in lawns, but in many other situations is a desirable source of fodder, honey and soil nitrogen. "Many gardeners will agree that hand-weeding is not the terrible drudgery that it is often made out to be. Some people find in it a kind of soothing monotony.

It leaves their minds free to develop the plot for their next novel or to perfect the brilliant repartee with which they should have encountered a relative's latest example of unreasonableness. Weeds have been found to represent a very important component of indigenous pharmacopoeias. The consumption of weedy greens has often been perceived to have a medicinal character. In ancient Indian literatures all plants were not considered as weeds and it is clearly mentioned that every plant on this earth is useful for human beings, animals and other plants.

It is ignorance of human beings as they consider some plants are useful and others as unwanted. Studies conducted by department of Agronomy. (IGAU), Raipur has revealed that weeds are a boon for tile farmers and industries. Uses of weeds of many important agricultural crops have been reported.

5.c.iii. The Nilgiris

The Nilgiri hills located in Western Ghats, Tamilnadu State, India have a history going back for many centuries. It is not known why they were called the Blue Mountains (Table 1). Several sources cite the reason as the smoky haze enveloping the area, while other sources say it is because of the kurunji flower, which blooms every twelve years giving the slopes a bluish tinge. It was originally tribal land and was occupied by the todas around what is now the Ooty area, and by the Kotas around what is now the Kotagiri (Kothar Keri) area. The Badagas are one of the major non tribal populations in the district who reside in the mountain. Although the Nilgiri hills are mentioned in the Ramayana of Valmiki (estimated by Western scholars to have been recorded in the second century BC), they remained all but undiscovered by Europeans until 1602.

The district has an area of 2,452.50 km². The district is basically a hilly region, situated at an elevation of 2000 to 2,600 MSL. Almost the entire district lies in the
Western ghats. Its latitudinal and longitudinal dimensions being 130 km (Latitude: 10 - 38 WP 11-49N) by 185 km (Longitude: 76° E to 77.15° E). The Nilgiris district is bounded by Mysore district of Karnataka and Wayanad district of Kerala in the North, Malappuram and Palakkad districts of Kerala in the West, Coimbatore district of Tamil Nadu in the South and Erode district of Tamil Nadu and Chamarajanagar district of Karnataka in the East. In Nilgiris district the topography is rolling and steep. About 60% of the cultivable land falls under the slopes ranging from 16 to 35%. The altitude of the Nilgiris results in a much cooler and wetter climate than the surrounding plains, so the area is popular as a retreat from the summer heat. The temperature remains to the maximum of 25°C and reaches a minimum of 0°C.

5.c.iv. Tribal communities in Nilgiris

The Nilgiris is gifted with richest flora in which lot of medicinally important plants are present. But many of these plants are considered as weeds or useless plants. But many of these weeds will grow wildly and in cultivated fields. Many of these weeds having ethno medicinal and pharmacodynamic importance but due to lack of proper guidance and scientific documentation, many of these weeds are under destruction due to their short term useless selfish benefits of mankind, but some tribal people like Todas, Kotas, Kurumbas, Paniyas and Kattunayakas are safeguarding this type of plants and using as tribal medicine to cure lot of diseases.

5.c.v. Tribes and weeds of Nilgiris

Todas:  
*Centella asiatica* (L) Urban (Apiaceae), locally known as “Vallarai”. Plant juice is considered as refrigerant to the body, when given orally.

Kotas:  
i. *Achyranthes aspera* L. (Amaranthaceae), locally known as “Uthrunk”. Leaf paste is applied on cuts, wounds and sores for quick healing.

ii. *Lantana camara* L. (Verbenaceae), locally known as “Thusik”. Leaf juice is applied to the gum to stop bleeding and to reduce tooth-ache.

iii. *Rubia cordifolia* L. (Rubiaceae), locally known as “Sappli Koth”. Decoction of stem is orally administered as a restorative tonic. Root juice is given orally to cure jaundice.

Kurumbas:  
i. *A. aspera* L. (Amaranthaceae), locally known as “Nayurvi Geeda”. Decoction of whole plant with root is orally given for ease child birth and to mitigate labour pain.

ii. *Ageratum conyzoides* L. (Asteraceae), locally known as “Nasar soppu”. Leaf juice is
orally given as a cure for cough and cold.

Paniyas:

*Oxalis corniculata* L. (Oxalidaceae), locally known as “Pulichen segae. The whole plant extract in water is orally given for piles and also used as a febrifuge.

Kattunayakas:

i. *A. aspera* L. (Amaranthaceae), locally known as “Cherukadalai”. The whole plant with water is made into paste and applied on body to relieve sprain ached in the Joints.

ii. *Centella asiatica* (L) Urban (Apiaceae), locally known as “Gottala”. Plant extract is orally given to allay toothache.

As a trail, in this research, we are tried to expose the important weeds and their pharmacodynamic importance and to educate the society to prevent the destruction of these important weeds and can be made them as economically important plants.

3. c.v. Economical importance of weeds

Generally, weeds are considered as nuisances in the garden and enemies to the farmer, as there is a misconception that they are useless. Many of the herbs used in Indian traditional medicine and tribal medicine are considered weeds by agriculturists and field botanists (for example, *Phyllanthus amarus* L., *Eclipta alba* L., *Centella asiatica* (L.) etc.).

Even though many of these weeds have high ethnopharmacological importance, they are being destroyed and there is a lack of scientific knowledge and guidance. In the Nilgiris many medicinally valuable weeds like *Achyranthes bidentata* Blume., *Artemisia nilagirica* Clarke., *Centella asiatica* L., are very prominent having good therapeutic values like diuretic, antimalarial and brain tonic.

It is a misconception in people minds to consider all weeds as useless or hurdles to public, as some of these weeds having good ethno medicinal values globally and is good sources for new drug discovery and grows naturally in bulk, no need of specialized good agricultural practices, easily available in all the seasons. It is our duty to safe guards these beautiful nature gifts.

Globally some of these weeds are used as ethnomedicinal aids in treatment of fevers, pains, inflammations, microbial infections, worm infestations, cancer, wounds etc. But very less scientific validation is available on this area so there is a great scope for the phytoscientists to work on this area in order to explore the phytochemical or pharmacological importance of weeds. It is the duty of phytoscientists establish the scientific validation for these medicinally important weeds, so that the misconception of
weeds as useless or public hurdle will convert to weeds as a pharmacologically and economically valuables\textsuperscript{22}.

5. c.vi. Weeds as major sources of pharmaceuticals

Natural products can be important sources for new pharmaceuticals. With regards to plants, primary tropical forest is often considered to be the most promising habitat for this search due to high biodiversity and endemism. Many researchers have combined this assumption with an ethnobotanical approach to drug discovery in order to maximize the chance of a successful drug discovery. However, this focus on tropical forests overlooks the fact that disturbed environments are preferred habitats for medicinal plant procurement by many traditional peoples\textsuperscript{23}.

Meanwhile, the role of weeds in the present pharmacopeia has been overlooked, despite significant evidence that weeds in particular, are an important source of medicines for indigenous peoples and have a highly significant over representation in indigenous pharmacopoeias in relation to other types of plants. The significant representation of weeds in the flora from which drugs are currently derived is reported here. While primary tropical forests likely contain many undiscovered novel compounds that could have medicinal applications, disturbed habitats may also hold promising leads for drug discovery. There are also policy implications in these findings related to conservation of medicinal plants utilized in traditional societies. The undervaluing and even destruction of weeds could have an impact on availability of certain medicinal plants. Also, the realization that medicinal plants are readily available in a “living pharmacy” right outside the door and along trail sides rather than deep in the forest could lead governments and NGO’s to encourage and promote traditional medical practices rather than discourage them\textsuperscript{24}.

As sources of new anti-cancer and anti-infective agents, natural products play an even larger role. From 1989 to 1995, over 60% of approved drugs and pre-NDA candidates in these disease areas were from natural products. While natural products drug discovery efforts increasingly focus more on micro-organisms and fungi, research continues with vascular plants and much of this work is in areas of high biodiversity and endemism such as the humid tropics\textsuperscript{25}.

5.c.vii. Weeds in the modern pharmacopeia

An analysis was undertaken to determine the role that weedy plant species currently play as source plants for modern pharmaceuticals. A species was considered to be a
weed if it was included in the standard reference for weeds worldwide, containing over 8000 species based on a global literature search. Because it is a global compendium based on a comprehensive survey of the literature, do not offer a singular definition of weeds. However, they do classify each species as to whether it is a serious, principal, or common weed; whether its rank is unknown; and whether a species is present in the flora but unconfirmed that it behaves as a weed. For the purpose of this analysis, only weeds that were considered to be serious, principal, or common are included. A good general definition of a weed that corresponds to those species included in this analysis is “a plant. If, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man, without, of course, being a deliberately cultivated plant.” Weeds are those plants that are successful in disturbed environments, short-lived, fast-growing and oftentimes, herbaceous.

Based on a World Health Organization survey and more recent review articles based on literature searches for drugs of natural plant product origin, there are 121 pharmaceutical compounds used as medicine worldwide that are derived from plants. Of these, 101 plant species are the primary sources for 119 of these compounds. The remaining two compounds (borneol and pinitol) are regularly obtained from dozens of different species and were not included in this analysis. Using a conservative estimate of 250,000 flowering plants and an estimate that 8000 of these plants are weeds, one would expect that 3% of these 101 species would be weeds.26 There are some notable exceptions. For example, Taxus brevifolia Nutt. is a long-lived understory tree of the Pacific Northwest. Even so, it shares some traits of a weed, in that it is aggressive and quickly colonizes a disturbed area. Prior to the development of paclitaxel (TaxolTM) it was considered to be a nuisance species by foresters because of its invasiveness. Other woody species on the list are Azadirachta indica Juss., Erythroxylum coca Lam., Larrea divaricata Cav., and Salix alba L. Despite their habit as trees or shrubs, these species are considered invasive enough in certain regions to warrant their inclusion in the list of world weeds. Also, a few of the species on the list are cultivated (Cannabis sativa L., Gossypium sp., Nicotiana tabacum L., and Thymus vulgaris L.)26.

5.c.viii. Weeds as sources of various lead molecules

There is increasing evidence to support the hypothesis that weeds are relatively high in bioactive secondary compounds and are thus likely to hold promise for drug discovery. Secondary compounds in weeds are important for a variety of ecological functions. Chief
among these are allelopathy, where secondary compounds inhibit germination and growth of other plants; and, as chemical defense against herbivory. At least 50 species of weeds have been shown to interfere with crops through allopathic secondary compounds. However, because allelopathy usually occurs through the complex chemical matrix of the soil it is difficult to conclusively show a causal relationship. The various leads isolated from various weeds were discussed in the Table 1.

<table>
<thead>
<tr>
<th>Weed species</th>
<th>Isolated Pharmaceutical Leads</th>
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<tbody>
<tr>
<td><em>Adonis vernalis</em> L.</td>
<td>Adoniside</td>
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<tr>
<td><em>Agrimonia eupatoria</em> L.</td>
<td>Agrimophol</td>
</tr>
<tr>
<td><em>Ammi visnaga</em> (L.) Lamk.</td>
<td>Khellin</td>
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<tr>
<td><em>Anabasis aphylla</em> L.</td>
<td>Anabasine</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em> Nees</td>
<td>Andrographolide</td>
</tr>
<tr>
<td><em>Artemisia annua</em> L.</td>
<td>Artemisinin</td>
</tr>
<tr>
<td><em>Atropa belladonna</em> L.</td>
<td>Atropine</td>
</tr>
<tr>
<td><em>Berberis vulgaris</em> L.</td>
<td>Berberine</td>
</tr>
<tr>
<td><em>Brassica nigra</em> (L.)</td>
<td>Allyl isothiocynate</td>
</tr>
<tr>
<td><em>Centella asiatica</em> (L.)</td>
<td>Asiaticoside</td>
</tr>
<tr>
<td><em>Cissampelos pareira</em> L</td>
<td>Cissampeline</td>
</tr>
<tr>
<td><em>Colchicum autumnale</em> L</td>
<td>Colchicine</td>
</tr>
<tr>
<td><em>Crotalaria spectabilis</em> Roth</td>
<td>Monocrotaline</td>
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<tr>
<td><em>Convallaria majalis</em> L</td>
<td>Convallatoxin</td>
</tr>
<tr>
<td><em>Cytisus scoparius</em> (L.)</td>
<td>Sparteine</td>
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<tr>
<td><em>Lobelia inflata</em> L.</td>
<td>Lobeline</td>
</tr>
<tr>
<td><em>Silybum marianum</em> (L.)</td>
<td>Silymarin</td>
</tr>
<tr>
<td><em>Rorippa indica</em> (L.)</td>
<td>Rorifone</td>
</tr>
<tr>
<td><em>Sophora pachycarpa</em> Schrenk ex</td>
<td>Pachycarpine</td>
</tr>
<tr>
<td><em>Urginea maritana</em> (L.) Baker</td>
<td>Scillaren A</td>
</tr>
</tbody>
</table>

Table 1. Various Pharmaceutical leads from different weeds

5. d. Cancer

5. d.1. Definition of cancer

Cancer is defined as a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by external factors, such as tobacco, infectious organisms, and an unhealthy diet,
and internal factors, such as inherited genetic mutations, hormones, and immune conditions. These factors may act together or in sequence to cause cancer. Ten or more years often pass between exposure to external factors and detectable cancer. Treatments include surgery, radiation, chemotherapy, hormone therapy, immune therapy, and targeted therapy (drugs that specifically interfere with cancer cell growth).  

5.d.2. Global scenario of cancer

Cancer is a major public health problem in the United States and many other parts of the world. It is currently the second leading cause of death in the United States, and is expected to surpass heart disease as the leading cause of death in the next few years. In this article, we provide the expected numbers of new cancer cases and deaths in 2015 in the United States. Nationally and for each state, as well as a comprehensive overview of cancer incidence, mortality, and survival rates and trends using the most current population-based data. In addition, we estimate the total number of deaths averted nationally during the past 2 decades and by state in 2011 as a result of the continual decline in cancer death rates.

Each year the American Cancer Society estimates the numbers of new cancer cases and deaths that will occur in the United States in the current year and compiles the most recent data on cancer incidence, mortality, and survival. Incidence data were collected by the National Cancer Institute (Surveillance, Epidemiology, and End Results [SEER] Program), the Centers for Disease Control and Prevention (National Program of Cancer Registries), and the North American Association of Central Cancer Registries. Mortality data were collected by the National Center for Health Statistics. A total of 1,658,370 new cancer cases and 589,430 cancer deaths are projected to occur in the United States in 2015. During the most recent 5 years for which there are data (2007-2011), delay-adjusted cancer incidence rates (13 oldest SEER registries) declined by 1.8% per year in men and were stable in women, while cancer death rates nationwide decreased by 1.8% per year in men and by 1.4% per year in women.

The overall cancer death rate decreased from 215.1 (per 100,000 populations) in 1991 to 168.7 in 2011, a total relative decline of 22%. However, the magnitude of the decline varied by state, and was generally lowest in the South (15%) and highest in the Northeast (20%). For example, there were declines of 25% to 30% in Maryland, New Jersey, Massachusetts, New York, and Delaware, which collectively averted 29,000 cancer deaths in 2011 as a result of this progress. Further gains can be accelerated by applying existing cancer control knowledge across all segments of the population.
different types of leading cancers in 2015 were represented in the form of figure 2 given below.

**Figure 2. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2015**

5. d.3. Need for new natural anticancer leads

Increasing recurrence of mammalian tumors and severe side-effects of chemotherapeutic agents reduce the clinical efficacy of a large variety of anticancer agents that are currently being used. Thus, there is always a constant need to develop alternative or synergistic anticancer drugs with minimal side-effects. One important strategy to develop effective anticancer agents is to study into anticancer agents derived from natural sources. Anticancer agents derived from plants and their derivatives have been proven to be effective for cancer prevention and therapeutics. Vinca alkaloid and their derivatives, alone and in combination with therapeutic agents, have been used for a long time for the treatment of various types of cancers. Polyphenols form one of the most important and extensively used classes of plant-derived therapeutics for cancer prevention or chemotherapy. The present review highlights a plethora of studies focused on the antineoplastic properties of plant-derived chemicals, such as alkaloids, saponins, and flavonoids.

Despite technological and social development, cancer has become one of the most common diseases of concern and a leading cause of human suffering and death. One in 4 deaths in the United States is due to cancer. A total of 1,638,910 new cancer cases and 577,190 deaths from cancer are reported in the United States in 2012. The alarming rise in incidence of new types of cancer and the public burden represents a real...
cancer. Despite their severe toxicity, chemotherapy, irradiation and immunotherapy are the gold standard approaches for the treatment of cancer worldwide\textsuperscript{31-33}.

Other than these classical ways, use of natural products from plants and animals and their derivatives have produced remarkable leads for the control of cancer. Due to the toxicity of currently used therapeutics for the treatment of various types of tumors, several natural products are being tried as an alternative. Being less toxic, many therapeutic compounds from animal and plant sources have been extensively studied. The newly discovered plant-derived chemicals exhibiting anticancer properties were described below\textsuperscript{34}.

5. d.4. Important secondary metabolites for discovery of new anticancer leads
5. d.4.1. Polyphenols

Fruit, vegetables and some drinks, such as tea and coffee, are particularly rich in polyphenols, and approximately 8,000 different naturally occurring polyphenols have been identified. It is widely accepted that dietary polyphenols are beneficial for cancer prevention. Notable examples of polyphenols with anticancer effects include green tea catechins, curcumin, resveratrol and genistein.

Possible mechanisms of anticancer effects of dietary polyphenols may be via removal of carcinogenic agents, modulation of cancer cell signalling and antioxidant enzymatic activities, and induction of apoptosis and cell cycle arrest\textsuperscript{35-36}.

A population-based cohort study of 74,942 Chinese women suggested that regular intake of green tea may delay the onset of breast cancer. Moreover, out of women under 50 years of age, those who consumed tea were 37\% less likely to develop breast cancer compared to women who did not. Wu \textit{et al.} reported the correlation between catechol-o-methyl transferase (\textit{COMT}) allele, intake of tea and occurrences of cancer. They observed that women who possess at least one low-activity \textit{COMT} allele had a reduction in breast cancer risk with intake of tea, but in the case of these homozygous for the high-activity \textit{COMT} allele, there was no effect of drinking tea on breast cancer onset.
An interesting study showed that green tea reduced malignancy in prostate cancer among green tea-treated individuals as compared to untreated ones. No significant side-effects or adverse effects were documented\textsuperscript{37}.

5. d.4.2. Saponins

Saponins, a class of bioactive compounds naturally present in many plants, are a major family of secondary metabolites containing a sugar moiety glycosidically linked to a hydrophobic aglycone (sapogenin). Saponins have emerged as natural detergents and foaming agents, with cardiac, immunostimulatory, and anticancer activity, and other health promoting functions.

Saponins allow plants to cope with environmental stress such as storing and conserving water, resisting predators, and surviving severe weather conditions. Saponins have detergent and surfactant properties because they contain both water-soluble (the sugar moiety) and fat-soluble (sapogenin) subunits. Plant sources of saponins include yucca, Christmas rose (\textit{Helleborus niger}), horse chestnuts (\textit{Aesculus hippocastanum}), asparagus fern (\textit{Asparagus officinalis}), daisies (\textit{Bellis perennis}), chickpeas, soybeans and alfalfa\textsuperscript{38-39}.

The polarity, hydrophobicity, and nature of the reactive groups of saponins are important determinants of their biological properties. The most potent compounds in soybean were shown to be the aglycones soya sapogenol A and B, inducing almost complete suppression of cell growth. Saponins from soybean suppressed the growth of HT-29 colon cancer cells. Soybean extracts also exhibited synergistic antiproliferative activity against an ovarian tumor cell line (OVCA 433). Several glycosides (naringin, rutin, and baicalin) of soybean origin exhibited anticancer activity. In humans, rutin attaches to iron ion (Fe\textsuperscript{2+}), preventing it from binding to hydrogen peroxide, which would otherwise create highly reactive free radicals that can damage cells. Baicalin had a cytotoxic effect on leukemia-derived T-cells. The aglycone sapogenol exhibited antiproliferative activity against MCF-7 breast cancer cells. Saponins isolated from \textit{Balanites aegyptica} exhibited cytostatic activity against P-388 lymphocytic leukemia cultured cells\textsuperscript{40}.

A mixture of the steroidal saponins balanitin-6 and balanitin-7 (Bal 6/7), isolated from \textit{B.aegyptiaca} kernels, demonstrated appreciable anticancer effects against A549 non-small cell lung cancer and U373 glioblastoma cell lines. Saponins from \textit{Agave schottii}, a Sonora Desert xerophytes plant of the Agavaceae family, were effective inhibitors of a Walker carcinoma 256 tumor system. Moreover, steroid saponins derived from Yucca
(Yucca schidigera), a xerophytes also belonging to the Agavaceae family, display carcinostatic and mutagenesis inhibitory effects, and are thus capable of inhibiting tumors. Another desert plant Quillaja (Quillaja saponaria), a Quillajaceae family drought-resistant evergreen tree native to warm-temperate central Chile, is used in folk medicine by the Andean people. The compounds SAP-1016 (3β-O-β-D-xylpyranosyl-(1-3)-β-D-glucopyranosyl-(1-4)-[α-L-rhamnopyranosyl-(1-2)]-β-D-glucopyranoside) exhibited potent antiproliferative activity against MCF-7 human breast cancer cells and HT-29 human colon cancer cells, as compared to a well-known anticancer agent, cisplatin. A recently patented anticancer preparation contains extracts from Schisandra, Trichosanthes, yucca plants and glycine and claims to induce apoptosis or cell-cycle stasis and inhibit angiogenesis or tumor cell metastasis, and to be useful for the treatment of cancer and cell proliferation disorders.

5. d.4.3. Alkaloids

The antitumor properties of Vinca alkaloids derive from their interaction with tubulin, the major component of microtubules in mitotic spindles. These drugs interfere with the dynamics and assembly of microtubules resulting in cell division arrest in metaphase. Vinorelbine and vinflunine, the second generation of Vinca alkaloids, suppress the rate and extent of microtubule growth and enlargement, affecting mitotic spindle functions, leading to modifications of cell-cycle progression and cell killing. Vinflunine is a specific inhibitor of tubulin that prevents microtubule assembly during mitosis and induces apoptosis.

Patients with metastatic breast cancer (MBC) whose anthracyclines and taxanes therapy failed, experienced promising antitumour activity when treated with the combination of vinflunine and capecitabine and this combination was found to be safe with minimal side-effects. Further clinical development of this combination is warranted.

5. d.4.4. Miscellaneous Secondary metabolites

Some rare compounds have also been exploited for their anticancer property. Nordihydroguaiaretic acid, a naturally occurring lignin from creosote bush (Larrea divaricata Cav.or Corillea tridentate), and its synthetic analogues are potentially useful in treating cancer. Remarkably, Terameprocol, a tetra-O-methyl derivative of nordihydroguaiaretic acid, is in phase I/II clinical trials as an anticancer agent. Thymol, piperitone, and methyleugenol, essential oils from the root of Anemopsis californica inhibited the growth of human endometrial cancer cell-line AN3CA and of the cervical...
cancer cell line HeLa. Iridoids, bioactive compounds in the roots and rhizomes of plants belonging to the genus Valeriana (Valerianaceae), are known to be inhibitors of cell migration\textsuperscript{46}.

\textit{Ammopiptanthus mongolicus} and its lipid, traditionally used in China have been shown to inhibit liver cancer. Ethyl acetate fractionated extracts of \textit{Calligonum comosum} (Polygonaceae) demonstrated anticancer properties\textsuperscript{47-48}.

Terpinen-4-ol, sabinene, α-terpinene, and β-myrcene isolated from \textit{P. tortuosus}, exhibited significant cytotoxicity towards against human cancer cell lines, namely, human hepatocellular liver carcinoma cell line HepG2, colon cancer cell line HCT116 and breast cancer cell line MCF7\textsuperscript{49}.

Haterumaimide J and K obtained from \textit{Lissoclinum} sp. exhibited cytotoxicity against murine leukemia P388 cells. Dichlorolissoclimide, chlorolissoclimide from \textit{Lissoclinum} sp. showed an antiproliferative effect due to blockage of G1 phase cells against the non-small cell bronchopulmonary carcinoma line NSCLC-N6. Cyclopentenones from \textit{Lissoclinum} sp. also showed significant cytotoxicity towards human colon carcinoma HCT116, epidermal cancer line A431 and the human alveolar basal epithelial adenocarcinoma line A549. Lissoclibadin and lissoclinotoxin, obtained from \textit{Lissoclinum cf. badium} showed a wide range of inhibitory effects against the human colon cancer lines DLD-1 and HCT116, the breast cancer line MDA-MB-231, the renal cancer line ACHN and the NSCLC line NCI-H460\textsuperscript{50-51}.

5. d.4.5. Natural molecules as leads: In demand for cancer drug discovery

Pharmacological activities associated with natural products have been recognized since the beginning of mankind; however only limited numbers of medicinal plants and other natural products have been scientifically evaluated so far. Many plant products and their chemical derivatives have been used in therapeutics of serious diseases such as cancer. Although a growing body of plant-derived products have been reported to prevent tumor growth the exact underlying molecular mechanisms of most of these agents remains to be elucidated, particularly with respect to kinetic approach towards active site of the target enzymes. Understanding the cross talk between these plant products and proteins in various signalling pathways would be a step forward in development of anticancer drugs. Different biochemical and biophysical approaches such as cocrystallization and three-dimensional structure determinations could be adopted for further dissecting their molecular mechanisms. In addition, \textit{in silico} approaches like molecular docking could also be of significant importance in
understanding the interaction of these products in different signalling pathways which can be further validated by various *in vitro* and *in vivo* studies.

There is a growing demand for testing these products in clinical trials, which is possible only after gaining the insight into the molecular interaction of these plant-derived chemicals with different signalling molecules\(^5\)\(^2\).

5.d.4.6. Some promising anticancer leads from plants

Plants since the time immemorial have been regarded as a source of medicines and numerous types of bioactive substances have been isolated and characterized as therapeutic agents. Most of these plants were considered as weeds by many of the botanists. A number of such molecules are under clinical studies.

A synthetic flavone favopiridol, originally derived from the plant alkaloid rohitukine and isolated from *Dysoxylum binectariferum* is currently under phase I and phase II clinical trials. This flavone has shown its broad activity against tumors, leukemia, lymphomas and solid tumors\(^5\)\(^3\).

Olomucine is a natural product isolated from *Raphanus sativus* (Brassicaceae), has converted into a synthetic agent roscovitine. Roscovitine is in Phase II and Phase III clinical trials in Europe\(^5\)\(^4\). Isolation studies on the bark of *Combretum caffrum* (Combretaceae) resulted in the characterization of combretastatins. Combretastatin A-4 has been found effective against lung and colon cancers, leukemia and it is presumed this is the most cytotoxic phytomolecule isolated so far\(^5\)\(^5\)-\(^5\)\(^6\).

Betulinic acid is a common pentacyclic triterpenoid found in the species of genus *Betula* has proved a significant growth inhibitory agent\(^5\)\(^7\). Betulinic acid was also isolated from *Zizyphus* species, like *Zizyphus mauritiana*, *Zizyphus rugosa* and *Zizyphus oenoplia* and exhibited selective cytotoxic activity against human melanoma cell lines\(^5\)\(^7\).

The efforts of National Cancer Institute to develop systemic and topical formulations for potential clinical trials are ongoing. Phytochemical studies on roots of *Erythroxylum privillei* (Erythroxylaceae) has resulted in the isolation of Pervilleine-A. In a study conducted on multidrug resistant oral epidermoid cancer cell lines, Pervilleine-A was found cytotoxic when administered with anticancer agent vinblastine and this compound is under preclinical development\(^5\)\(^8\)-\(^5\)\(^9\).

Silvesterol was isolated from the fruits of Meliaceae family plant *Aglaila sylvestre* exhibited cytotoxicity against breast and lung cancer cells\(^6\)\(^0\). Isolation studies on the seeds of *Centaurea schischkinii* and *Centaurea Montana* has been resulted in the
isolation of two novel alkaloids schischkinnin and montamine. Schischkinnin and montamine both exhibited anticancer activity against human cancer cells.

The molecular skeleton of these novel alkaloids can be exploited for synthesizing compound to enhance cytotoxic activity. The following Table 2 represents the plants or weeds, where the recent anticancer leads were isolated.

<table>
<thead>
<tr>
<th>Botanical name of plant with family name</th>
<th>Part used</th>
<th>Parts used and their main active components</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agave americana</em> (Agavaceae)</td>
<td>Leaf</td>
<td>Steroidal saponin, alkaloid, coumarin, isoflavonoid, heckogenin and vitamins (A, B, C)</td>
</tr>
<tr>
<td><em>Agropyron repens</em> (Poaceae)</td>
<td>Rhizomes</td>
<td>Rhizome contains essential oil, polysaccharide and mucilage</td>
</tr>
<tr>
<td><em>Agrimonia pilosa</em> (Rosaceae)</td>
<td>Herb</td>
<td>Agrimonalide, flavonoid, triterpene, tannin and coumarin</td>
</tr>
<tr>
<td><em>Ailanthus altissima</em> (Simaroubaceae)</td>
<td>Bark</td>
<td>Triterpene, tannin, saponin and quercetin-3-glucoside</td>
</tr>
<tr>
<td><em>Akebia quinata</em> (Lardizabalaceae)</td>
<td>Fruit</td>
<td>Flavonoid and saponin</td>
</tr>
<tr>
<td><em>Alpinia galanga</em> (Zingiberaceae)</td>
<td>Rhizomes</td>
<td>Kaempferide and flavones</td>
</tr>
<tr>
<td><em>Aristolochia contorta</em> (Aristolochiaceae)</td>
<td>Root and fruit</td>
<td>Lysicamine and oxaaporphine</td>
</tr>
<tr>
<td><em>Aster tataricus</em> (Asteraceae)</td>
<td>Whole plant</td>
<td>Triterpene, monoterpene and epifriedelanol</td>
</tr>
<tr>
<td><em>Bryonia dioica</em> (cucurbitaceae)</td>
<td>Root</td>
<td>Cucurbitacin and glycoside</td>
</tr>
<tr>
<td><em>Cannabis sativa</em> (Cannabinaceae)</td>
<td>Leaf</td>
<td>Stereo isomers of cannabitriol</td>
</tr>
<tr>
<td><em>Chelidonium var. asiaticum</em> (Papaveraceae)</td>
<td>Herb</td>
<td>Alkaloids (sanguinarine, chelerythrine, berberine)</td>
</tr>
<tr>
<td><em>Chimaphila umbellata</em> (Ericaceae)</td>
<td>Whole plant</td>
<td>Ericolin, arbutin, urson and tannin</td>
</tr>
<tr>
<td><em>Coix lachryma jobi</em> (Poaceae)</td>
<td>Seed</td>
<td>Trans-ferulyl stigmasterol</td>
</tr>
<tr>
<td><em>Dryopteris crassirhizoma</em> (Polypodiaceae)</td>
<td>Rhizomes</td>
<td>Filicin and filicic acids, aspidinol and aspidin</td>
</tr>
</tbody>
</table>

**INTRODUCTION**
<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Part Used</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Echinops setifer</em> (Asteraceae)</td>
<td>Whole plant</td>
<td>Echinopsine</td>
</tr>
<tr>
<td><em>Erythronium americanum</em></td>
<td>Whole plant</td>
<td>Alpha-methylenebutyrolactone</td>
</tr>
<tr>
<td>(Liliaceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Euonymus alatus</em> (Celastraceae)</td>
<td>Whole plant</td>
<td>Triterpene, euolatin, steroid and sesquiterpene alkaloid</td>
</tr>
<tr>
<td><em>Eupatorium cannabinum</em></td>
<td>Whole plant</td>
<td>Sesquiterpene, lactone, pyrrolizidine alkaloidand flavonoid</td>
</tr>
<tr>
<td>(Asteraceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fragaria vesca</em> (Rosaceae)</td>
<td>Leaf and</td>
<td>Flavonoid, tannin, borneol and ellagic acid Asia, Europe</td>
</tr>
<tr>
<td></td>
<td>fruit</td>
<td></td>
</tr>
<tr>
<td><em>Fritillaria thunbergii</em></td>
<td>Whole plant</td>
<td>Alkaloid and peimine</td>
</tr>
<tr>
<td>(Liliaceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Galium aparine</em> (Rubiaceae)</td>
<td>Cleaver</td>
<td>Iridoid, polyphenolic acid, tannin, anthraquinoneand flavonoid</td>
</tr>
<tr>
<td><em>Hydrastis canadensis</em></td>
<td>Whole plant</td>
<td>Isoquinoline alkaloids (hydrastine, berberine, berberastine, candaline), resin and lactone</td>
</tr>
<tr>
<td>(Ranunculaceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Juncus effuses</em> (Juncaceae)</td>
<td>Whole plant</td>
<td>Tridecanone, effusol, juncanol, phenylpropanoid and α-tocopherol</td>
</tr>
<tr>
<td><em>Lantana camara</em> (Verbenaceae)</td>
<td>Whole plant</td>
<td>Alkaloids (camerine, isocamerine, micranine,lantanine,lantadene)</td>
</tr>
<tr>
<td><em>Larrea tridentate</em> (Zygophyllaceae)</td>
<td>Whole plant</td>
<td>Resins</td>
</tr>
<tr>
<td><em>Lonicera japonica</em> (Caprifoliaceae)</td>
<td>Whole plant</td>
<td>Tannins, saponins and carotenoids</td>
</tr>
<tr>
<td><em>Olea europaea</em> (Oleaceae)</td>
<td>Leaf and</td>
<td>Oleic acid and polyphenol</td>
</tr>
<tr>
<td></td>
<td>oil</td>
<td></td>
</tr>
<tr>
<td><em>Panax quinquefolium</em> (Araliaceae)</td>
<td>Roots</td>
<td>Ginsenoside, sesquiterpene, limonene vitamins (B1, B2, B12)</td>
</tr>
<tr>
<td><em>Phaleria macrocarpa</em></td>
<td>Fruits</td>
<td>Gallic acid</td>
</tr>
<tr>
<td><em>Polygonatum multiflorum</em></td>
<td>Whole plant</td>
<td>Saponin, flavonoid and vitamin A</td>
</tr>
<tr>
<td>(Liliaceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Potentilla chinensis</em> (Rolsaaceae)</td>
<td>Whole plant</td>
<td>Gallic acid and tannin</td>
</tr>
<tr>
<td><em>Pygeum africanum</em> (Boraginaceae)</td>
<td>Bark</td>
<td>Phytosterol, triterpene and tannin</td>
</tr>
<tr>
<td><em>Rhus chinensis</em> (Anacardiaceae)</td>
<td>Leaf</td>
<td>Apigenin and glycoside</td>
</tr>
</tbody>
</table>
INTRODUCTION

| **Scrophularia nodosa** *(Scrophulariaceae)* | Aerial parts | Iridoid, flavonoid and phenolic acid |
| **Smilax chinensis** *(Liliaceae)* | Rhizomes | Tannin, saponins and flavonoid |
| **Tabebuia** spp. *(Bignoniaceae)* | Bark | Quinine, bioflavonoid and co-enzyme Q |
| **Thuja occidentalis** *(Cupressaceae)* | Whole plant | Flavonoid, tannin, volatile oil and mucilage |
| **Thymus vulgaris** *(Lamiaceae)* | Whole plant | Volatile oil, flavonoid and tannin |
| **Trifolium pratense** *(Fabaceae)* | Flower | Glucosides (trifolin, trifolitin, trifolianol), flavonoid |
| **Vitex rotundifolia** *(Verbenaceae)* | Whole plant | Camphene, pinene and diterpene |

**Table 2. Plants reported for their anticancer activity**

Natural products discovered from medicinal plants have played an important role in the treatment of cancer. They have exhibited anticancer activity in animal models of leukemia, skin cancer and sarcomas. Through generating awareness regarding usage of herbs and exploring natural product properties, healthcare professionals, can play significant clinical roles as knowledge resources for masses.

Selected plants have been explored for biological activity and further investigations into anticancer activity of the plants showing promising activity, must be undertaken. Vinca rosea alkaloids, Vinblastine and Vincristine, are one of the most potent anticancer drugs known. Taxol isolated from *Taxus brevifolia* has figured high in the therapeutic segment of cancer. Cancer being associated with high mortality rates, if herbs can be used even in the palliative care or to reduce the side effects associated with cancer would be of great relief for the sufferer\(^6\).

5. e. Wound healing and cancer

The relationship between wound healing and cancer has long been recognized. The mechanisms that regulate wound healing have been shown to promote transformation and growth of malignant cells. In addition, chronic inflammation has been associated with malignant transformation in many tissues. Recently, pathways involved in inflammation and wound healing have been reported to enhance cancer stem cell (CSC) populations. These cells, which are highly resistant to current treatments, are capable of repopulating the tumor after treatment, causing local and systemic recurrences\(^6\).
INTRODUCTION

Tumors have been described as wounds that do not heal. Recently, inflammatory processes that occur during normal wound healing have been linked to the pathological state of many tumors. Normal epithelial tissue exists in a state of homeostasis where tissue regeneration is tightly regulated by epithelial stem cells located within highly specialized niches. During tissue injury, replenishment of epithelial cell loss is ensured by the proliferation of these stem cells and their progeny in response to proinflammatory cytokines.  

5.1. Wound healing, chronic inflammation and the tumor microenvironment

Wound healing is a dynamic process that consists of an inflammatory phase followed by epithelial cell proliferation and tissue remodeling. In normal tissue, the inflammatory phase is limited, lasting only 3–14 days. Tissue injury induces immediate recruitment of neutrophils, which are later replaced by macrophages and lymphocytes. Infiltrating leukocytes play a major role in secretion of inflammatory cytokines, growth factors, and chemokines, which stimulate proliferation of progenitor cells and recruitment of keratinocytes and endothelial cells during the proliferative phase of wound healing. At this stage, granulation tissue forms, angiogenesis is induced, and new extracellular matrix (ECM) is secreted. Epithelial cells undergo epithelial–mesenchymal transition (EMT) and migrate to the edges of the wound to impart re-epithelialization of the damaged tissue.  

In the final phase of wound healing, the maturation phase, wound contraction, and differentiation of fibroblasts to myofibroblasts result in the formation of scar tissue. Failure to exit the inflammatory stage results in improper tissue remodeling and is associated with impaired wound healing in many disorders including diabetes mellitus, pressure necrosis, and vasculitis.  

During the course of malignancy, tumor cells invade neighboring tissues, stimulate angiogenesis, remodel the ECM, undergo EMT, and metastasize. In doing so, they activate a chronic inflammatory response involving numerous cytokines, developmental pathways, and growth factors involved in the normal wound healing process. The presence of these factors within the tumor microenvironment is linked to an increase in the proportion of cells bearing stem-like phenotypes, enhanced tumor-initiating properties, and increased resistance to standard therapies.  

It is currently unclear whether the tumor microenvironment confers a survival advantage through selection of resistant CSC (Cancer stem cell) or through up-regulation of stem-like properties in non-stem cells. Although evidence for cells bearing...
stem cell signatures in resistant breast cancer is compelling, the origin of these cells is controversial. The question remains as to whether signals within the tumor microenvironment lead to proliferation of a small population of cells arising from genetically altered stem cells, or do they induce a stem-like phenotype in differentiated cells through up-regulation of developmental signaling pathways and stem cell markers. Further, does this change in phenotype confer a functional change in differentiated cells so that they now behave as CSC, exhibiting self-renewal and tumor-initiating properties?

As shown in Figure 3, we propose that when normal homeostasis is disrupted, either by tissue injury or by the tumor microenvironment, activation of inflammatory and developmental pathways alters the ratio of stem cells to non-stem cells in two possible ways. The first is by driving existing slow-cycling, quiescent stem cell populations into the proliferative phase of the cell cycle.67

**Figure 3. Cellular plasticity in normal vs altered homeostasis.**

(Left panel) Under normal conditions, stem cell/non-stem cell ratios are maintained at a consistent level via slow cycling stem cells (blue), which undergo a unidirectional differentiation to lineage restricted cells. (Right panel) In conditions of altered homeostasis, the ratio of stem cell/non-stem cell is increased by proliferation of stem cells and a bidirectional differentiation whereby lineage restricted cells are able to dedifferentiate and acquire stem-like features. In both wound healing and the tumor microenvironment, this cellular plasticity is driven by inflammatory and developmental factors. However, in wound healing, expression of these factors is transient, homeostasis returns, and the ratio of stem/non-stem cells returns to normal levels. In the tumor microenvironment, continuous expression of these factors may lead to a permanent expansion of stem-like cells.
The second is by altering plasticity of differentiated cells so that they obtain stemness properties. The latter of these processes is supported by recent evidence showing significant cellular plasticity in normal tissue during wound healing processes. Lineage tracing experiments have shown that committed progeny in the lung and intestine can revert to a stem-like phenotype and contribute to tissue regeneration. Likewise, interconversions between stem-like and differentiated states have been reported in glioblastoma and breast cancer cells after chemotherapy. Although both wound healing and the tumor microenvironment represent states of disrupted homeostasis in which stem cell numbers may be increased, they differ significantly in that the level of stemness during wound healing is tightly regulated, with a number of stem-like cells returning to normal during the final phases of the healing process. In the tumor microenvironment, stem cell plasticity may be constant with an overall increase in stem-like cells.

The effect of inflammatory signals within the microenvironment on the cellular plasticity of tumor cells has yet to be fully determined. Thus, it will be important to investigate whether blocking specific inflammatory signals, alone or in combination with other stem cell pathway inhibitors, can decrease CSC populations in a therapeutic setting and promote better responses to standard therapies.

The answer to this question would help to uncover a link between inflammation and CSCs and determine whether, together or separately, they impact the response to therapy.

5.f. Examples of plant-derived anti-cancer compounds currently involved in clinical trials

Although relatively few plant-derived drugs have been launched onto the market during the last 6 years, many plant-derived compounds are currently undergoing clinical trial for the potential treatment of various diseases. The majority of such drugs under clinical development are in the oncological area, including new analogs of known anticancer drugs based on the camptothecin, taxane, podophyllotoxin, or vinblastine type skeletons.

Examples of compounds with carbon skeletons different from the existing plant-derived drugs used in cancer chemotherapy will be discussed below, namely, betulinic acid, ceftalonine (homoharringtonine), combretastatin A4 phosphate, ingenol-3-angelate, phenoxodiol, and protopanaxadio.
In 1995, a research group from the University of Illinois at Chicago reported that betulinic acid (1) selectively inhibited human melanoma in both *in vitro* and *in vivo* model systems, and induced apoptosis in Mel-2 human melanoma cells.

This compound was further developed under the Rapid Access to Intervention Development program of the United States National Cancer Institute, and is currently undergoing phase I/II clinical trials for treatment of dysplastic melanocytic nevi, a preliminary symptom that may lead to melanomas of the skin\textsuperscript{70-71}.

\begin{center}
\begin{tikzpicture}
\node (compound1) at (0,0) {\includegraphics[width=1.5in]{compound1.png}};
\node (compound2) at (5,0) {\includegraphics[width=1.5in]{compound2.png}};
\node (compound3) at (10,0) {\includegraphics[width=1.5in]{compound3.png}};
\end{tikzpicture}
\end{center}

1. **Betulinic acid**, R=H

Several antineoplastic compounds isolated from plants, such as podophyllotoxin (2) and camptothecin (3), are too toxic or not water soluble enough for clinical application, and analogs with higher therapeutic indices such as etoposide (4) and topotecan (5) have been developed in consequence. Due to their unique modes of anticancer activities, there is much interest in the clinical development of further derivatives of paclitaxel (6) and camptothecin (3) as anticancer therapeutic drugs\textsuperscript{72}.

According to a recent review, of the 2255 cancer clinical trials recorded as of August 2003, (or 13.7 \%) and (or 5.4 \%) of the trials involved taxane- and camptothecin-derived drugs, respectively. In 2002, it was estimated that the combined sales of paclitaxel and docetaxel (both taxanes), and topotecan and irinotecan (both based on the parent molecule camptothecin) constituted over 30 \% of the total global sales of cytotoxic drugs\textsuperscript{73}.

\begin{center}
\begin{tikzpicture}
\node (compound4) at (0,0) {\includegraphics[width=1.5in]{compound4.png}};
\node (compound5) at (5,0) {\includegraphics[width=1.5in]{compound5.png}};
\end{tikzpicture}
\end{center}

2. **Podophyllotoxin**, R= H
3. **Camptothecin**, R\textsubscript{1} = H, R\textsubscript{2} = H
INTRODUCTION

4. Etoposide

5. Topotecan, $R_1 = \text{OH}, R_2 = \text{CH}_2\text{N(CH}_3)_2$

6. Paclitaxel

Ceflatonine (7), a synthetic version of homoharringtonine produced by ChemGenex Pharmaceuticals (Menlo Park, CA, USA), is currently undergoing phase II/III clinical trials for the treatment of patients with chronic myeloid leukemia that is resistant to the first-line therapy\textsuperscript{74}.

7. Ceflatonine

Combretastatin A4 phosphate (8, CA4P) is a disodium phosphate pro-drug of the natural stilbene combretastatin A4 (9) isolated from the South African tree \textit{Combretum caffrum} Kuntze. CA4P is being developed by OXiGENE (Waltham, MA, USA) to treat anaplastic thyroid cancer in combination with other anticancer drugs and also for myopic macular degeneration, both in phase II clinical trials. Combretastatin is a vascular targeting agent that functions by destroying existing tumor vasculature by inducing morphological changes within the endothelial cells\textsuperscript{75-76}.
8, $R = \text{OP}_3\text{Na}_2$. 9, $R = \text{OH}$

Ingenol 3-angelate (10, PEP005) is a diterpene ester isolated from the medicinal plant *Euphorbia peplus* L., a species used traditionally to treat skin conditions such as warts and actinic keratoses. PEP005 kills tumor cells via two mechanisms: (1) by inducing primary necrosis of tumor cells, and (2) by potently activating PKC. This is also associated with an acute T-cell-independent inflammatory response that is characterized by a pronounced neutrophil infiltration. PEP005, developed by Peplin (Brisbane, Australia), is currently undergoing phase II clinical trials as a topical formulation for the treatment of actinic keratosis and basal cell carcinoma.

10. Ingenol 3-angelate

Phenoxodiol (11) an isoflavone from soybean (*Glycine max* Merr.), is being developed by Marshall Edwards (North Ryde, Australia) for the treatment of cervical, ovarian, prostate, renal, and vaginal cancers. Phenoxodiol is a broad-spectrum anticancer drug that induces cancer cell death through inhibition of antiapoptotic proteins including XIAP and FLIP. Phase III clinical trials of phenoxodiol as a treatment for ovarian cancer has started in Australia, with phase II trials currently underway in the USA.

11. Phenoxodiol

Protopanaxadiol (12), a triterpene aglycone hydrolyzed from various Korean ginseng (*Panax ginseng* C. A. Mey.) saponins, has been shown to exhibit apoptotic effects on cancer cells through various signaling pathways, and has also been reported to be cytotoxic against multidrug-resistant tumors. PanaGin Pharmaceuticals (British
Columbia, Canada) is developing protopanaxadiol (Pandimex) for the treatment of lung cancer and other solid tumors, and is currently undergoing phase I clinical study in the USA. Pandimex has been marketed in the People’s Republic of China under conditional approval for the treatment of advanced cancers of the lung, breast, pancreas, stomach, colon, and rectum.

5. g. Recent trends and future directions in plant drug discovery

Plant-derived and other natural product secondary metabolites have provided many novel prototype bioactive molecules, some of which have led to important drugs that are available on the market today. In spite of this, in the last 10 years or so, most large pharmaceutical companies have either terminated or scaled down their natural products drug-discovery programs, largely in favor of performing combinatorial chemistry, which can generate libraries consisting of millions of compounds. The roles of large pharmaceutical companies in the field of natural products have now been taken over to some extent by small biotechnology companies, which are specializing in lead identification from natural product extracts and the development of these leads into drugs.

Many of the plant-derived drugs currently undergoing clinical trials were obtained and promoted by these emerging “biotech” companies, some of which were mentioned in the previous section.

In the past, drug discovery of bioactive compounds from plants was time consuming, and the process of identifying the structures of active compounds from an extract could take weeks, months, or even years, depending on the complexity of the problem. Nowadays, the speed of bioassay-guided fractionation has been improved significantly by improvements in instrumentation such as high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS/MS), liquid chromatography, LC-MS), higher magnetic field-strength nuclear magnetic resonance (NMR) instruments, and robotics to automate high-throughput bioassays. The introduction of capillary NMR (cap-NMR) spectroscopy is a recent major breakthrough for the characterization of compounds that are extremely limited in quantity in their organisms of origin.
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The high sensitivity of the cap-NMR probe has allowed for the combination of NMR spectroscopy with other analytical “hyphenated” techniques, such as LC-NMR-MS and LC-solid phase extraction (SPE)-NMR. The LC-NMR-MS technique generally requires deuterated solvents during the chromatographic separation, or alternatively, solvent suppression can be used for nondeuterated solvents. In contrast, the LC-SPE-NMR technique does not require deuterated solvents during the chromatographic separation, and, furthermore, it allows for sample enrichment through repeated chromatographic runs using SPE before NMR analysis.

A state-of-the-art integrated system for LC-NMR-MS and LC-SPE-NMR-MS has been developed and the hardware can be switched from LC-NMR-MS to LC-SPE-NMR MS with minimal reconfiguration. LC-SPE-NMR in combination with HPLC-electrospray ionization mass spectrometry (ESIMS) has been used for the rapid identification of compounds present in crude extracts of plants, as exemplified by the identification of sesquiterpene lactones and esterified phenylpropanoids in *Thapsia garganica* L. and the characterization of constituents of *Harpagophytum procumbens*.

The development of automated high-throughput techniques has allowed for rapid screening of plant extracts; thus, the biological assay is no longer the rate-limiting step in the drug-discovery process. With advances in data handling systems and robotics, 100,000 samples can be assayed in just over 1 week when using a 384-well format.

Screening of plant extract libraries can be problematic due to the presence of compounds that may auto-fluoresce or have UV absorptions that interfere with the screen readout, but pre-fractionation of extracts can be used to alleviate some of these types of problems. Also, most high-throughput screening assay methods have been developed with computational filtering methods to identify and remove potentially problematic compounds that can give false-positive results.

In the future, the routine use of NMR “hyphenated” techniques will allow for quick “de-replication” (a process of eliminating known and active compounds in the plant extracts that have been studied previously), and high-throughput screening will permit the rapid identification of the active compounds.

For example, duplicate SPE plates can be generated during the HPLC separation, with one plate used to prepare samples for high-throughput screening, while the other plate is kept as a reference. The structure(s) of compounds in wells of these plates that show(s) activity can be determined by cap-NMR and MS, and known compounds can be ruled out quickly based on their NMR spectroscopic and MS information.
In instances where the active compound has a new structure, further isolation can be carried out from the plant material, provided there is enough samples. Alternatively, the compound can be synthesized for further bioassay, and combinatorial chemistry can be used to design new analogs based on the parent molecules.

Adequate and continuous supplies of plant-derived drugs are essential to meet the market demand. For compounds that are uneconomical to synthesize, and only available in a small quantities from plants, the use of plant cell cultures is an alternative production method. Plants accumulate secondary metabolites at specific developmental stages, and by manipulating the environmental conditions and medium, many natural products have been synthesized in cell cultures in larger percentage yields than those evident in whole plants. The total drug discovery process is a complex process and is explained in the following Figure 4.

**Figure 4. New Drug Discovery and Development process**

5. h. Flash chromatography

A column chromatography technique designed to rapidly separate individual chemical compounds from a crude mixture using a pressurized mobile phase and a 50 micron particle size stationary phase.
Flash chromatography is defined as a type of preparative purification for rapid isolation of compounds including reaction mixtures, where the target (synthesized) molecule must be separated from excess reagents, by-products, and side-products; natural products – compound of interest have be separated from matrix and impurities.

- Sample range varies from several mg to over 150 g
- Linear flow rates up to 15 cm/min.
- Pressure 10-100 psi.

5.h.i. Advantages of Flash chromatography

a) Purify crude synthetic and natural products
b) Very effective purification technique
c) Very fast technique, hence “flash”
d) Scalable from low mass (mg) to large mass (kg)
e) Inexpensive

5. h.ii. Column vs Flash chromatography

<table>
<thead>
<tr>
<th>Column chromatography</th>
<th>Flash chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation is very slow – flow based on gravitational force</td>
<td>Separation is very fast – flow based on pump</td>
</tr>
<tr>
<td>Manual Column packing – a tedious process – should not pack with air bubble – affect the resolution and separation – disposal is also difficult</td>
<td>Pre packed columns to improve the separation – plug and play- disposal is easy</td>
</tr>
<tr>
<td>Manually change the strength of solvents – collect the fraction manually – check the TLC for all the fraction</td>
<td>System will separate by gradient as per method – automatically collect the fraction based on time or volume – has in build UV detector – no need to check all fractions.</td>
</tr>
</tbody>
</table>

Table 3. Column chromatography vs Flash chromatography

In traditional column chromatography a sample to be purified is placed on the top of a column containing some solid support, often silica gel. The rest of the column is then filled with a solvent (or mixture of solvents) which then runs through the solid support under the force of gravity. The various components to be separated travel through the column at
different rates and then can be collected separately as they emerge from the bottom of the column. Unfortunately, the rate at which the solvent percolates through the column is slow.

In flash chromatography however air pressure is used to speed up the flow of solvent, dramatically decreasing the time needed to purify the sample, therefore making the column and running the separation could take less than 10-15 minutes. Flash chromatography is basically an air pressure driven hybrid of medium pressure and shorter column chromatography which has been optimized for particularly rapid separation.

Flash chromatography is a technique used to separate mixtures of molecules into their individual constituents, frequently used in the drug discovery process.

Flash chromatography, also known as medium pressure chromatography, was popularized several years ago by Clark Still of Columbia University, as an alternative to slow and often inefficient gravity-fed chromatography. Flash chromatography differs from the conventional technique in two ways:

1. Slightly smaller silica gel particles (250-400 mesh) are used
2. Due to restricted flow of solvent caused by the small gel particles, pressurized gas (10-15 psi) is used to drive the solvent through the column of stationary phase.

Automated flash chromatography systems include components normally found on more expensive HPLC systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent. Typically these automated systems separate samples from a few milligrams up to an industrial kg scale and offer much cheaper and quicker solution to doing multiple injections on prep-HPLC system. The software controlling an automated system coordinate the components, allow a user to only collect the factions that contain their target compound and help the user to find the resulting purified material within the fraction collector. The software also saves the resulting chromatograph from the process for archival and/or later recall purposes.

5. h.ii. Principle of flash chromatography

The principle is that the eluent which is a liquid, under gas pressure (normally nitrogen or compressed air) rapidly pushed through a short glass column. The glass column is packed with an adsorbent of defined particle size with large inner diameter. The most used stationary phase is silica gel 40 – 63 uM but obviously packing with other particle sizes can be used as well particles smaller than 25 um should only be used with very low viscosity mobile phases, because otherwise the flow rate would be very low. Normally gel beds are about 15 cm high with working pressures of 1.5 – 2.0 bars. Originally only unmodified silica was used as the stationary phase, so that only normal phase
chromatography was possible. In the meantime, however, and parallel to HPLC, reversed phase materials are used more frequently in flash chromatography.

5. h.iii. Isolera flash chromatography system
It is an automated flash purification system consists of state-of-the-art automated flash purification system, Single cartridge, 4-cartridge sequential, Touch screen graphic user interface with new, intuitive software, Dual piston pump, Two detector options Biotage fixed 254 nm UV, Biotage variable UV detector, Large fraction collection volume, Standard (4.8-L maximum), Extended bed (9.6-L maximum), Molded plastic racks Uses SNAP, Flash+, and Flash 75 cartridges, Elevated solvent reservoir platform (Figure 5).

5. h. iv. Key features of the instrument
i. Click-&-drag gradients simplify method building and on-the-fly modification.
ii. Fractionation using two wavelengths ensures compounds will be collected even if not detected using the primary detection wavelength.
iii. Dual-binary gradient capability allows users to elute strongly-bound compounds with up to four solvents without changing methods.
iv. Isocratic additive during binary gradient helps improve purification by increasing compound solubility or decreasing secondary compound-silica interactions.
v. 200 mL/min maximum flow rate speeds purification when using SNAP 340g, Flash 65, and Flash 75 cartridges.
vi. Large fraction capacity (up to 9.6L with extended bed) reduces number of rack changes.
vii. Real-time method changes without manually pausing the pump add to system intelligence and increases customer satisfaction.
viii. Elevated solvent rack increases bench space.
ix. Cartridge air flush dries cartridges for disposal.
x. Real-time chromatogram zoom allows detailed look at a peak or area of the chromatogram.
xii. Fractionate using auxiliary, non-UV based detection systems.
xii. Accurate TLC-to-gradient method building based on one TLC.

5.h.v. Converting TLC to flash chromatography
Thin layer chromatography (TLC) is relatively rapid, economical and easy to use, and provides qualitative data about the progress of organic reactions. The largest drawbacks of TLC are that it does not provide accurate quantitative data about the relative purity of the compounds being monitored, and it is not suitable for use in large-scale purification.
Chemists can use TLC separations to help determine effective solvent compositions for flash chromatography. In planar techniques such as TLC, the solvent flow rate cannot be controlled and is not constant throughout the separation. As a result, retention needs to be measured as a distance rather than in retention times or column volumes. Rf, a common TLC unit, is known as the Retention Factor and ΔRf is the distance between compound.

These can be defined as:

$$ R_F = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}} $$

$$ \Delta R_F = R_{F1} - R_{F2} $$

The ideal solvent system for TLC is one that moves the compound of interest in the mixture to an Rf of 0.15-0.35 and will separate this component from the others nearest to it by a ΔRf value of at least 0.15.

In contrast to TLC, flash chromatography separations are governed by column volumes (CV). CV is the number of column volumes required to elute the compound of interest from the column, and ΔCV is the number of column volumes between two compounds eluting from the column. A column volume is defined as the volume of solvent required to fill all the sorbent pores and interstitial spaces between sorbent particles in a given column. The ideal flash chromatography solvent system is one that elutes the desired compound of interest in a CV of 3-6 and will separate this component from the others nearest to it by a ΔCV value of greater than 1.

$$ CV = \frac{1}{R_F} $$

$$ \Delta CV = \frac{1}{R_{F1}} - \frac{1}{R_{F2}} $$

For a particular set of separation conditions, a weakly retained, fast-eluting component with an Rf value of 0.9 can be eluted in just over 1 column volume, whereas a strongly retained, slow-eluting component with an Rf of 0.10 requires 10 column volumes for complete elution (see Table 4).

<table>
<thead>
<tr>
<th>RF</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.90</td>
<td>1.10</td>
</tr>
<tr>
<td>0.70</td>
<td>1.40</td>
</tr>
<tr>
<td>0.50</td>
<td>2.00</td>
</tr>
<tr>
<td>0.30</td>
<td>3.33</td>
</tr>
<tr>
<td>0.10</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 4. Conversion of R_F and CV
Due to factors such as changes in the TLC solvent flow rate with respect to time and interference from adhesives used to bind TLC sorbents, solvent conditions that provide an acceptable TLC separation will not necessarily work effectively for flash chromatography without modification.

5. h.vi How to start flash
   a. The primary requirement for flash is TLC data.
   b. Calculate the Rf from TLC (Thin layer Chromatography).
   c. Calculate the CV for flash.
   d. Transfer the TLC Rf to Flash by converting Rf to CV.

Figure 5. Biotage (Isolera one), Flash Chromatography System

Purification of drug is an important step in any branch of research. Preparative chromatography is used to separate the components of a mixture for more advanced use and is thus a form of purification. Flash Chromatography can be alternative to preparative HPLC as it saves time and solvent. Extrapolation of TLC results on preparative scale can be achieved by Flash chromatography. Modern Flash chromatography with disposable cartridges and advanced detection techniques is applicable to a wide range of compounds.

5. i. Natural product isolation

5. i.i. General concept of natural product isolation

Plant or animal tissues always contain several classes of compounds with markedly different structures. Each class usually contains several or a lot of compounds closely
related in the structure. Natural product chemistry usually begins from the separation and isolation of single pure compound from such many similarly related ingredients.

The compounds essential for living body, which exist beyond species, such as glycogen, proteins, nucleic acids, some enzymes, and etc. are called “primary metabolite”. The other compounds present in relatively narrow class as specific to each species of the plants are called “secondary metabolite”. The role of these compounds in living body is still obscure.

Target of natural product chemistry is usually such secondary metabolite. Historically, isolation of secondary metabolites from the target plant begins by extraction with solvents. For example, alkaloids are extracted by acidic water, and after basification of the extract, they are again extracted with organic solvent such as chloroform. Sometimes, the methanol extract was suspended in water, and re-extracted with successive increase of solvent polarity, from hexane to chloroform.

Afterward, each extract is subjected to separation or purification by the other methods such as chromatography.

By the above method, many of water-soluble compounds, such as saponins, tannins, and carbohydrates are elusive. In view point of this, and by the development of carrier material in chromatography, the isolation method has been changed into the following manner. The methanol extract is made first. After concentration of the extract, the residue is put on the top of silica gel column, and then eluted with solvent gradually increasing its polarity, starting from hexane to chloroform-methanol. By this method, the range of compounds for isolation was widened from non-polar hydrocarbons to some water-soluble compounds such as saponins. However, isolation of very polar compounds was still difficult.

Later, variety of new packing material for column chromatography such as Amberite XAD-2, Diaion HP-20, MCI gel, and Sephadex LH-20 were introduced. Those new materials effected to fractionate highly polar compounds with greater efficacy and improved reproducibility. For example, chromatography on Sephadex LH-20 effected isolation of highly polar tannins.

5. i.ii. Modern isolation methods

Recent introduction of HPLC technique, particularly Recycling HPLC technique, made to allow the separation of structurally very close compounds, those which were inseparable or hardly separated by usual column chromatography. Thus, general strategy of isolation of natural products must be revised from classical simple
(stereotype) chromatography to modern multi-conceptual method. But, the weak issue of HPLC is in its non-applicability to the sample of large quantity. Usually, one treatment is about 10 to 30 mg, at most 0.1 g even if a preparative column is used. Therefore, modern method of isolation adopts multi-concept approach; combination of all separation and fractionation methods and techniques.

5. i.iii. General strategy for isolation of secondary metabolites

a. Preliminary fractionation of the extract has to be made by using various solvents depending on difference of solubility (i.e. fractionation by solubility class). After preliminary fractionation, water-soluble (hydrophilic) fraction and water-insoluble (hydrophobic) fraction must be treated by different concepts.

b. Each of water-insoluble (organic solvent-soluble) fractions is subjected to column chromatography on silica gel or ODS to separate the mixture as possible as one may can. This is Step 1 for isolation, which is the same with classical chromatographic separation.

c. Water-soluble fraction must be treated differently. Recommendable treatment is as follows. Firstly separate the mixture by the degree of hydrophobic (lipophylic) nature of the compounds shaking with n-butanol to divide into butanol layer (more lipophylic) and water (hydrophilic) layer. Then each layer is concentrated, dissolved in water, and passed through a column of Diaion HP-20 or Amberite XAD-2 resin. This is a mode of separation with the aid of p-p interaction between resin and compound. Compounds containing double bond(s) and/or aromatic group are held back by the resin and those without such group are eluted through the column. Carbohydrates are separated by this procedure. By increasing percentage of MeOH, more lipophylic compounds are eluted. Column chromatography on Sephadex LH-20 and/or polyamide is used as an option of sub-fractionation. Particularly, polyamide is effective for separation of phenolic compounds. Special care must be taken for tannins, which are hard to elute from the column. The above procedure is Step 1 toward separation of water-soluble fraction.

d. The procedure adopted in Step 1 not only fractionates the mixture into the compounds if similar chromatographic behavior, but also reduces the amount of each resulting the fraction making them easier and convenient for handling during purification stages. When each fraction amounts to 0.1 to 1 g, it is further separated by MPLC (medium Pressure HPLC) to yield compounds of very close chromatographic behavior. Sometimes you can obtain single compound in this stage. This is Step 2 of Pre-treatment.
e. Finally, each of above semi-pure fractions (usually less than 0.1 g) is subjected to a Recycling HPLC to separate or to purify into single pure compound. This is Step 3 of isolation.

5.i.iv. Types of isolation

Based on the purpose of isolation of natural products is broadly classified into one of the following three categories.

a. Activity directing isolation (What is the origin of that activity?)

b. Structure directing isolation (Searching new or novel structure)

c. Chemotaxonomical study (Relationship between histo- and chemo-type)

a. Activity directing isolation

The work of this type is done only when the plant is known as bio-active (such as traditional medicines) or an activity under investigation is shown by the crude extract. The work is done to disclose what ingredient(s) is responsible to the relevant activity. In any of the above two cases, one needs the method of bioassay, or to find out the method to evaluate the fractionated products. For the bioassay to screen active natural product the following criteria are necessary: simplicity (small quantity), rapidity, comparability (clear-cut result), and reproducibility. Those will be discussed in next session. Without such bioassay method, one cannot start activity directing isolation. When such method and co-worker are available, then proceed into the followings:

Start with 100 g of the plant material or 10 g of extract. Extract the pulverized material successively with each 200 ml of hexane (3 times), chloroform or AcOEt (2 times), and MeOH (2 times) under reflux for 3-4 h. Each fraction is concentrated (check quantity), and a part of residue is supplied to bioassay.

Compare TLC and activity of above three extracts, and judge which spot(s) may be responsible to the activity. Is the activity increased when compared to the original extract?

Make chromatography of the strongest active fraction. Elute with hexane, hexane-AcOEt (1:1), AcOEt, and MeOH (check quantity of each fraction). Supply a portion of them to bioassay. You need not necessary to obtain single or a pure compound at this stage. But the above procedure is essentially necessary to judge what type of compound is responsible to the activity.

After this stage, make chromatography carefully to obtain pure compounds. Choose the
solvent system according to the hitherto obtained knowledge. Detailed isolation will be done on the basis of the preliminary extraction. You may increase the amount of initial plant material or the extract.

c. Structure-directing isolation

You are searching new compound or hopefully novel structure. Why did you choose this plant? Does it have a possibility to yield novel structure? Can you confidently defend if asked why you choose this plant? Even if it is not chosen with yourself, make literature search as possible as much as possible: family, genus, species, local name and local uses. Search all references and compounds which has been isolated previously from the target plant. Are the structures known or unknown? Search literatures for plants of the same Genus of a similar use?

If there is some work on this plant, read that work carefully and critically. This literature survey will very much help your, or otherwise, your work will be of little fruits. When you start your work with poor knowledge, collect all information during the work. The more the knowledge is acquired more it promises the fruits in work. Once getting the sufficient information, start the work as follows.

Preliminary Work

a. Start your work with 100–200 g of the material repeating the previous investigators work, if it is present. Or if it is not, follow to the analogous isolation, with considering why they took such procedures. And observe how easy it is or how difficult it is. At the same time what compound(s) were neglected or discarded. Then you will find out, if your work is the second one, what work is remained to you and which part of work is unexplored.

b. At this stage, check to specify the chemical class of your compound in hand! Check the presence of alkaloid by Dragendorf reagent. Are you going to work low polar, medium polar, or high polar compound? By the class of compound, strategy of isolation is different and varies significantly.

c. [For Low–medium polar (water-insoluble) compounds] Prepare MeOH extract. Mix it with celite (if you do not have it, use material of low adsorption capability such as coarse silica gel) and dry. Extract it successively with hexane, EtOAc, and MeOH. Compare TLC of each extract. EtOAc extract (medium polar fraction): Filter if there is a precipitate, and shake the filtrate with water to remove water-soluble material from this fraction, then subject to chromatography as follows. Add the same volume of hexane to the above AcOEt fraction, and filter if any precipitate is present. Pour the solution onto silica gel column and make elution with hexane-EtOAc (1:1), EtOAc,
CHCl₃, acetone, and CHCl₃-MeOH (1:1). Compare TLC of each fraction. MeOH Extract and water washing of EtOAc extract contains high polar compounds.

d. When you get single compound (that usually means single spot on TLC), try to crystallize it from appropriate solvent, describe crystalline forms (prisms, needles, leaflets), and measure mp. Consult with the literature (the same plant, the same mp), if it is Known or not? Take UV, IR, MS, 1H-NMR, 13C-NMR spectra, if necessary, to know possible structures. Is it identical to any of the reported compound? If it is possible to be a known compound, compare the all reported physical and spectral data. Do not compare the structure (it is sometimes wrong), simply compare the exported data. If it is unknown (new) compound, go into structure determination (see identification and Structure Determination).

e. [High polar (water-soluble) compound] The MeOH extract and water-layer from c, contain high polar compound(s). They are usually glycosides, carbohydrates, amino acids, and tannins. These must be treated by different concept from low to medium polar compounds. Each class of compounds is separated by some special techniques, for example, chromatography over Sephadex LH-20 is useful for separation of tannins. Before going into such special separation, I recommend the following general method.

f. Combine the high polar fractions, dissolve it in water, and extract with n-butanol to divide into butanol-soluble and insoluble (water-soluble) fractions. Each of them is treated separately.

g. BuOH extract is concentrated and subjected to chromatography on Diaion HP-20 column, and treated as shown in h. Usually glycosides containing saponins come into this fraction. For Direct chromatography choice of the solvent is the most important factor.

h. Water layer (after removal of all organic solvent contaminated in this fraction under reduced pressure) is passed though Diaion HP-20 column. Wash the column with water. This will give simple carbohydrate (non-lipophilic molecule). Following elutes with water-MeOH (2:1), water-MeOH (1:1), water-MeOH (1:2), and MeOH (100%) give the fractions where lipophylicity is increasing in this order. Tannins are difficult to be eluted. They are eluted with the use of much powerful solvent, such as acetone containing acetic acid. Usually such solvent must be avoided because of damage of the column.

c. Isolation for Chemo-taxonomical Study

This work is, more or less, the same with type B. But, particularly in this work, do not bother or stick to new compound: the known and new compounds are treated in
equal weight and you have to clarify all constituents together with (preferably) relative existing amount.

A general scheme for isolation of secondary metabolites has been explained in the following figure 6.

### Figure 6. A General Scheme for isolation of secondary metabolites from plants

5.j. Drugging Topo-isomerases

Topoisomerases are nuclear enzymes that play essential roles in DNA replication, transcription, chromosome segregation and recombination. All cells have two major forms of topoisomerases: type I, which makes single – stranded cuts in DNA, and type II enzymes, which cut and pass double stranded DNA. Topoisomerases are important targets of approved and experimental anticancer agents and wound healing agents. Topoisomerases are ubiquitous enzymes that control DNA supercoiling and entanglements. They are essential during transcription and replication and topoisomerase inhibitors are among the most effective and most commonly used anticancer and antibacterial drugs. Topoisomerases are universal and present in
eukaryotes, archaebacteria and eubacteria. Human cells encode six topoisomerases whereas bacteria generally contain only 4 topoisomerases and lack the type IB enzymes.

Topoisomerase inhibitors are effective chemotherapies that should only be prescribed to patients who should benefit from the drugs. Otherwise, ineffective regimens delay access to the correct treatment, select for drug resistance and produce costly side effects. Because of the redundant repair pathways involved in the survival of cancer cells targeted by topoisomerase inhibitors, it has been difficult to pinpoint single determinants of response to anticancer topoisomerase inhibitors. However topoisomerase are required for the treatment of cancer (Figure 9)\(^{100–104}\).

The different catalytic mechanism of topoisomerases is explained in the Figure. 7 & 8 and the challenges and discovery of new topoisomerases is given in the table 5.

**Figure 7. Differential catalytic mechanisms of topoisomerases.**

Reactions are represented from left to right. Type I enzymes cleave one strand to process DNA entanglements whereas type II cleave both strands by concerted action of each Top2 monomer. Type IA and IIA enzymes (panels A and C) cleave DNA by covalently attaching their catalytic tyrosine to the DNA 5’-end. Type IA enzymes cleave a single-stranded segment and let another single-strand pass through the break, whereas type IIA let a duplex pass through the concerted breakage of both strands. For both type IA and IIA enzymes, the 3’-ends are tightly bound during strand passage, which keeps the passing DNA in an enzyme cavity before resealing of the ends. By contrast to type IA and IIA enzymes, type IB topoisomerases (panel B) form 3’-phosphotyrosine bonds and relax DNA supercoiling by controlled rotation of the broken 5’-end around the intact strand.
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![Diagram of Topoisomerase-DNA complexes]

**Figure 8.** Schematic representation of the two main repairs pathways removing Topoisomerase-DNA complexes.

<table>
<thead>
<tr>
<th>Challenges</th>
<th>Possible answers (new approaches)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. New topoisomerase targets</td>
<td>Type IA (TopA and Top3) inhibitors</td>
</tr>
<tr>
<td></td>
<td>Top2β-specific inhibitors</td>
</tr>
<tr>
<td>2. New topoisomerase inhibitors (in addition to #1 above)</td>
<td>Chemically stable camptothecins</td>
</tr>
<tr>
<td></td>
<td>Non-camptothecin Top1 inhibitors</td>
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<tr>
<td></td>
<td>Top1 catalytic inhibitors</td>
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<tr>
<td></td>
<td>New Top2 inhibitors with novel structures</td>
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<tr>
<td></td>
<td>Orally bioavailable inhibitors</td>
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<tr>
<td></td>
<td>Targeted delivery (nanoparticles for time-staggered and tumor-specific delivery)</td>
</tr>
<tr>
<td>3. Pharmacodynamic (PD) biomarkers to rapidly evaluate tumor drug response</td>
<td>Top1 and Top2 cleavage complexes induction</td>
</tr>
<tr>
<td></td>
<td>Top1 and Top2 down-regulation</td>
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<tr>
<td></td>
<td>DNA damage (γ-H2AX)</td>
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<tr>
<td></td>
<td>Apoptotic response (caspase activation)</td>
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<tr>
<td></td>
<td>Additional PD biomarkers based on further elucidation of the molecular DNA repair pathways and DNA damage responses (DDR) downstream from topoisomerase poisoning in model systems</td>
</tr>
<tr>
<td>4. Cancer patient selection</td>
<td>Identification and implementation of predictive biomarkers and “response signatures” (based on OMIC tools: tumor gene expression and somatic mutations)</td>
</tr>
</tbody>
</table>
proteomic and metabolomic) for patient stratification
- New predictive biomarkers based on molecular biology pharmacology studies in model systems
- High tumor Top1 and Top2 levels
- Pharmacogenomics tests (germline mutations affecting drug pharmacokinetics and metabolism)

| 5. Optimize drug combinations | Based on the further elucidation of the molecular DNA repair pathways and DNA damage responses (DDR) downstream from topoisomerase poisoning in model systems
- Based on synthetic lethality related to tumor-specific defects (ERCC1-deficiency for combining Top1 and PARP inhibitors)
- Based on system pharmacology in model systems to reveal the pathways (molecular networks) and novel genetic and molecular determinants that drive tumor response
- Based on experimental data obtained in model systems |

**Table 5. Challenges for the discovery and use of topoisomerase inhibitors**

Therefore, the dual topo drugging is an important phenomenena in the cancer treatment. Even though many synthetic drugs as topo-poisons have been developed and used in the clinical trials, but having many challenges and are not successful due to cancer resistance. Hence there is a current demand for the discovery of new human dual topo-poisons I & II, which is possible from phytochemically unexplored plants.  

5. k. Molecular docking studies

Computer-aided drug discovery/design methods have played a major role in the development of therapeutically important small molecules for over three decades. These
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methods are broadly classified as either structure-based or ligand-based methods. Structure-based methods are in principle analogous to high-throughput screening in that both target and ligand structure information is imperative. Structure based approaches include ligand docking, pharmacophore and ligand design methods.  

5. k.i. a. Position of computer-aided drug design in the drug discovery pipeline

CADD is capable of increasing the hit rate of novel drug compounds because it uses a much more targeted search than traditional HTS and combinatorial chemistry. It not only aims to explain the molecular basis of therapeutic activity but also to predict possible derivatives that would improve activity. In a drug discovery campaign, CADD is usually used for three major purposes: (1) filter large compound libraries into smaller sets of predicted active compounds that can be tested experimentally; (2) guide the optimization of lead compounds, whether to increase its affinity or optimize drug metabolism and pharmacokinetics (DMPK) properties including absorption, distribution, metabolism, excretion, and the potential for toxicity (ADMET); (3) design novel compounds, either by "growing" starting molecules one functional group at a time or by piecing together fragments into novel chemotypes. Figure 10 illustrates the position of CADD in drug discovery pipeline. 

![Diagram of CADD in drug discovery/design pipeline]

Fig. 10. CADD in drug discovery/design pipeline

CADD can be classified into two general categories: structure-based and ligand-based. Structure-based CADD relies on the knowledge of the target protein structure to calculate interaction energies for all compounds tested, whereas ligand-based CADD exploits the knowledge of known active and inactive molecules through chemical similarity searches or construction of predictive, quantitative structure-activity relation...
(QSAR) models. Structure based CADD is generally preferred where high-resolution structural data of the target protein are available, i.e., for soluble proteins that can readily be crystallized. Ligand based CADD is generally preferred when no or little structural information is available, often for membrane protein targets. The central goal of structure based CADD is to design compounds that bind tightly to the target, i.e., with large reduction in free energy, improved DMPK/ADMET properties, and are target specific, i.e., have reduced off-target effects. A successful application of these methods will result in a compound that has been validated in vitro and in vivo and its binding location has been confirmed, ideally through a co-crystal structure.

5. k.2. Target data bases for computer-aided drug discovery/design

The knowledge of the structure of the target protein is required for structure-based CADD. The Protein Data Bank (PDB) (2013), established in 1971 at the Brookhaven National Laboratory, and the Cambridge Crystallographic Data Center, are among the most commonly used databases for protein structure. PDB currently houses more than 81,000 protein structures, the majority of which have been determined using X-ray crystallography and a smaller set determined using NMR spectroscopy. When an experimentally determined structure of a protein is not available, it is often possible to create a comparative model based on the experimental structure of a related protein. Most frequently the relation is based in evolution that introduced the term "homology model." The Swiss-Model server is one of the most widely used web-based tools for homology modeling.

Initially, static protein structures were used for all structure-based design methods. However, proteins are not static structures but rather exist as ensembles of different conformational states. The protein fluctuates through this ensemble depending on the relative free energies of each of these states, spending more time in conformations of lower free energy. Ligands are thought to interact with some conformations but not others, thus stabilizing conformational populations in the ensemble. Therefore, docking compounds into a static protein structure can be misleading, as the chosen conformation may not be representative of the conformation capable of binding the ligand. Recently, it has become state of the art to use additional computational tools such as molecular dynamics and molecular mechanics to simulate and evaluate a protein’s conformational space. Conformational sampling provides a collection of snapshots that can be used in place of a single structure that reflect the breadth of fluctuations the ligand may encounter.
in vivo. This approach was proven to be invaluable in CADD by Schames et al. (2004) in the 2004 identification of novel HIV integrase inhibitors\textsuperscript{110}.

Some methods, such as ROSETTALIGAND, are capable of incorporating protein flexibility during the actual docking procedure, omitting the need for snapshot ensembles. The collection of events that occurs when a ligand binds a receptor extends far beyond the noncovalent interactions between ligand and protein. Desolvation of ligand and binding pocket, shifts in the ligand and protein conformational ensembles, and reordering of water molecules in the binding site all contribute to binding free energies.

Consideration of water molecules as an integral part of binding sites is necessary for key mechanistic steps and binding. These water molecules shift the free energy change of ligand binding by either facilitating certain non covalent interactions\textsuperscript{111}.

5.k.3. Ligand-based computer-aided drug design

The ligand-based computer-aided drug discovery (LB-CADD) approach involves the analysis of ligands known to interact with a target of interest. These methods use a set of reference structures collected from compounds known to interact with the target of interest and analyze their 2D or 3D structures. The overall goal is to represent these compounds in such a way that the physicochemical properties most important for their desired interactions are retained, whereas extraneous information not relevant to the interactions is discarded. It is considered an indirect approach to drug discovery in that it does not necessitate knowledge of the structure of the target of interest.

The two fundamental approaches of LB-CADD are (1) selection of compounds based on chemical similarity to known actives using some similarity measure or (2) the construction of a QSAR model that predicts biologic activity from chemical structure. The difference between the two approaches is that the latter weights the features of the chemical structure according to their influence on the biologic activity of interest, whereas the former does not. The methods are applied for \textit{in silico} screening for novel compounds possessing the biologic activity of interest, hit-to-lead and lead-to drug optimization, and also for the optimization of DMPK/ADMET properties. LB-CADD is based on the Similar Property Principle, published by Johnson et al. (1990), which states that molecules that are structurally similar are likely to have similar properties. LB-CADD approaches in contrast to SB-CADD approaches can also be applied when the structure of the biologic target is unknown. Additionally, active compounds identified by ligand-based virtual high-throughput screening (LB-vHTS) methods are often more potent than those identified in SB-vHTS\textsuperscript{112}.
5.k.4. Scoring functions for evaluation protein-ligand complexes

Docking applications need to rapidly and accurately assess protein-ligand complexes, i.e., approximate the energy of the interaction. A ligand docking experiment may generate hundreds of thousands of target-ligand complex conformations, and an efficient scoring function is necessary to rank these complexes and differentiate valid binding mode predictions from invalid predictions. More complex scoring functions attempt to predict target-ligand binding affinities for hit-to-lead and lead-to-drug optimization.

Scoring functions can be grouped into four types: (1) force-field or molecular mechanics-based scoring functions, (2) empirical scoring functions, (3) knowledge-based scoring functions, and (4) consensus scoring functions

Docking methods including Glide, GOLD, Surflex, and FlexX were used to dock ligands into rigid target crystal structures obtained from PDB. A single ligand was used as a reference for ligand-based similarity search strategies such as 2D (fingerprints and feature trees) and 3D [rapid overlay of chemical structures (ROCS; OpenEye Scientific Software, Santa Fe, NM)], a similarity algorithm that calculates maximum volume overlap of two 3D structures.

However, with the implementation of in-silico designing tools, the natural product research towards discovery of anticancer molecules had been easier and faster. These tools in herbal medicine research as a means: to seek out potential mechanisms of action of their constituents; to identify putative new leads for drugs; and to summarize and/or visualize the complex herbal medicine. Knowledge of the computational specialist is essential in guiding decisions made here, and it is imperative that the tools should be applied in herbal medicine research through close collaboration between computational specialists and natural product research.

Keeping all these facts and challenges in focus, the current research is focused on phytochemical and pharmacological exploration of unexplored weed species for the discovery of novel human dual topo poisons with the help of chromatographic, spectroscopic and in-silico designing tools. The isolated fractions and molecules proved that these weeds are good sources for the discovery of new lead molecules which are good human dual Topo-poisons I & II. Hence these weeds can be good sources of medicinal values and can be commercially utilized like other medicinal plants.