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Drug Discovery of Anticancer Agents from the Weeds of the Niligiris Using High Throughput Screening Techniques

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Abstract

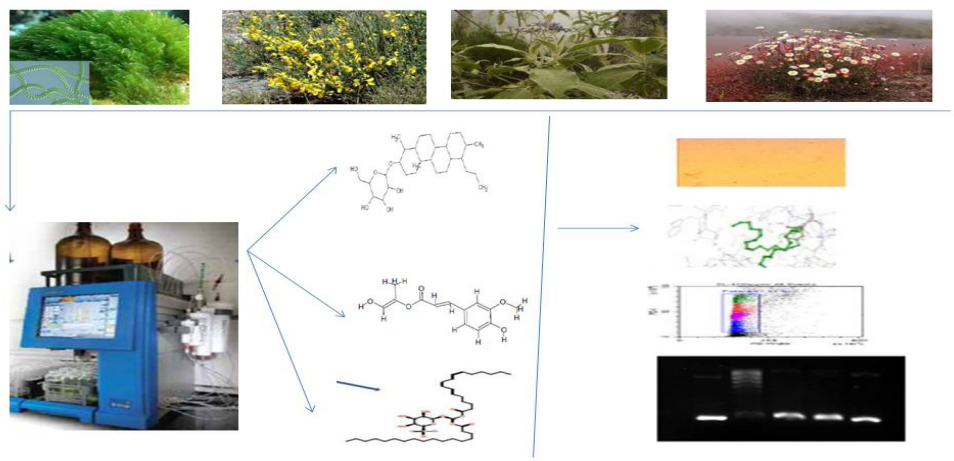
Background: Weeds are store houses of complex bioactive secondary compounds which are the lead molecules for the discovery of pharmaceuticals like Adoniside from *Adonis vernalis* L., Asiaticoside from *Centella asiatica*, Silymarin from *Silybum marianum* (L.) Gaertn etc., which proved that weeds are the richest sources of new pharmaceuticals against many resistant diseases like cancer and tuberculosis. The Nilgiris is gifted with richest flora in which lot of medicinally important plants are present

Objective: The main objective of this research work is to identify any six prominent weed plants of Nilgiris and to establish the phytochemical and pharmacological importance as a human dual topo-poisons I & II and to make them to utilize commerciall

Methods: After a thorough field survey and literature survey six major weeds of the Nilgiris from 56 identified weeds have been taken for phytochemical and biological studies (Anticancer and wound healing), Molecular docking studies, cell cycle analysis and human topopoison I & II assays.

Results: All the weeds proved to have interesting secondary metabolites like phenols (36.9 to 119.9 µg/g gallic acid equivalents), alkaloids (106.7 to 154.8 µg/g atropine equivalents) and flavonoids (43.0 to 159.6µg/g quercetin equivalents) and saponins (14.2 to 25.0 % w/w). First time two flavonoids (Narangenin and 7 Methoxy Hesperetin) were isolated from the aerial parts of *Erigeron karvinskianus* (Asteraceae). A novel saponin glycoside (Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3, 4, 5-triol was isolated from the aerial parts of *Solanum mauritianum* (Solanaceae) and a novel ferulic acid derivative 1E-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoate) was isolated from the aerial parts of *Cytisus scoparius* (Fabaceae). All the isolated compounds showed good *in vitro* cytotoxic activity on MCF-7, HCT-116, and A-549 cell lines 116 with CTC_{50} values ranging from 20.0 to 92.0 µg/ml and proved to be safe on Vero cell lines with CTC50 values 383 to 472 µg/ml in compare to standard quercetin and the compounds also showed good *in-vitro* wound healing activity on HDF cell lines with active concentration level of 50- 12.5 µg/ml. Based on the molecular docking studies, the isolated compounds 2 & 4 showed dual human topo-poison activity at 400 µg/ml dose. All the compounds arresting the cells at S-phase and G2 M –phase on HELA cell lines.

Conclusion: The isolated compounds can be good lead molecules in anticancer drug discovery. The topo-poison I and II studies proved that the compounds II & IV only having Dual topo-poisoning activity. However, further in-depth studies have to be carried out on these identified molecules and the plants in-order to increase its clinical significance through structure modification of functional groups. The current research work may give a platform for the discovery of novel dual human topo I & II isomerase poisons, where there is a current demand in the anticancer research. Hence the research work proved that these weeds have good medicinal values and can be utilized commercially and be good economical sources to farmers as they have many good novel molecules.



Drug discovery process of Human Dual Human Topo poisons I & II.

Keywords: Weeds; Human dual topopoisons I & II; Cell cycle analysis & cancer

Introduction

Herbal medicine

a. **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 study [2].

b. **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 is either direct extracts of plants or is 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 medicine [3].

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 [3].

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" [4].

c. **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].

d. **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].

Natural Products

a. **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 [9]. 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 Eicones (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 [12].

b. **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.

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, and capsaicin) could be isolated from their natural sources [13]. 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 [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 *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 *Galanthus nivalis* L. and the important antimalarial and potential anti-cancer agent artemisinin originally derived from the traditional Chinese herb *Artemisia annua* L [15].

c. **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 (Figure 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].

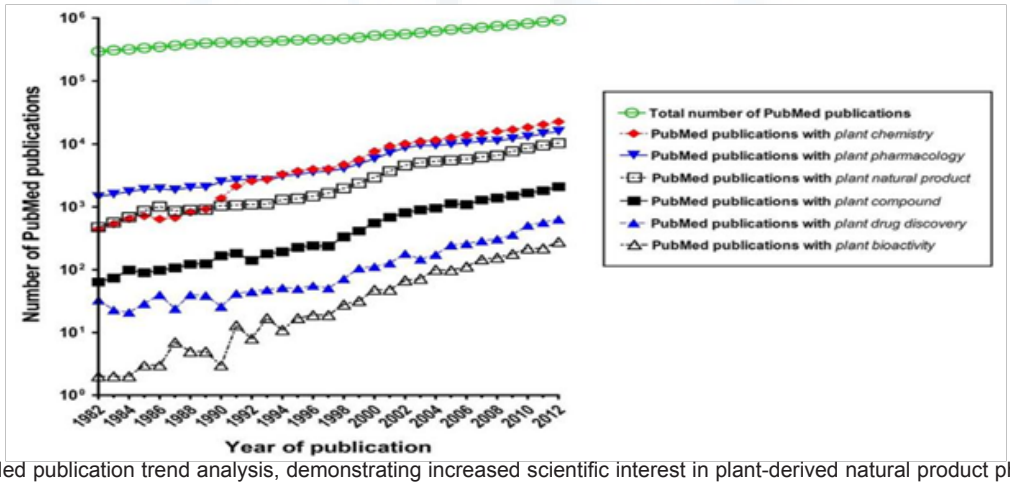


Figure 1: PubMed publication trend analysis, demonstrating increased scientific interest in plant-derived natural product pharmacology, chemistry, and drug discovery.

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).

Weeds

a. **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.

b. **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 feed 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 [17].

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 wildness? Let them be left. let them be left; wildness 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 lappais* 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 [18]. 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 [18]. 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 [19].

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 km^2. 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 [20].

Tribal communities in Nilgiris

The Niligiris 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.

Tribes and Weeds of Nilgiris

a. **Todas**

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

b. **Kotas**

i. ***Achyaranthes 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.

c. **Kurumbas**

i. ***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), locallyknown as "Nasar soppu". Leaf juice is orally given as a cure for cough and cold.

d. **Paniyas**

i. ***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.

e. **Kattunayakas**

i. *aspera* L. (Amaranthaceae), locally known as "Cherukadalai". The whole plant with water is made into paste and applied on body to relive 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 [21].

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 [22].

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 [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 indigeous 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 [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 [25].

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].

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 [27].

Table 1: Various Pharmaceutical leads from different weeds.

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

Cancer

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) [28].

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 diseases 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 [29]. The different types of leading cancers in 2015 were represented in the form of Figure 2 given below.

Estimated Deaths

Males			Females		
Lung & bronchus	86,380	28%	Lung & bronchus	71,660	26%
Prostate	27,540	9%	Breast	40,290	15%
Colon & rectum	26,100	8%	Colon & rectum	23,600	9%
Pancreas	20,710	7%	Pancreas	19,850	7%
Liver & intrahepatic bile duct	17,030	5%	Ovary	14,180	5%
Leukemia	14,210	5%	Leukemia	10,240	4%
Esophagus	12,600	4%	Uterine corpus	10,170	4%
Urinary bladder	11,510	4%	Non-Hodgkin lymphoma	8,310	3%
Non-Hodgkin lymphoma	11,480	4%	Liver & intrahepatic bile duct	7,520	3%
Kidney & renal pelvis	9,070	3%	Brain & other nervous system	6,380	2%
All Sites	**312,150**	**100%**	**All Sites**	**277,280**	**100%**

Figure 2: Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States [29].

Need for New Natural Anticancer Leads

Increasing recurrence of mammalian tumors and severe side-effects of chemo-therapeutic 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 [30].

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 crisis for public health and health systems worldwide. Detailed analyses of pathways and mechanisms and structures of antitumor compounds have led to significant developments in the prevention and treatment of cancer. Establishment of tumor cell lines and analysis of the effect of many natural and synthetic antitumor compounds have achieved remarkable success. Despite their severe toxicity, chemotherapy, irradiation and immunotherapy are the gold standard approaches for the treatment of cancer worldwide [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 [34].

Important Secondary Metabolites for Discovery of New Anticancer Leads

a. 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 [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 et al. [37] reported the correlation between catechol-o-methyl transferase (*COMT*) allele, intake of tea and occurrences of cancer. They observed that women who possess at least one low-activity *COMT* allele had a reduction in breast cancer risk with intake of tea, but in the case of these homozygous for the high-activity *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 [37].

b. Saponins

Saponins, a class of bioactive compounds naturally presents in many plants, are a major family of secondary metabolites containing a sugar moiety glycosidically linked to a hydrophobic a glycone (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 (*Helleborus niger*), horse chestnuts (*Aesculus hipppcastanum*), asparagus fern (*Asparagus officinalis*), daisies (*Bellis perennis*), chickpeas, soybeans and alfalfa [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 (Fe2+), 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 *Balanites aegyptica* exhibited cytostatic activity against P-388 lymphocytic leukemia cultured cells [40].

A mixture of the steroidal saponins balanitin-6 and balanitin-7 (Bal 6/7), isolated from *B.aegyptiaca* kernels, demonstrated appreciable anticancer effects against A549 non-small cell lung cancer and U373 glioblastoma cell lines. Saponins from *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 [41].

The compounds SAP-1016 (3β-*O*-β-Dxylopyranosyl-(1-3)-β-D-glucopyranosyl-(1-4)-[α Lrhamnopyranosyl-(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 [42].

c. 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 [43,44].

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 [45].

d. **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 [46].

Ammopiptanthus mongolicus and its lipid, traditionally used in China have been shown to inhibit liver cancer. Ethyl acetate fractionated extracts of *Calligonum comosum* (Polygonaceae) demonstrated anticancer properties [47,48]. Terpinen-4-ol, sabinene, α- terpinene, and β-myrcene isolated from *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 [49] .

Haterumaimide J and K obtained from *Lissoclinum* sp. exhibited cytotoxicity against murine leukemia P388 cells. Dichlorolissoclimide, chlorolissoclimide from *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 *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 *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 [50,51].

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, *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 [52].

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 [53]. 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 [54]. 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 [55,56].

Betulinic acid is a common pentacyclic triterpenoid found in the species of genus *Betula* has proved a significant growth inhibitory agent [57]. Betulinic acid was also isolated from *Zizyphus* species, like *Zizyphus mauritiana, Zizyphusrugosa* and *Zizyphus oenoplia* and exhibited selective cytotoxic activity against human melanoma cell lines [57].

The efforts of National Cancer Institute to develop systemic and topical formulations for potential clinical

trials are ongoing. Phytochemical studies on roots of *Erythroxylum prvillei* (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 [58,59].

Silvesterol was isolated from the fruits of Meliaceae family plant *Aglaila sylvestre* exhibited cytotoxicity against breast and lung cancer cells [60]. 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 [61].

The following Table 2 represents the plants or weeds, where the recent anticancer leads were isolated.

Table 2: Plants reported for their anticancer activity.

Botanical Name of Plant With Family Name	Part Used	Parts Used and their Main Active Components
*Agave americana (*Agavaceae)	Leaf	Steroidal saponin, alkaloid, coumarin, isoflavonoid, hecogenin and vitamins (A, B, C)
Agropyron repens (Poaceae)	Rhizomes	Rhizome contains essential oil, polysaccharide and mucilage
Agrimonia pilosa (Rosaceae)	Herb	Agrimonolide, flavonoid, triterpene, tannin and coumarin
Ailanthus altissima (Simaroubaceae)	Bark	Triterpene, tannin, saponin and quercetin-3-glucoside
Akebia quinata (Lardizabalaceae)	Fruit	Flavonoid and saponin
Alpinia galanga (Zingiberaceae)	Rhizomes	Kaempferide and flavones
Aristolochia contorta (Aristolochiaceae)	Root and fruit	Lysicamine and oxaaporphine
Aster tataricus (Asteraceae)	Whole plant	Triterpene, monoterpene and epifriedelanol
Bryonia dioica (cucurbitaceae)	Root	Cucurbitacin and glycoside
Cannabis sativa (Cannabinaceae)	Leaf	Stereo isomers of cannabitriol
Chelidonium var. *asiaticum* (Papaveraceae)	Herb	Alkaloids (sanguinarine, chelerythrine, berberine)
*Chimaphila umbellate (*Ericaceae)	Whole plant	Ericolin, arbutin, urson and tannin
Coix lachryma jobi (Poaceae)	Seed	Trans-ferulyl stigmasterol
Dryopteris crassirhizoma (Polypodiaceae)	Rhizomes	Filicinic and filicic acids, aspidinol and aspidin
Echinops setifer (Asteraceae)	Whole plant	Echinopsine
Erythronium americanum (Liliaceae)	Whole plant	Alpha-methylenebutyrolactone
Euonymus alatus (Celastraceae)	Whole plant	Triterpene, euolatin, steroid and sesquiterpene alkaloid
Eupatorium cannabinum (Asteraceae)	Whole plant	Sesquiterpene, lactone, pyrrolizidine alkaloidand flavonoid
Fragaria vesca (Rosaceae)	Leaf and fruit	Flavonoid, tannin, borneol and ellagic acid Asia, Europe
Fritillaria thunbergii (Liliaceae)	Whole plant	Alkaloid and peimine
Galium aparine (Rubiaceae)	Cleaver	Iridoid, polyphenolic acid, tannin, anthraquinoneand flavonoid
Hydrastis canadensis (Ranunculaceae)	Whole plant	Isoquinoline alkaloids (hydrastine, berberine, berberastine, candaline), resin and lactone
Junchus effuses (Juncaceae)	Whole plant	tridecanone, effusol, juncanol, phenylpropanoid and a- tocopherol

Lantana camara (Verbenaceae)	Whole plant	Alkaloids (camerine, isocamerine, micranine, lantanine, lantadene)
Larrea tridentate (Zygophyllaceae)	Whole plant	Resins
Lonicera japonica (Caprifoliaceae)	Whole plant	Tannins, saponins and carotenoids
Olea europrae (Oleaceae)	Leaf and oil	Oleic acid and polyphenol
Panax quinquefolium (Araliaceae)	Roots	Ginsenoside, sesquiterpene, limonene vitamins (B_1, B_2, B_{12})
Phaleria macrocarpa	Fruits	Gallic acid
Polygonatum multiflorum (Liliaceae)	Whole plant	Saponin, flavonoid and vitamin A
Potentilla chinensis (Rolsaaceae)	Whole plant	Gallic acid and tannin
Pygeum africanum (Boraginaceae)	Bark	Phytosterol, triterpene and tannin
Rhus chinensis (Anacardiaceae)	Leaf	Apigenin and glycoside
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

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 [60].

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 in-flammation 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 [61].

Tumors as wounds

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 [62,63].

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 [64,65].

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 [66].

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]. 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 glioblastoma26 and breast cancer180 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 [68].

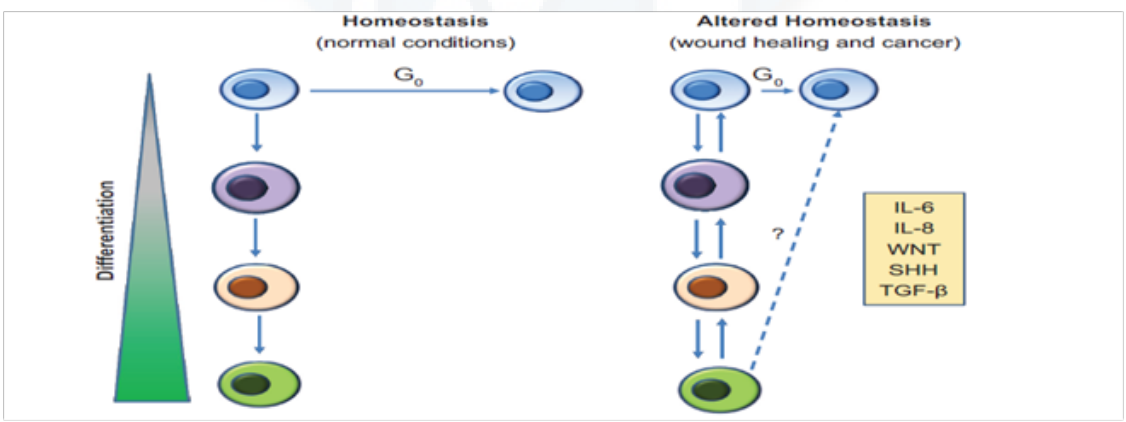


Figure 3: Cellular plasticity in normal vs altered homeostasis.

(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.

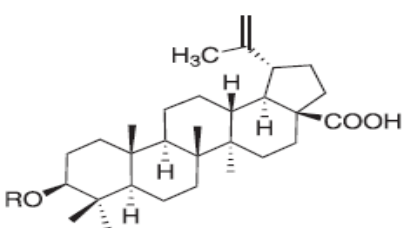
The effect of inflammatory signals within the microenvironment on the cellular plasticity of tumor cells has yet to be fully determined. Thus, it will be important to investigate whether blocking specific inflammatory signals, alone or in combination with other stem cell pathway inhibitors, can decrease 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 [68].

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 [69].

Examples of compounds with carbon skeletons different from the existing plant-derived drugs used in cancer chemotherapy will be discussed below, namely, betulinic acid, ceflatonine (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.

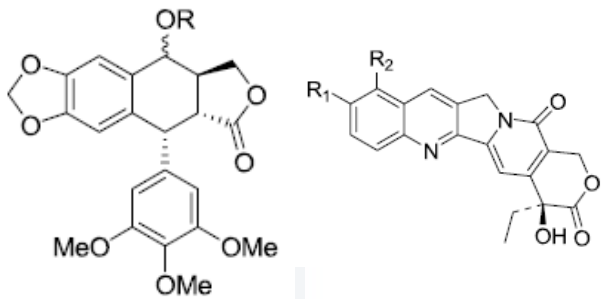
This compound was further developed under the Rapid Access to Intervention Development program of the United States National Cancer Institute, and is currently undergoing phase I/II clinical trials for treatment of dysplastic melanocytic nevi, a preliminary symptom that may lead to melanomas of the skin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



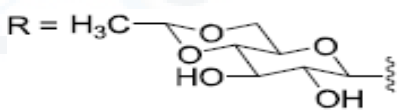
Betulinic acid, R=H

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 [72].

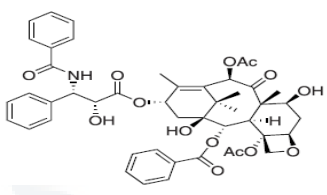
According to a recent review, of the 2255 cancer clinical trials recorded as of August 2003, (or 13.7 %) and (or 5.4 %) of the trials involved taxane- and camptothecin-derived drugs, respectively. In 2002, it was estimated that the combined sales of paclitaxel and docetaxel (both taxanes), and topotecan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



2. Podophyllotoxin, R= H 3. Camptothecin, R_1 = H, R_2 = H

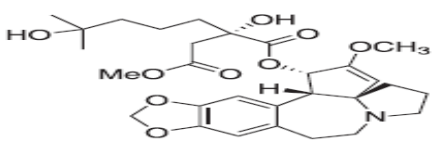


Etoposide



Topotecan, R_1 = OH, R_2 = $CH_2N(CH_3)_2$

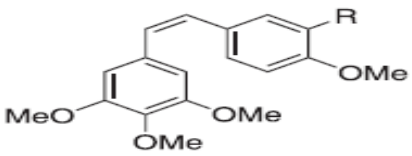
Ceflatonine a synthetic version of homoharringtonine produced by ChemGenex Pharmaceuticals (Menlo Park, CA, USA), is currently undergoing phase II/III clinical trials for the treatment of patients with chronic myeloid leukemia that is resistant to the first-line therapy [74].



Ceflatonine

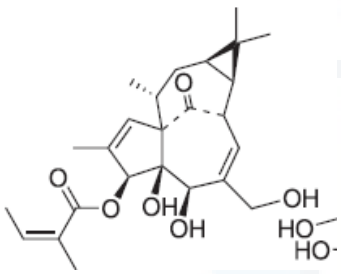
Combretastatin A4 phosphate (8, CA4P) is a disodium phosphate pro-drug of the natural stilbene combretastatin A4 (9) isolated from the South African tree *Combretum caffrum* Kuntze. CA4P is being developed by OXiGENE (Waltham, MA, USA) to treat anaplastic thyroid cancer in combination with other anticancer 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 [75,76].



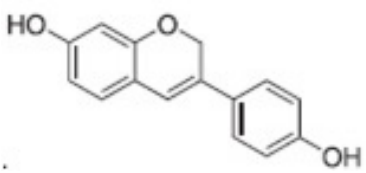
R = OPO_3Na_2, 9, R = OH

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 Kerasotes. 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 [77,78].



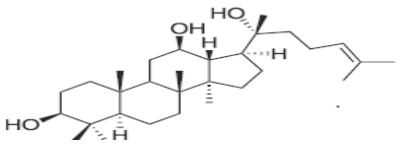
Ingenol 3-angelate

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 [79].



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 [80-82].



Protopanaxadiol

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 [83].

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 [84].

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 [85].

In instances where the active compound has a new structure, further isolation can be carried out from the plant material, provided there are 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 [86]. The total drug discovery process is a complex process and is explained in the following Figure 4.

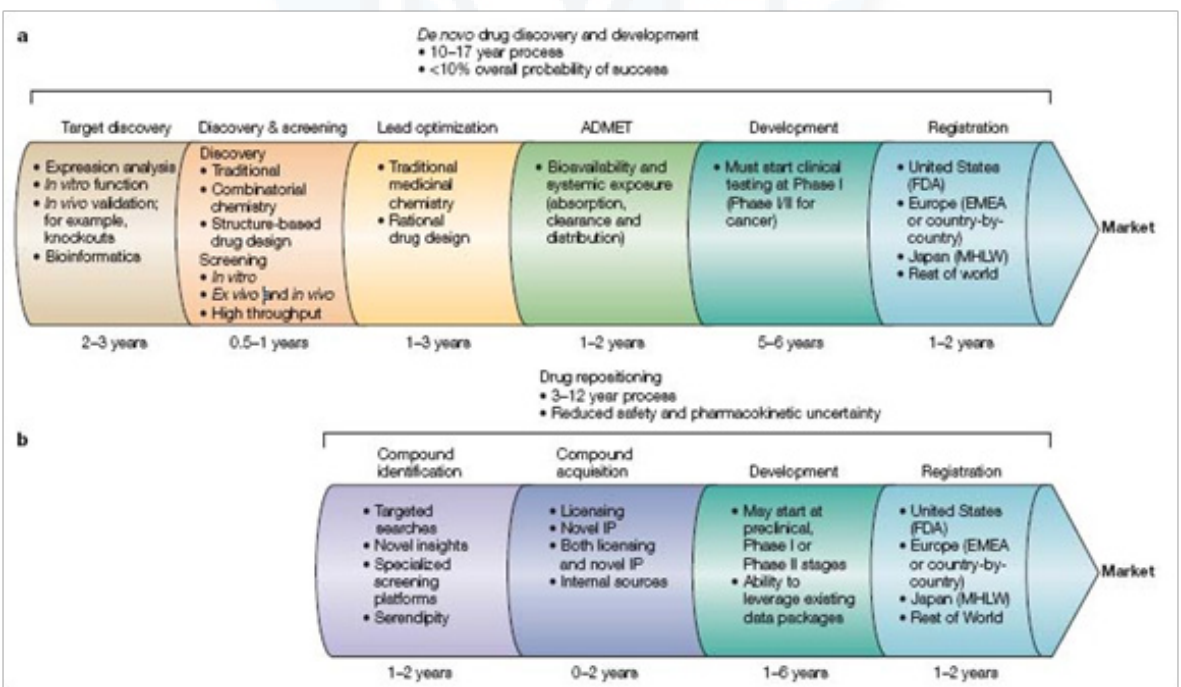


Figure 4: New Drug Discovery and Development process.

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 [87].

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

i. **Advantages of Flash chromatography (Table 3)**

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.

ii. **Column 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:

a. Slightly smaller silica gel particles (250-400 mesh) are used
b. 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 [88].

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- 63uM 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.

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).

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. 200mL/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 are of the chromatogram.

xi. Fractionate using auxiliary, non-UV based detection systems.

xii. Accurate TLC-to-gradient method building based on one TLC.

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

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 = Distance travelled by solute/ Distance travelled by solvent

$$\ddot{A}R_F = R_{F1} - R_{F2}$$

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.

$$CV = 1 / R_F$$

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

For a particular set of separation conditions, a weekly 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 R_F of 0.10 requires 10 column volumes for complete elution (Table 4).

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 [89].

How to start flash

a. The primary requirement for flash is TLC data.

b. Calculate the R_f from TLC (Thin layer Chromatography).

c. Calculate the CV for flash.

d. Transfer the TLC R_f to Flash by converting R_F to CV (Figure 5).

Table 3: Column chromatography vs Flash chromatography.

Column Chromatography	Flash Chromatography
Separation is very slow- flow based on gravitational force	Separation is very fast- flow based on pump
Manual Column packing- a tedious process-should not pack with air bubble- affect the resolution and separation- disposal is also difficult	Pre packed columns to improve the separation- plug and play- disposal is easy
Manually change the strength of solvents- collect the fraction manually- check the TLC for all the fraction	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.

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.

Natural Product Isolation [90]

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.

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.

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 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 1g, it is further separated by MPLC (medium Pressure HPLC) to yield compounds of very close chromatographic behavior. Some- times 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 0.1g) is subjected to a Recycling HPLC to separate or to purify into single pure compound. This is Step 3 of isolation.

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, and 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 3-4h. 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.

b. 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 you, make literature search as possible as much as possible: family, genus, species, and 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.

i. Preliminary work

Start your work with 100-200g 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.

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. [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.

a. 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).

[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.

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. 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.

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.

ii. **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.

iii. **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 [91].

Table 4: Conversion of R_F and CV.

RF	CV
0.90	1.10
0.70	1.40
0.50	2.00
0.30	3.33
0.10	10.0

Topoisomerases are ubiquitous enzymes that control DNA super coiling 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 [92-99].

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 topoisomerases are required for the treatment of cancer (Figure 7) [100-104].

The different catalytic mechanism of topoisomerases is explained in the Figures 8 & 9 and the challenges and discovery of new topoisomerases is given in the Table 5.

Therefore, the dual topo drugging is an important phenonomena 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 [105].

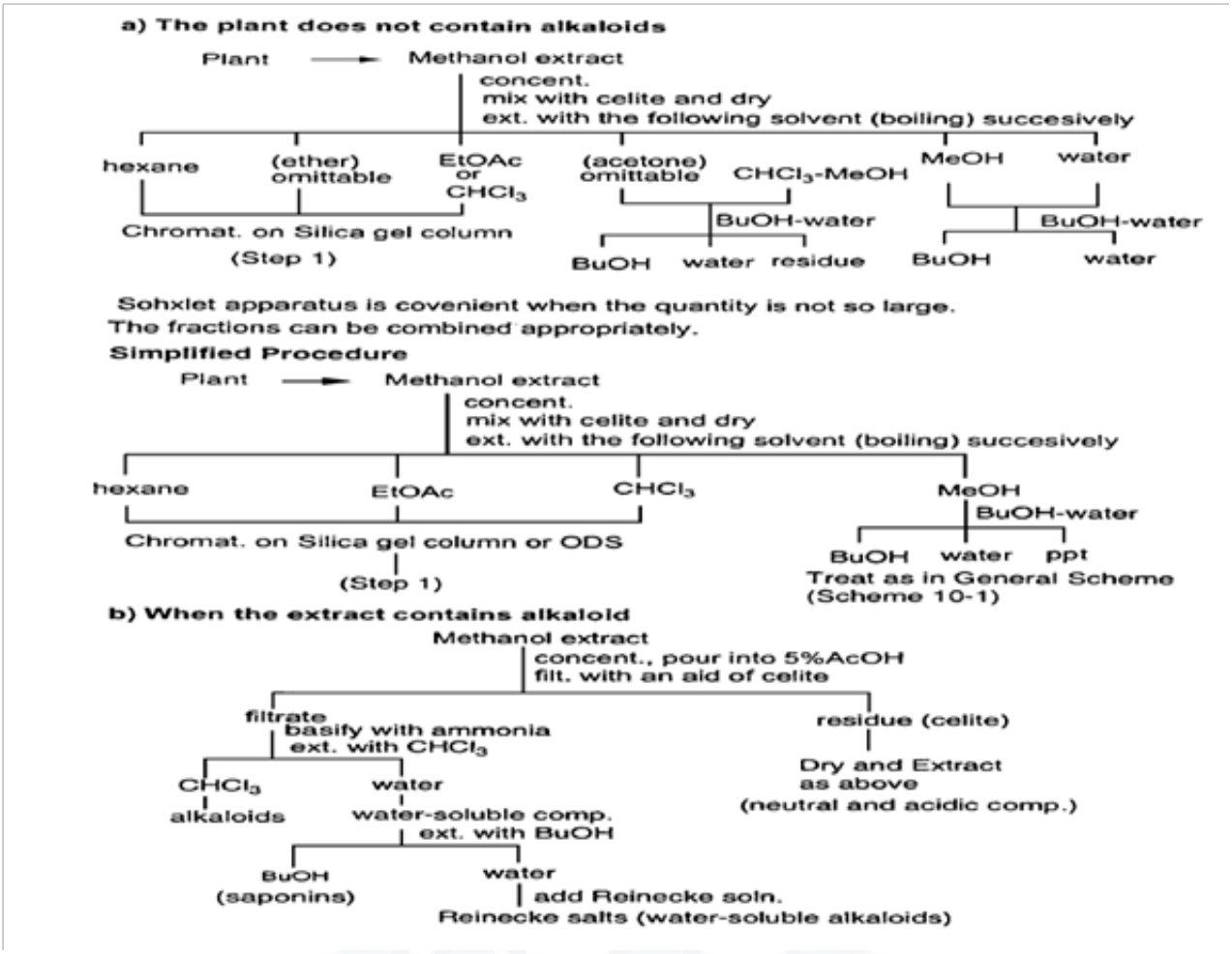


Figure 6: A General Scheme for isolation of secondary metabolites from plants.

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

Challenges	Possible Answers (New Approaches)
1. New topoisomerase targets	Type IA (TopA and Top3) inhibitors Top2β-specific inhibitors
2. New topoisomerase inhibitors (in addition to #1 above)	Chemically stable camptothecins Non-camptothecin Top1 inhibitors Top1 catalytic inhibitors New Top2 inhibitors with novel structures Orally bioavailable inhibitors Targeted delivery (nanoparticles for time-staggered and tumor-specific delivery)
3. Pharmacodynamic (PD) biomarkers to rapidly evaluate tumor drug response	Top1 and Top2 cleavage complexes induction Top1 and Top2 down-regulation DNA damage (γ-H2AX) Apoptotic response (caspase activation) 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

4. Cancer patient selection	Identification and implementation of predictive biomarkers and "drug response signatures" (based on OMIC tools: tumor gene expression and somatic mutations, proteomic and metabolomic) for patient stratification New predictive biomarkers based on molecular biology and 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

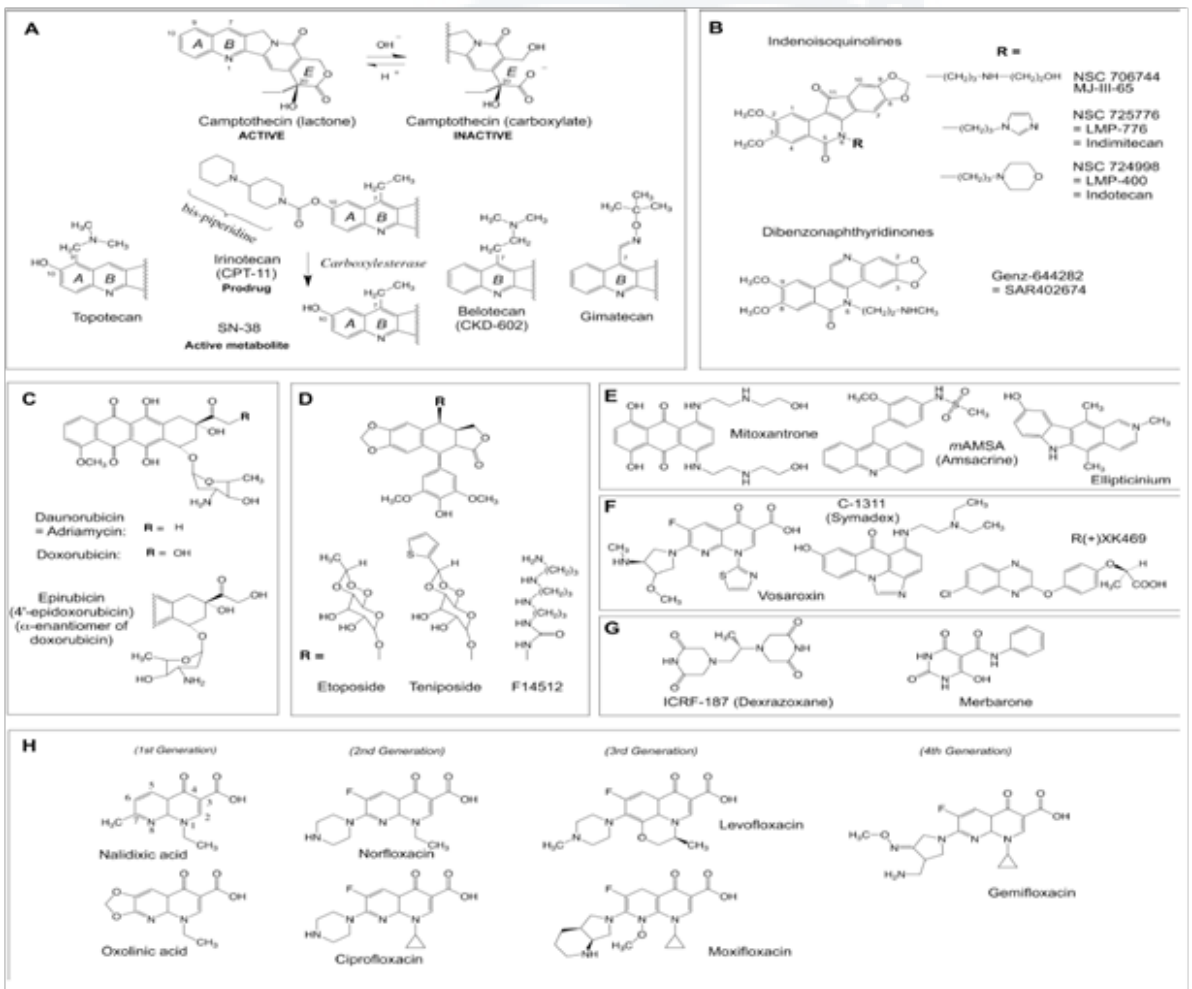


Figure 7: Structure of anticancer and antibacterial topoisomerase inhibitors.

A: Camptothecins. B: Non-camptothecin Top1 inhibitors in clinical trials. C: Anthracyclines. D: Demethylepipodophyllotoxin derivatives, including the clinical trial drug F14512 with its spermine side chain. E: Other Top2cc-targeted intercalative drugs. F: Three Top2cc-targeted drugs in clinical trials in addition to F14512 shown in panel D. G: Top2 catalytic inhibitors. G: Quinolone antibacterials.

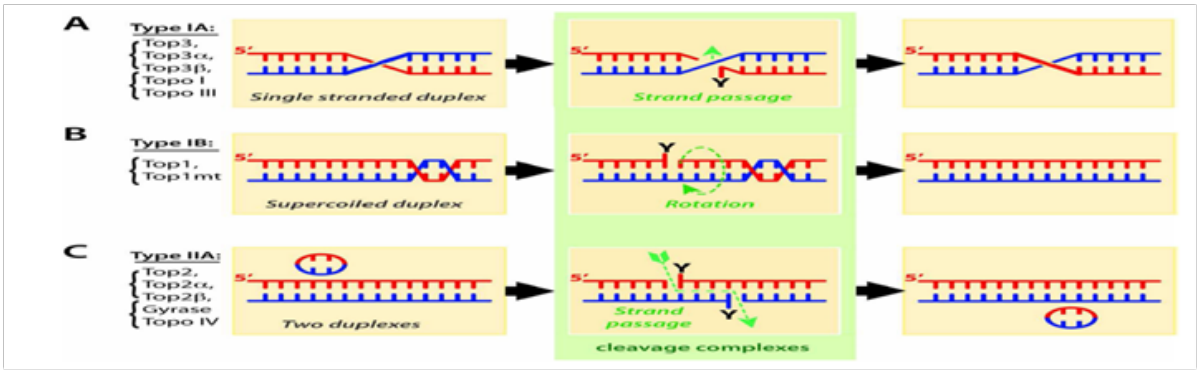


Figure 8: Differential catalytic mechanisms of topoisomerases.

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 super coiling by controlled rotation of the broken 5'-end around the intact strand.

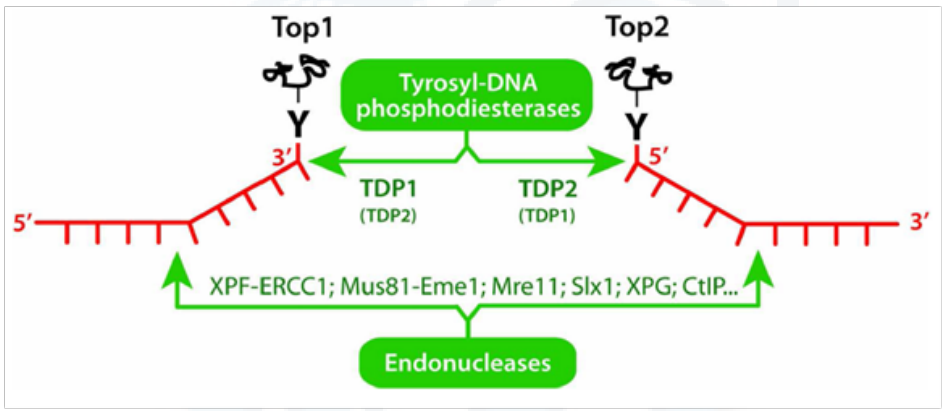


Figure 9: Schematic representation of the two main repairs pathways removing Topoisomerase-DNA complexes.

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 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 [106].

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 chemo types. Figure 10 illustrates the position of CADD in drug discovery pipeline [107].

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 [108].

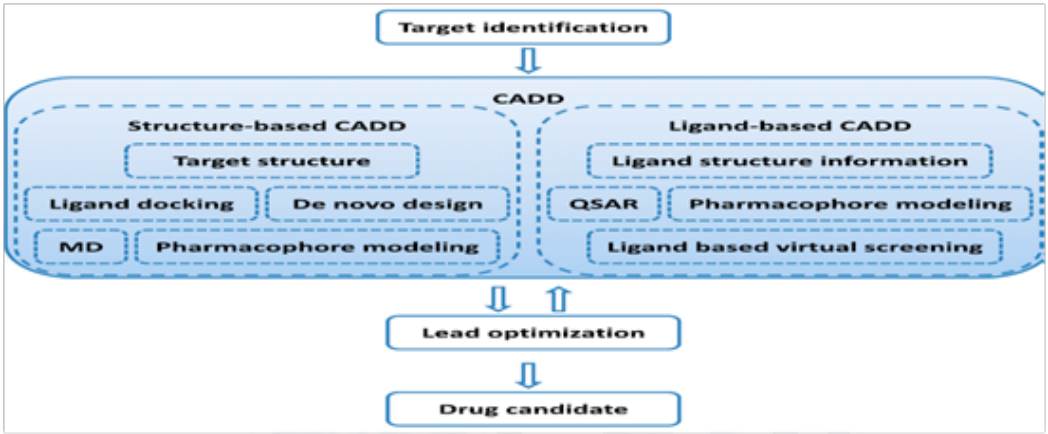


Figure 10: CADD in drug discovery/design pipeline.

b. **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 data bases 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 [109].

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. in the 2004 identification of novel HIV integrase inhibitors [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 non-covalent 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 [111].

c. **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 *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, 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 [112].

d. **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 [113].

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 [114].

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.

Review of Literature

I. Pharmacognostical reviews on selected plants

i. **Vijayalakshmi S et al. [115]:** Investigated on macro and microscopical characters of leaf and stem, powder microscopy, fluorescence analysis, leaf constants like vein islet number, vein termination number, stomatal number, stomatal index and study of physical constants like ash values, extractive values and preliminary phytochemical evaluation on the stems and leaves of *Cnicus wallichii* DC family (Asteraceae).

ii. **CCRH (Central Council for Research in Homeophaty), quarterly bulletin [116]:** Investigated first time the Pharmacognostical and histochemical characters of the stems of *Cytisus scoparius* Linn (Fabaceae).

II. Phytochemical reviews on selected plants

a. **Prasad MP et al. [117]:** The three *Cestrum species C. aurantiacum, C.nocturnum and C. diurnum* were investigated for phytochemicals and revealed presence of Alkaloids, Anthraquinones, Cardiac Glycosides, Carbohydrates, Flavonoids, Phenolic compound, Tannins and Terpenoids. Antioxidant potential was estimated by DPPH and FRAP assay and *C. diurnum* showed higher anti-oxidant potential than that of the other two species in both assays. Different solvents such as ethanol, methanol, butanol, propanol and acetone were used to extract the bioactive compounds from the leaves of these plant species.

b. **Vijayalakshmi S et al.** [118]**:** Investigated the antioxidant studies of *Anaphalis neelgerriana* DC and *Cnicus wallichi* DC belonging to the family Asteraceae found that the ethyl acetate extract of both plants showed significant antioxidant activity by DPPH, Nitric oxide and Hydrogen peroxide methods.

c. **Vijayalakshmi S et al.** [119]: Investigated the antimicrobial activity of seven different fractions of *Cnicus wallichi* DC belonging to Asteraceae and found that the Ethyl acetate fraction proved to have significant antioxidant and wound healing activity.

d. **Singh KN et al.** [120]**:** Isolated Isorhamnetin 7-glucoside and was characterized from *Cnicus wallichi*. This is the first report of this glycoside in the Compositae and the second in nature.

e. **Suzuki H et al.** [122]**:** Identified a novel O-tigloyltransferase enzyme from *Cystisus Scoparius* (Fabaceae) and is very important for quinolizidine alkaloid metabolism [123].

f. **Young NM et al.** [122]**:** Isolated three lectin fractions from seeds of the leguminous plant *Cytisus scoparius* (Scotch broom) by means of affinity chromatography on a N acetyl-D-galactosamine medium. The first fraction, termed CSIa, was equally well inhibited in haemagglutination experiments by D-galactose and by N-acetyl-D-galactosamine and consisted of a group of isolectins formed from closely related polypeptide chains of approx. Mr 30000. The second fraction, CSIb, was closely related to CSIa in specificity, c.d. and other properties. The third fraction contained a homo- geneous lectin, CSII, formed from subunits again of approx. Mr 30000. CSII was 100 times more readily inhibited by N- acetyl-D-galactosamine than by D-galactose. Despite the similarity in specificity, comparative studies of their amino acid composition, c.d. and N-terminal amino acid sequence showed that the CSIa and CSII lectins diverged considerably in structure. The lectin from *Cytisus sessilifolius*, specific for chitobiose, was also examined and resembled CSIa in composition and c.d. properties [122].

g. **Kang SS et al. [123]:** Two new monodesmosidic saponins (Phytolaccosides F and D(2)) have been isolated from the roots of *Phytolacca americana* and identified as 3- O-[alpha- L-rhamnopyranosyl(1-->2)-beta- D-glucopyranosyl (1-->2)- beta-D xylopyranosyl]phytolaccagenic acid and 3- O-[beta-D-glucopyranosyl(1-->2)- beta- D- xylopyranosyl] phytolaccagenic acid, respectively

III. Pharmacological reviews

i. **Vijayalakshmi S et al. [124]:** Anti-tumour study was carried out by short term toxicity studies using Dalton's Lymphoma Ascites (DLA) cells. The ethyl acetate extract of *Cnicus wallichi* DC showed significant activity.

ii. **Nirmal J et al. [125]:** Observed that methanolic extract of *Cytisus scoparius* showed anxiogenic and depressive behaviour in experimental rats with metabolic disturbance. Significant increase in lipid peroxidation level was observed in stressed rats. Administration of extract for 21 days during stress exposure significantly increased the ambulatory behaviour and decreased the freezing time in open field behavior. The extract also improved SOD, CAT, and ascorbic acid level and controlled the lipid peroxidation in different tissues.

iii. **Gowthamarajan K et al. [126]:** Observed that all the microorganisms used were sensitive to the *Cytisus scoparius* extracts. The minimum inhibitory concentrations (MIC) were 250µg/ml for *B. Subtilis* and 500µg/ml for all the other strains used. However, *in comparison* the ampicillin (MIC: 62.5µg/ml), and Amphotericin-B (MIC: 125µg/ml), the activity of the extract was less.

iv. **Das J et al. [127]:** Tested relative efficacy of the extract of *Phytolacca decandra* (PD) and its PLGA nano-encapsulated form (NPD) in mice intoxicated with benzo[a]pyrene (BaP) (25mg/kg b.w.) plus sodium-arsenite (SA) (10mg/kg b.w.) and on A549 lung cancer cells in vitro. We characterized nanoparticles by physico-chemical and morphological studies using dynamic light scattering, scanning electron and atomic force microscopies and also conducted FTIR and (1)H NMR studies to determine if NPD had a co-polymeric nature and analyzed drug-DNA interaction through circular dichroism spectra (CD) and melting temperature profiles (T(m)) taking calf thymus DNA as target. An oral dose of 0.3mg/kg b.w. for NPD and 30mg/kg b.w.for PD in mice showed chemopreventive effects in regard to DNA fragmentation, comet tail length and toxicity biomarkers like ROS generation, NFκβ, p53, PARP, CYP1A1 and caspase 3. NPD showed greater effects than that by PD. Results of in vivo studies showed similar effects on A549 in regard to cell viability, DAPI and PI staining, Comet tail length, DNA fragmentation. To further confirm the biological molecule present in PD we analyzed its chromatographic fraction through mass spectroscopy, NMR and FT-IR studies and characterized it to be a tri-terpenoid, a derivative of betulinic acid with a molecular formula C(30)H(46)O(2.) Thus, overall results suggest that nano-encapsulation of PD (NPD) increases drug bioavailability and thereby has a better chemo-preventive action against lung cancer in vivo and on A549 cells in vitro than that of PD.

v. **Bhattacharyya SS et al. [128]:** Prepared *Phytolacca Decandra* precipitated silver nanoparticles in ambient conditions. The nano-particles had 91 nm particle size, with polydispersity index of 0.119 and zeta potential of -15.6 mV. The nano particles showed anticancer and antibacterial properties, but no clear antifungal activity.

vi. **Lahiri SC et al. [129]:** Isolated the glycoalkaloid fraction from the whole plant of *Solanum mauritianum* and observed that the fraction is responsible for various pharmacological activities on CNS and Asthma.

vii. **Ochwangi DO et al. [130]:** Performed the ethno-medicinal survey in Kenya and found that the people uses aqueous decoction of *Solanum mauritianum* for the treatment of cancer and the proposed mechanism is may be due to prostaglandin inhibition.

viii. **Abraham z et al. [131]:** Explained the importance of *Erigeron Karvinkianus* aerial parts hydro-alcohol extract for its antibacterial activity especially on *Mycobacterium* species.

Weeds Profile

I. Cytisus scoparius

i. Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Cytisus

Species: *C. scoparius*

ii. **Synonyms**

Spartium scoparium (Linn.). *Genista scoparius* (Lam.). *Sarothamnus scoparius* (Koch). Broom Tops. Irish Tops. Basam. Bisom. Bizzom. Browme. Brum. Breeam. Green Broom.

iii. **Part used**

Tops.

iv. **Habitat**

The densely-growing Broom, a shrub indigenous to England and common in this country, grows wild all over temperate Europe and northern Asia, being found in abundance on sandy pastures and heaths. It is sparingly naturalized in sandy soil in North America. Plants of Cytisus scoparius typically grow to 1-3 m (3-9 ft) tall, rarely to 4m (13 ft), with main stems up to 5cm (2 in) thick, rarely 10cm (4 in). The shrubs have green shoots with small deciduous trifoliate leave 5-15mm long, and in spring and summer is covered in profuse golden yellow flowers 20-30 mm from top to bottom and 15-20 mm wide. Flowering occurs after 50-80 growing degree days. In late summer, its legumes (seed pods) mature black, 2-3 cm long, 8 mm broad and 2-3 mm thick; they burst open, often with an audible crack, forcibly throwing seed from the parent plant [132].

v. **Chemical constituents**

Spiraeoside, Scoparoside and Lupanine [120].

vi. **Traditional uses**

Jaundice [120]

II. Solanum mauritianum

a. **Classification**

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: Solanum

Species: *S. mauritianum*

b. **Synonyms**

Solanum auriculatum, Tobbaco bush weed.

c. **Part used**

Aerial Parts

d. **Habitat**

Solanum mauritianum Scop also known as *Solanum auriculatum* is a small tree or shrub native to South America & India which is commonly known as woolly nightshade, ear-leaved nightshade (or “ear leaf nightshade”), flannel weed, bug weed, tobacco weed, tobacco bush, wild tobacco and kerosene plant. The plant has a life of up to thirty years, and can grow up to 10m (33 ft) tall. Its large oval leaves are grey-green in color and covered with felt-like hairs. The flower is purple with a yellow center. The plant can flower year round but fruiting occurs in late spring to early summer. It is tolerant of many soil types and quickly becomes established around plantations, forest margins, scrub and open land [133].

e. **Chemical constituents**

Glycoalkaloid fraction [129]

f. **Traditional uses**

No claims

III. Erigeron karvinskianus

a. **Classification**

Kingdom: Plantae

Division: Magnoliophyta

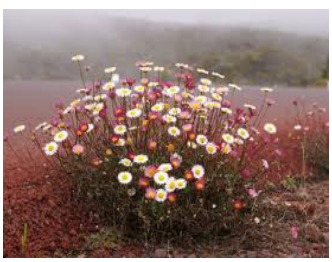
Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: Erigeron

Species: *E. karvinskianus*



b. **Synonyms**

Mexican fleabane, Latin American fleabane, Santa Barbara daisy or Spanish daisy

c. **Part used**

Whole Plant

d. **Habitat**

Erigeron Karvinkianus DC is also known as Mexican fleabane, Latin American fleabane, Santa Barbara daisy or Spanish Daisy is a Mexican flora and predominantly distributed all over the Nilgiris town. It is a perennial herbaceous plant belonging to the family Asteraceae, high 20-40 centimeters; it has prostrate stems and numerous erected branches that form pretty and compact pillows. It requires very little maintenance, a well-drained soil, a sunny location and temperatures that do not drop for a long time under 0 °C [134].

e. **Chemical constituents**

Not Reported

f. **Traditional uses**

Antimicrobial [131].

IV. *Phytolacca dodecandra*

a. **Classification**

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Caryophyllales

Family: Phytolaccaceae

Genus: Phytolacca

Species: *P. decandra*

b. **Synonyms**

African soap berry, West African: Ghana Akan-twi ahoro (auctt.) guinea: kissi fundelo un'do

c. **Part used**

Aerial Parts

d. **Habitat**

Phytolacca dodecandara is an evergreen weedy herb having brownish red colour fruits. also used as an ornamental plant in the Phytolaccaceae family. These terms supplant the Algonquian term for the plant, pocan, meaning "blood red." Its informal name, poke root or poke week, also descends from the Algonquin. It is used in herbalism to stimulate the flow of lymph and is emetic in anything other than small doses [135].

e. **Chemical constituents**

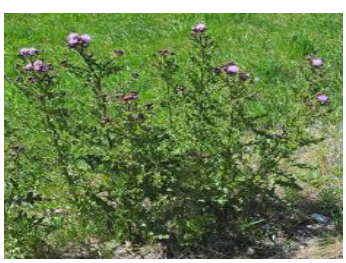
Phytolaccosides [123]

f. **Traditional uses**

Vermifuge, dye and laxative [135]

V. *Cnicus wallichi*

a. **Classification**



Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Asterales

Family: Asteraceae

Genus: Cnicus

Species: C. wallichi

b. **Synonyms**

Wallich's Thistle, Hindi: Bungsee, Nepali: थकाल Thakal, kanta

c. **Parts used**

Aerial Parts

d. **Habitat**

Wallich's Thistle is an extremely variable plant, 4-10 ft tall, with spreading branches. White to purplish-white flower-heads, clustered or solitary, are borne on leafless stalks, or are stalkless. They are 2-3.8 cm across, with florets about 1.6 cm. There are lanceshaped bracts ending in erect or recurved spines. Stalkless leaves are pinnately lobed, with margins having very long, stout spines. Leaves are hairless above and cottony beneath. Stems are hairy and leafy. Wallich's Thistle is found in the Himalayas, from Afghanistan to SW China, at altitudes of 1200-3300 m.

e. **Flowering**

May-August [124].

f. Chemical constituents

Isorhamnetin 7-glucoside [120].

g. **Traditional uses**

Wound healing activity [119].

VI. *Cestrum aurantiacum*

a. Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus: Cestrum

Species: *C. aurantiacum*

b. **Synonyms**

Orange Cestrum, Yellow Cestrum, Yellow Shrub Jessamine.

c. **Parts used**

Aerial Parts

d. **Habitat**

Native to North and South America, Orange Cestrum is an evergreen, half-climbing shrub reaching 10 ft tall. Leaves are single, alternately arranged, dark green with lighter midrib. They are lance like to ovate in shape, with entire margin. Stems and leaves bruise easily, emitting an unpleasant smell. The plant sports clusters of tubular dark

yellow or orange flowers, which look very much like Night Blooming Jasmine. The flowers have an overpowering citrus-like smell, particularly at night. Flowers attract butterflies. The flowers are followed by white berries [117].

e. **Chemical constituents**

Phenolics [117]

f. **Traditional uses**

Wound healing [117].

Materials and Methods

Materials

a. **Chemicals**: Glacial acetic acid, Bromo-cresol green, Phosphate buffer (7.4) was procured from S D Fine - Chem Limited, Mumbai, India. Acetone AR, Ethanol (99.5 %) AR, N- Butanol AR, Chloroform AR, Dichloromethane AR, Ethyl acetate AR, Methanol AR, Conc. Hydrochloric acid, Petroleum ether (60^0 - 80^0) AR, Potassium Iodide , Iodine, Picric acid, Strong Ammonia solution AR, Conc. Sulphuric acid, Sodium hydroxide, Sodium chloride were procured from RANKEM, Gurgaon, India. Ascorbic acid, Fehlings solution A and B, Silica gel 60 GF_{254} Pre-coated Aluminum plates, Silica gel for column chromatography 230-400 were procured from Merck Life Science Private Limited, Mumbai, India. Mayer's reagent, Lead acetate, Pyridine, Sodium Nitro-prusside, Millon's reagent, Folin-ciocalteu reagent, Sodium Carbonate, Aluminum chloride, Tri-chloro acetic acid, Glutaraldehyde, crystal violete were purchased from LOBA Chemie, Mumbai, India. Minimal Essential Medium, L-glutamine, Fetal Bovine Serum, Dulbecco's modified eagle medium (DMEM), Phosphate buffered saline (PBS) was procured from Hi-media, Mumbai, India. Standard Atropine, Standard Gallic acid, Standard Quercetin, Standard Rutin, 2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) assay kit, 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH), P-iodo nitro tetrazolium violet, Sulpharhodamine B, Sephadex LH-20, standard lead and cadmium, Penicillin, amphotericin B, 1XPBS, 1X Trypsin, Rnase, PI (were procured from Sigma-Aldrich, Mumbai, India. A-549, MCF-7, HCT-119, HELA Vero & Human HDF cell lines were procured NCCS, Pune, India, *Staphylococcus aureus* (MCC 2408) was procured from National Center for Cell Science, Pune, India and the topoisomerase assay kits was procured from TopoGEN, Inc., USA, and DMEM media was procured from (Gibco, Mumbai, India).

b. **Instruments**

S.No	Name of the Instrument	Make/Model
1.	Flash Chromatography	Biotage, Isolera one
2.	Rotary Evaporator	Buchi, R-120
3.	HPTLC	Camag, Linomat-IV
4.	Auto Analyser	Merck, Microlab200
5.	Electron Microscope	Nikon, Photo lab 2
6.	Atomic Absorption Spectroscopy	Shimadzu, AA 6300
7.	UV-Spectrophotometer	Perkin Elmer, Lambda-5
8.	LCMS	Shimadzu, SCL-10A
9.	Elisa, Micro plate Reader	Bio-rad, 550
10.	Schrödinger Docking software	Schrodinger 9.9/2014-3 suite
11.	Flow cytometer	FACS, Aria-II

Methods

A. **Field survey:** A field survey was carried out in and around the Nilgiris with the help of Dr. S.Rajan (Field botanist), CCRAH, Department of AYUSH, Survey of Medicinal Plants and Collection Unit, Indira Nagar, Emerald, in-order to trace out the important distributed weeds having traditional claims.

B. **Pharmacognostical studies**

i. **Microscopical authentication:** The weed material (Whole plant & aerial parts) used in the present study was collected from Udhagamandalam in June 2013, The Niligiris district, Tamilnadu, India and was authenticated by the field taxonomist Dr. S. Rajan, Survey of Medicinal Plants and collection unit, Department of Ayush, Emarald. The Microscopical studies of the collected fresh material (leaf and stem) were carried out at Plant Anatomy Research Centre, Chennai, Tamilnadu. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations of bright field was used [136,137].

ii. **Determination of physicochemical constants**

a. **Ash values:** The ash remaining after ignition of medicinal plant materials is determined by three different methods, which measure total ash, acid-insoluble ash and water-soluble ash followed by the procedure explained by W.H.O. guidelines of standardization [138].

i. Total ash - Physiological (Plant tissue) & Non physiological (sand/soil)

ii. Water soluble ash.

iii. Acid insoluble ash and

iv. Sulphated ash

The total ash value method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

iii. **Significance**

i. Find out the excess calcium oxalate crystals present in a drug.

ii. To find out adulteration and determine the silica impurities admixed with the drug.

iii. Extractive values

This technique determines the amount of active constituents in a drug when extracted with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used.

iv. **Types of extractive values**

a. Water soluble extractive

b. Alcohol soluble extractive

c. Ether soluble extractive

d. Hexane soluble extractive

v. **Significance**

a. It is used to give preliminary information on quality of drugs.

b. Water-soluble extractive gives information about the drugs containing tannins, sugars and plant acids.

c. Alcohol soluble extractive gives information about the drugs containing resins and tannins.

d. Ether soluble extractive gives information about the drugs containing fixed oils and coloring matter presents.

e. Hexane soluble extractive gives information about the drugs containing fatty materials [136,137].

C. **Heavy metal analysis**

Heavy metal analysis was carried out by using flame atomic absorption spectroscopy (SHIMADZU AA 6300) instrumentation and method followed was standard calibration method for estimation of mercury and cadmium levels in the crude drugs.

a. **Sample preparation:** 5g of air dried powder from each weed sample was placed in dried crucibles and subjected to incineration and converted in to ashes through muffle furnace at 650 **°C**. The obtained ashes were treated with 10 ml of 6 M nitric acid and evaporated on hot plate until it became half, the resulting saluting was filtered in to 25 ml volumetric and make up the volume with double distilled water. The obtained ash suspension was then filtered through Whatman filter paper No. 41.

b. **Standard preparation:** Cadmium and Lead were used as standards and prepared a calibration curve using various concentrations ranging from 1 to 15ppm [138,139].

c. **Extraction:** 500 g of finely powdered of selected six weeds were subjected to triple maceration using 2 L of 70% v/v of hydro-alcohol as menstrum for 72 hours. The obtained filtrates were pooled together individually and were filtered through vacuum. The obtained filtrates were subjected to Buchi rotary evaporation (R-210). The concentrated extracts were dried in a vaccum desiccator until constant weight was obtained. The percentage yield was calculated for each weed extract [140,141].

d. **Qualitative phytochemical screening:** The preliminary qualitative phytochemical screening was performed for the extracts and prepared fractions based on standard protocols [142,143]. The presence of various secondary metabolites was determined in this method.

e. **Quantitative phytochemical screening**

a) **Sample preparation:** 1mg/ml of sample extract was prepared by using double distilled water and used for the total phenol content and total flavanoid content.

b) **Total phenol content:** The total phenolic content of the dry crude drug was determined with Folin- Ciocaletau assay. An aliquot (1ml) of sample or a standard solution of gallic acid (10, 20, 40, 60, 80 and 100 Mg/l) was added to a 25ml volumetric flask,1ml of 1mg/ml of sample and standard was added to 10 ml volumetric flask , to this 1 ml of 20% sodium carbonate was added , then 1 ml of Phenol -FC reagent was added, then diluted with 7 ml of double distilled water and incubated for 3 hours at 37 **°C** . A reagent blank was prepared and the absorbance was measured at 765nm. Based on the standard calibration curves, the amount of phenol was calculated [144].

c) **Total flavonoid content:** The total flavanoid content was measured with an aluminum chloride colorimetric assay. An aliquot (1ml) of extracts or a standard solution of standard quercitin (10, 20, 40, 80 and 100mg/l) was added to a 10ml volumetric flask, containing 4 ml of distilled deionised water. To the flask was added 0.3ml of 5% sodium nitrite. After 5 min, 0.3% Alcl3 was added. To this 2ml of 1 M NaoH was added and volume was made up to 10 ml with double distilled water. A blank was prepared and absorbance was measured at 510 nm [145].

d) **Total alkaloid content**

A. **Sample preparation**

0.5 g of extract was hydrolyzed with conc. HcL and was filtered. 1 ml of this filterate was fractionated with 10 ml of chloroform and was adjusted to neutral with 0.1 N NaoH. Then 5 ml of phosphate buffer of P^H 7.4 and 5 mL of bromo cresol green solution was taken and the mixture was shaken with 10 ml of chloroform (1, 2, 3 and 4 ml chloroform) and diluted to 10 ml with chloroform.

B. **Preparation of standard curve**

An aliquot (1ml) of extracts or a standard solution of Atropine (10, 20, 40, 60, 80 and 100 µg/ml) was added to a separating funnel individually, then 5 ml of PH 4.7 phosphate

buffer and 5 ml of BCG solution was taken and the mixture was shaken with 10 ml (1,2,3 and 4 ml) of chloroform and then diluted to 10ml with chloroform absorbance of the complex in chloroform was measured at spectrum of 470 nm [146].

C. **Liquid-Liquid fractionation**

20g of above obtained extract was dissolved in 50 ml of double distilled water and subjected to liquid-liquid fractionation using different solvents like 200 ml of n-hexane, 500 ml of chloroform, and 500 ml of ethyl acetate. The obtained filtrate was subjected to vacuum distillation using Buchi Rotavap (R-210) and the percentage yield of obtaining fractions was calculated on dried basis [147,148].

D. **TLC Finger printing of 2 °C metabolites from various fractions**

a. TLC of fractions containing cardiac glycosides: 1g of substance was treated with 3mL of 50% ethanol, sonicated for 10 minutes and add 10mL of 10% lead acetate solution, cool it and filter, then the solution was gently shake with 1.5mL of dichloromethane, The dichloro methane fraction was dissolved in 0.1mL of dichloromethane/iso-propanol(3:2) and 10µl of sample was applied on TLC plate, using Silica gel $60GF_{254}$ pre-coated aluminum plates as stationary phase and Ethyl acetate : Methanol : Water (100:13.5:10) as mobile phase [149,150].

b. TLC of fractions containing flavanoids: 5mL of hydro-alcohol extract is concentrated to about 2mL to this add 1mL of water and 10mL of ethyl acetate is added. The ethyl acetate layer was concentrated and used for TLC Analysis after defatting with 1 ml of petroleum ether and 10µl of sample was applied on TLC plate. , using Silica gel $60GF_{254}$ pre-coated aluminum plates as stationary phase and Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) as a mobile phase [151].

c. TLC of fractions containing alkaloids: 1g of substance is treated with 1 ml of 10% sodium carbonated and then extracted with 6 ml of chloroform and the chloroform residuals was subjected to thin layer chromatography and 10ul of sample was applied on TLC plate and finger print was developed using Ethyl acetate: Methanol: Water (100: 13.5:10) as a mobile phase [151].

d. Isolation of total saponin fractions from the selected weeds: The total saponin content was isolated from the 100 g of crude drug using the gravimetric separation technique. 100g sample crude drug powder with 90% v/v methanol (500ml) and was refluxed for half an hour. The residue was extracted two more times by 500 ml methanol. The methanol extract was combined and distilled off the solvent. The soft extract left after distillation of alcohol, was refluxed with petroleum ether 60-80 °C, 500 ml for half an hour. The solvent was removed by decantation. The same soft extract was treated successively with chloroform 500ml and ethyl acetate 500ml and the solvents poured out after cooling, the soft extract was kept in the same flask. The soft extract (after three extractions cited above) was dissolved in 500 ml of butanol and was Filtered and concentrated. To this 500ml of acetone was added in order to precipitate the saponins. The precipitates are filtered, collected and dried to a constant weight at 105 **°C** [152-153].

e. LCMS and HPTLC finger printing of total saponin fractions

f. LCMS finger printing of total saponin fractions: Shimadzu HPLC, Japan with system controller (SCL -10A), twin pump (LC-10AT VP), UV-Vis detector (SPD-10A VP) and rheodyne injector with 100µl injection loop and the separation was done using lichrosorb micro bondapack C-18 Column (Merck). The data processing was done by using shimadzu HPLC Software class -VP (V5.03). The LC-MS experiment was carried out on a micromass quadro II triple quadrupole mass spectrometer. And the ESI capillary was set at 3.5 KV and the voltage was 40 V. The total positive mode spectrum and negative mode spectrum was carried out and the identity of saponins in fractions was done by the known mass values which was detected based on mass ion peaks [153,154].

g. HPTLC finger printing of total saponin fractions: 1g of the isolated fraction was dissolved in 5ml of methanol and evaporated to about 1ml, mixed with 0.5 ml water and then extracted with 3ml of n-Butanol. The n-Butanol layer was collected and evaporated used for TLC analysis by using 20µl as sample was applied on aluminum pre-coated silica gel 60 GF 254 TLC plate using Chloroform : Methanol : Water (65 : 35 : 10) and the eluted saponin spots were identified under visible light & 366 nm in a TLC Visualiser (CAMAG) [155].

h. Isolation of 2 °C metabolites using flash chromatography

i. Isolera flash chromatography system: Isolera flash chromatography system having a touch screen display, which is a solvent-resistant, color LCD screen with a resolution of 800 x 600 pixels. It serves both as a display and as the system's input device via on-screen touch controls. A fraction collector, which collects fractions into a wide variety of collection racks and vessels. A pump module, which directs the liquid flow through the system. A default flow rate is specified for each cartridge but, if desired, the flow rate can be changed. If the flow rate is increased, the system will start the run at the default flow rate and then regulate towards the flow rate defined in the method. Note that the system regulates on both flow rate and pressure. If 90% of the maximum allowed pressure is reached before the defined flow rate, the flow rate at 90% pressure will be used. The different fractions can be collected through an automated fraction collector based on the R_f the compound of interest can be identified and pooled together after performing thin layer chromatographic analysis [156,157].

ii. Isolation of flavanoids (Compound-I & Compound-III) from *Erigeron Karvinskianus* ethyl acetate fraction using flash chromatography system)

A novel method for isolation of flavanoids was developed

using flash chromatography system of 1.0 g of ethyl acetate fraction obtained above was directly applied on 10g samplet and was dried under vacuum in a rotary evaporator (Buchi R 120).The dried samplet was packed in 50g KPSil Biotage SNAP cartridge. A gradient flash chromatography method was developed based on blank method. A constant flow rate 50 ml/min of mobile phase (N-butanol: glacial acetic acid: water) is used. A total no of 70 fractions, each 18 ml was collected in different test tubes at the wave length of 366nm. Each individual fraction was subjected to TLC analysis and pooled together. Fraction No 5-22. The pooled fractions were subjected to rota-vap evaporation and a brownish yellow powder was obtained and washed with pet -ether 40-60 **°C** and air dried leaving a 63mg of yellow crystals (Compound-I). 1µg/ml of sample solution was subjected to TLC analysis using Toluene: Ethyl acetate (2: 6).

The fraction numbers 34-44 were pooled together and evaporated under vaccum using a rotary evaporator and the obtained soft brownish mass was precipitated using acetone leaving a white color powder of 20 mg (Compound-III). 1µg/ml of sample solution was subjected to TLC analysis using n- Hexane: Acetone (60: 40).

iii. Isolation of compound-IV from *Cytisus scoparius* ethyl acetate fraction using flash chromatography system

5g of the 70% hydro-ethanol extract was dissolved in 20mL of water and sonicated for 25 minutes. The obtained mixture was filtered under vacuum and 10mL of filtrate was partitioned with 60mL of ethyl acetate (3 x 20mL). The Ethyl acetate fractions were pooled together and evaporated under Buchi rotavap (R210) leaving a brownish mass. 500mg of the dried powder was directly applied on 10g samplet and samplet was dried under vacuum in rotary evaporator (Buchi R 120).The dried samplet was packed in 50g KPSil Biotage SNAP Cartridge filled tightly or compactly with 50g of Sephadex gel (LH-20). A gradient flash chromatography method was developed based on blank method. A constant flow rate 50ml/min of mobile phase (Water: Methanol) is used. The creamy mass is acidified with 1N sulphuric acid and dissolved in 2mL of hot water and on evaporation leaving 30 mg of white crystals.

iv. Isolation of compound- II from *Solanum mauritianum* aerial parts using flash chromatography

5g of 70%v/v hydro-alcohol extract was dissolved in 50mL of water and sonicated for 25 minutes and filtered through whatman filter paper. The filtrate was partitioned with 90mL of chloroform 9 (3 X 30) mL and pooled together. The chloroform fraction was partitioned with 180mL of n-Butanol (3x 60) mL and pooled together. The obtained n-Butanol fraction was concentrated to 20mL under vaccum using rotavap (R-120). The n-Butanol concentrate was precipitated with 3mL of acetone added drop wise. The precipitate was dried to get 1gm of dried brown glittery flakes.

1gm of flakes subjected to flash chromatography directly applied to 10 gm samplet and was dried. A constant flow rate of 50mL/min of mobile phase (n-Butanol: acetic acid: water) is used. A total no of 70 fractions each 16mL was collected in different test tubes at wave length 366 nm. Based on TLC profile, the fractions 21-24 were pooled together, concentrated and crystalised with 0.5mL of diethyl ether to get buff colour crystals and was characterized by using TLC, IR, NMR and MASS analysis.

v. Characterization of isolated molecules

The isolated compounds were identified by phytochemical test, melting point; TLC analysis and structural elucidation were characterized by using IR, NMR & LC-MS Spectral studies.

vi. *In-vitro* antioxidant studies

vii. DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging activity

1ml of test solution was added to 1 ml of DPPH in methanol (0.33%) incubated for 30 minutes at 37 **°C** the absorbance at 518nm was measured using UV spectrophotometer and corresponding blanks were taken [158].

viii. ABTS(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay

The ABTS free radical scavenging activity was carried out using procedure Trolox assay kit method [159]. 10µL of test drug was mixed with 1 ml of diluted $ABTS.^{+}$ solution and was incubated at 37 **°C** for 45 minutes. The absorbance was measured at 734 nm. A calibration curve was made with the given trolox standard and the antioxidant activity was measured and was expressed in microgram/ml of extracts [159].

ix. *In- vitro* cyto-toxic studies using sulphorhodamine b (srb) assay

In- vitro cyto-toxic studies were carried on the isolated compounds and fractions based up on the standard protocol and compared with standard quercetin. The cell cultures used in this research were procured from National Center for Cell Sciences, Pune, India. The cells were grown in Earls Minimal Essential Medium supplemented with 2mmol L-glutamine, 10 % Fetal Bovine Serum, Penicillin (100 µg/mL) and amphotericin B (5µg/mL) and the cells were maintained at 37^{0}C in a humidified atmosphere with 5 % CO2 and subculture twice a week.

The sulphorhodamine B assay measures whole cellular protein content, which is proportional to the cell number. Cell cultures are stained with a protein staining dye, sulphoramide B (SRB). SRB is a bright pink anionic dye that binds to basic amino acids of cells. Unbound amino acid is then removed by washing with acetic acid, and protein bound dye extracted using unbuffered Tris base for determination of optical density in a computer interfaced 96- well micro titer plate reader. Since dead cells either lyse or are lost during the procedure, the amount of SRB binding is proportional to the number of live cells left in a culture

after drug exposure. 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. 100µl of different drug dilutions (1000 to 15.6µg/ml) were added to the cells in microtitre plates containing monolayers. The plates were then incubated at 37 **°C** for 3 days in 5% CO2 atmosphere, and microscopic examination was recorded every 24 hours and After 72 hours, 25µl of 50% trichloro acetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form an overall concentration of 10%. The plates were incubated at 4 **°C** for one hour. The air diried plates were stained with 0.4 % of SRB dye prepared in acetic acid for 30 minutes. The absorbance was calculated by using Elisa Reader at a wave length of 540 nm [160].

x. *In-vitro* wound healing studies

The Human Dermal Fibroblast cell lines (HDF) were grown in Dulbecco's modified eagle medium supplemented with 10% Fetal bovine serum (FBS) medium, then seed the cells into 24-well tissue culture plate at a density that after 24h of growth and should reach ~70-80% confluence as a monolayer. Scratch the monolayer with a new 1mL pipette tip across the center of the well. Replenish the well with fresh medium and Grow cells for additional 48 h (or the time required if different cells are used). The cells were washed twice with 1x PBS, then fix the cells with 3.7% Para formaldehyde for 30 min. The fixed cells were stained with 1% crystal violet in 2% ethanol for 30 min and photos were taken for the stained monolayer on a microscope [161,162].

Xi. TLC based direct Bio-autography against Methicillin resistant *Staphylococcus aureus* (MCC 2408)

10µl of (1mg/ml) of isolated compounds were loaded onto TLC plates and eluted using the developed mobile solvent systems .The developed plates were dried under a stream of fast moving air to remove traces of solvent on the plates. The densities of bacterial organism used for *S. aureus* were approximately d 3 $\times 10^{12}$ cfu/ml, respectively. The prepared chromatograms were sprayed with the fungal or bacterial suspension until wet then sprayed with a 2 mg/ml solution of p-iodonitrotetrazolium violet. White bands represent the inhibition growth of microbes [163,164].

xi. *In-silico* molecular docking studies

The molecular docking studies were carried out using the X-ray crystal structures of Human topo isomerases I (PDB ID: 1K4T at 2.10 A°, PDB ID: 3AL2 at 2.0 A°) and Human topo isomerases II (PDB ID: 1ZXN at 2.51 A°, PDB ID: 3QX3 at 2.16 A°). The docking studies were carried out between the selected isomerase proteins and energetically minimized isolated compounds using GLIDE Program (Grid Based Ligand Docking from Energetics from Schrodinger 9.9/2014-3 suite). The Epik studies were carried out for the selected compounds using the same software in order to know their pKa values. The ADMET studies were carried out using QikProp43-44. The binding free energy of inhibitors in the catalytic domain enzyme (1K4T) was calculated by Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) VSGB 2.0 method. The calculated inhibitor-enzyme complex was relaxed using the local optimization feature in Prime, version 4.1, Schrödinger 2014, and the energies of the system were calculated using the VSGB 2.0 (solvation model) method available in Schrödinger suite 2014 (Schrodinger 9.9/2014-3 suite) [165,166].

xii. Topo drugging studies (Human Topoisomerases I & II)/ Topoisomerase I & II inhibition assay

The compound -2 (Tetrahydro-2- (hydroxymethyl)-6-(octadecahydro-2,7,10a-trimethyl-1-propylchrysen-8-yloxy)-2HPyran- 3,4,5-triol and compound -4 (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate) was subjected to topo-isomerase- I&II inhibition assay.

Stock of the compound was prepared at a concentration of 1mg/mL in water. The Corresponding sub-stocks were prepared such that addition of 5ul of the stock given a working concentration of 50, 100, 200 and 400µg/mL for Topo-I& 100,250& 400µg/mL for Topo-II. The compounds (different concentrations) were incubated with 1unit of topo1 enzyme and 4 units of topo-2 for 15 min at room temperature. To this 0.5ug of pBR329 was added and the volume was made up to 20ul with relaxation buffer and kept at 37 **°C** for 30 min and the relaxation was terminated by adding 5µl of loading buffer. The reaction was analysed in 0.8% agarose gel prepared and the gel was run at 120V for 2hrs, then the gel was stained with Ethidium Bromide solution for 1hr and destained with water overnight and photo was taken [166].

xiii. Cell cycle analysis

Cell lines were washed with 1X PBS and trypsinised using 1X Trypsin. The Cells were gently suspended in DMEM Media and centrifuged at 3000rpm for 5 minutes at 4 **°C**. The supernatant was discarded and the pellet was re suspended in 1ml 1X PBS. Then, it is centrifuged at 3000rpm for 5 minutes at 4 **°C**. The supernatant was discarded. Add 1ml ice cold 1X PBS and centrifuged at 3000rpm for 5 minutes at 4 **°C**. The supernatant was discarded and 1ml of ice cold 70% ethanol was added through the sides of the tube. Incubate at 4 **°C** for 45 minutes. Add 1ml of ice cold 1X PBS and centrifuged at 3000rpm for 5 minutes at 4**°C.** The supernatant was discarded. Add 1X PBS and centrifuged at 3000rpm for 5 minutes at 4 **°C**. The supernatant was discarded. Add 250 µl of 0.4mg/ml RNase to each tube and incubated at 37 **°C** for 30 minutes. Add 10µl of 1mg/ml PI to each tube and incubate it in dark for 5 minutes. The samples are filtered through cell strainer in to FACS tube. The samples were analysed in Flow cytometer (FACS Aria II).

Results and Discussion

A. Field survey

After a thorough field survey, total 55 weeds from 26 different families were identified, from these six weeds were

selected for the current research. The pharmacodynamic uses and ethno-medicinal uses were published as a Review article. The identified plants weeds were represented below in the Figure 11 [22].

B. Microscopical authentication of selected weed species

The selected weed species (aerial parts) were subjected to microscopical authentication. The transverse sections of the selected weeds were proved to have the desired microscopical characters of the specified families.

1. *Achyranthes bidentata* Blume

2. *Acalypha indica* L.

3. *Aegeratum conyzoides* L

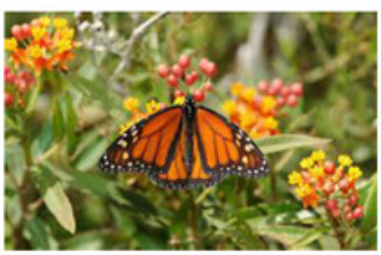
4. *Amaranthus spinosus* L

5. *Anthoxnthum odoratum* L

6. *Argemone mexicana* L

7. *Artemisia nilagirica* Clarke.

8. *Artemisia parviflora* Roxb



9. *Asclepias curassavica* L

10. *Bidens biternata* (Lour.) Merr.

11. *Borreria latifolia* Aubl

12. *Brassica juncea* L.

13. *Capsella bursapastoris* L.

14. *Centella asiatica* L.

15. *Cardiospermum halicacabum* L

16. *Crassocephalum Crepidioides* B

17. *Chenopodium ambrosioides* L.

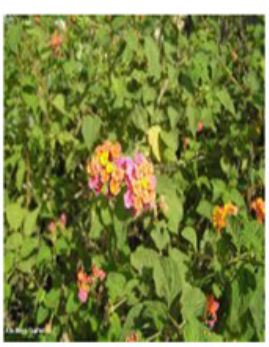
18. *Chromolaena odorata* L

19. *Cirsium wallichii* DC

20. *Commelina benghalensis* L.

21. *Dodonea viscose* L.

22. *Kalanchoe pinnata* L



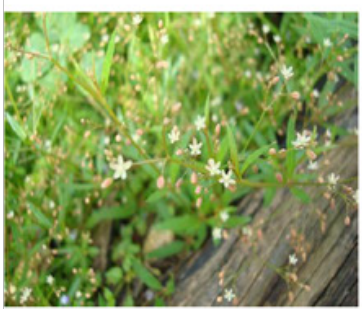
23. *Lantana camara* L.

24. *Leucas aspera* L

25. *Lobelia nicotianaefolia* R

26. *Melilotus indica* L

27. *Mirabilis jalapa* L



28. *Mollugo pentaphylla* L

29. *Nicandra physaloides* L

30. *Oenothera rosea* L

31. *Opuntia stricta* K.H.

32. *Oxalis corniculata* L

33. Persicaria nepalensis M.H.

34.*Plantago erosa* R

35. *Plectranthus barbatus* A

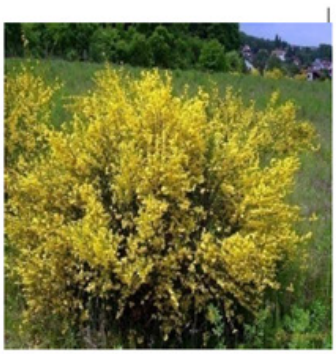
36. *Polygonum chinense* L

37. *Prinsepia utilis* R.

38. *Ricinus communis* L

39. *Rubus ellipticus* S

40. *Rumex nepalensis* S



41. *Sarothamnus scoparius* L.

42. *Silybum marianum* L

43. *Stellaria media* L.

44. Taraxacum officinale F.H.

45. *Tephrosia purpurea* L

46. *Tithonia diversifolia* A.G.

47. *Trifolium repens* L.

48. *Urtica parviflora* Roxb

49. *Verbascum thapsus* L

50. *Cytisus scoparius* 51. *Solanum mauritianum* 52. *Erigeron karvinskianus*

53. *Phytolacca dodecandara* 54. *Cnicus wallichi* 55. *Cestrum aurantiacum*

Figure 11: Identified weed species through field survey [22].

C. Microscopical authentication of *erigeron karvinskianus* (Stem& Leaf)

The leaf is slightly curved on the adaxial side; it has less prominent midrib and thin dorsiventral lamina. Mid rib is plano convex with flat adaxial side and short and broad conical adaxial part. The mid rib is 300 µ m wide. The palaside layer is adaxially transcurrent and extends in between the adaxial epidermis and the vascular bundle. The vascular strand is single, circular and 170 µm in diameter. It includes about four short parallel xylem lines and equal number of small units of phloem. The xylem and phloem are collateral. The vascular bundle is surrounded by a single layer of large circular parenchyma cells. Short wide two celled glandular trichomes are sporadically seen on the abaxial epidermis and are 30 µm in height, represented below in the Figure 12.

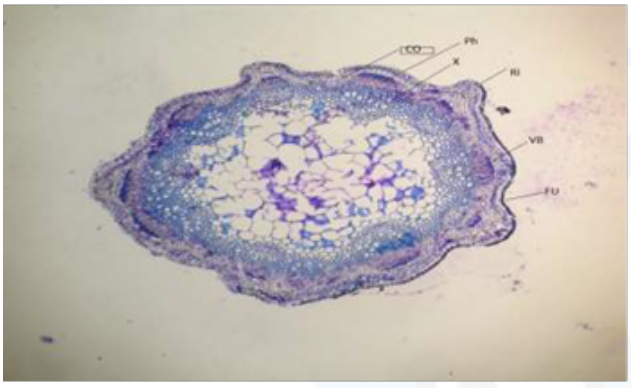


Figure 12: Transverse section of *Erigeron karvinskianus stem.*
(Co- Cortex; Ph: Phloem; X: Xylem; Ri: Ridge; VB: Vascular Bundle; Pi: Pith).

The stem is five winged and appear star shaped in sectional view. It is 1.4 mm thick. The wings are 300 x 400 µm thick. The epidermal layer of the stem is thin and the cells are small and square shaped. The cuticle is very thick, the cortex is parenchymatous, the cells are varied in shape and size; these are small masses gelatinous fibres which occur within the wing and in radial row in the wing. Secondary phloem is in thin continous cylinder encircling the xylem cylinder. The phloem elements are small and compact. Secondary xylem is thick and solid hollow cylinder. It includes small clusters of vessels in the outer part of the xylem cylinder. It includes small clusters of vessels in the outer part of the xylem cylinder. The pith is fairly wide and parenchymatous represented below in the Figure 13.

D. Microscopical authentication of *Solanum mauritianum* (stem & leaf)

The transverse section of the stem showed a circular and even in sectional view and it is 3.6 mm thick and it consists of epidermal layer of radially elongated cells with thick cuticle. The epidermal cells have undergone tangential division forming an intitial periderm cells towards inside, represented below in the Figure 14.

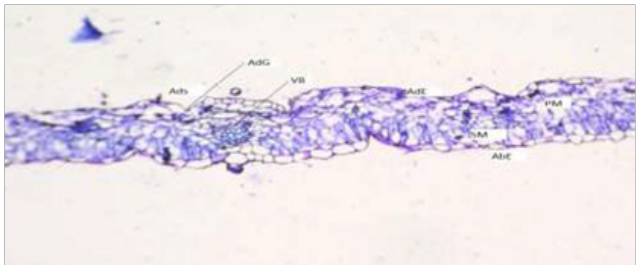


Figure 13: Transverse section of *Erigeron karvinskianus* Leaf.
(Ads: Ad axial Side; Abe: Abaxial Epidermis; Adg: Adaxial Groove; VB: Vascular Bundle; PM: Palaside Mesophyll; SM: Spongy Mesophyll)).

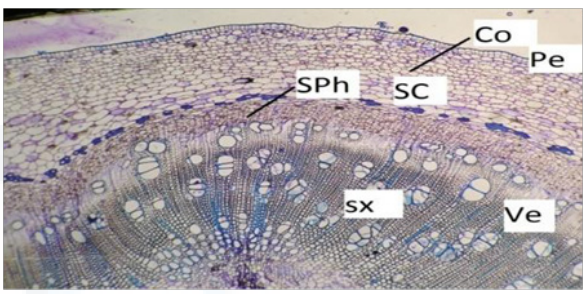


Figure 14: Transverse Section of *Solanum mauritianum* stem.
(Co: Cortex; pe: Periderm; phr: Phloem Ray, Sc: Sclerenchyma; Sph: Secondary Phloem; sx: secondary xylem; ve: Vessels, XF: Xylem Fibre, XR: Xylem Ray).

The leaf consists of a wide and thick midrib. It consists of a short and thick adaxial hump and wide lobed abaxial part. It is 850µm and the abaxial part is 1.15µm wide. The midrib consists of a thin wavy epidermal layer of small squarish cells. Inner to the epidermis are two or three layers of collenchyma cells followed by a wide zone of large thin walled compact parenchymatous ground tissue. The vascular strand is single, shallow, wide are of bicollateral xylem and phloem. It consists of diffusely distributed angular, thick walled wide xylem elements. Several discrete nests of phloem elements occur both on the adaxial and abaxial sides of the xylem strand. No sclerenchyma cells are seen on the adaxial and abaxial phloem units.

The lamina is thick and bifacial. It is 90µm thick. The adaxial epidermis consists of dilated circular or elliptical thin walled cells. The abaxial epidermis has thin and cylindrical cells. The palaside cells are single layered thin and have wide gaps inbetween the cells. The abaxial zone of spongy parenchyma includes about five layers of lobed cells and form air chambers. Both on the abaxial and adaxial surfacial surfaces of the lamina occur dense stellate epidermal trichomes. The trichomes has thick, short stack which consists of three or four vertical mass of cylindrical cells. At the apex of the stalk are situated circular readiating lateral trichomes. These trichomes appear star shaped in surface view and hence they are called stellate trichomes. The trichome is 100µm in height and the lateral trichomes are up to 350 µm long, represented in the below Figure 15.

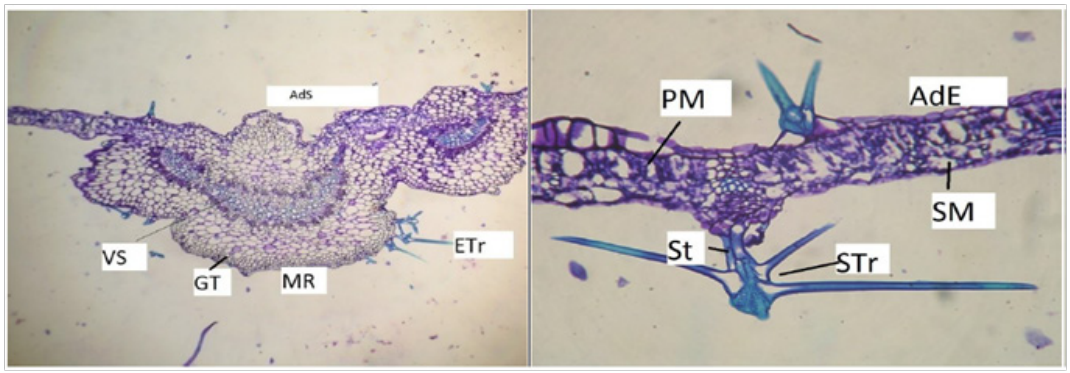


Figure 15: Transverse Section of *Solanum Mauritianum* leaf.

(AdS: Adaxial Side; ETr: Epidermal Trichomes; VS: Vascular Strands; GT: Ground Tissue; MR: Mid Rib; VS: Vascular Strand; AdE: Adaxial Epidermis; PM: Palaside Mesophyll; STr: Stellate Trichome; SM: Spongy Mesophyll and St: Stalk).

E. Microscopical authentication of *Cytisus Scoparius* (stem & leaf)

The stem is five winged and appear star shaped in sectional view. It is 1.4 mm thick. The wings are 300 x 400µm thick. The epidermal layer of the stem is thin and the cells are small and square shaped. The cuticle is very thick the cortex is parenchymatous, the cells are varied in shape and size; these are small masses gelatinous fibres which occur within the wing and in radial row in the wing. Secondary phloem is in thin continous cylinder encircling the xylem cylinder. The phloem elements are small and compact. Secondary xylem is thick and solid hollow cylinder. It includes small clusters of vessels in the outer part of the xylem cylinder. It includes small clusters of vessels in the outer part of the xylem cylinder. The pith is fairly wide and parenchymatous represented below in the Figure 16.

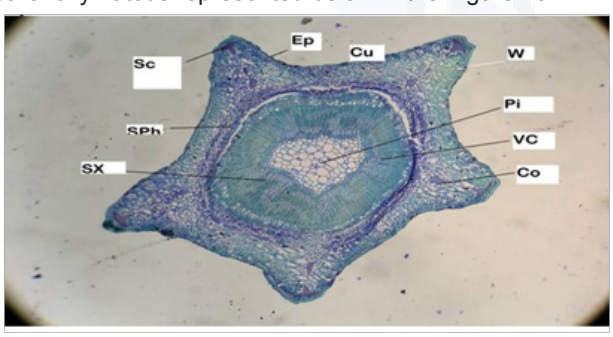


Figure 16: Transverse Section of *Cytisus scoparius* stem.

Co: Cortex; Cu- Cuticle; Ep-Epidermis; Sc: Sclerechyma; SPh: Secondary Phloem; SX: Secondary Xylem; Ve: Vessel; XF: Xylem Fiber; VC- Vascular Cylinder; W: Wing.

The leaf is slightly curved on the adaxial side; it has less prominent midrib and thin dorsiventral lamina. Mid rib is plano convex with flat adaxial side and short and broad conical adaxial part. The mid rib is 300µ m wide. The palaside layer is adaxially transcurrent and extends in between the adaxial epidermis and the vascular bundle. The vascular strand is single, circular and 170µm in diameter. It includes about four short parallel xylem lines and equal number of small units of phloem. The xylem and phloem are collateral. The vascular bundle is surrounded by a single layer of large circular parenchyma cells. Short wide two celled glandular trichomes are sporadically seen on the abaxial epidermis and are 30µm in height, represented below in the Figure 17.

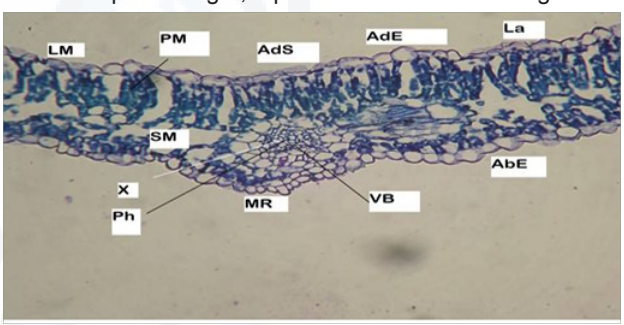


Figure 17: Transverse Section of *Cytisus scoparius* leaf.

AbE: Abaxial Epidermis; Ads: Adaxial Side; AdE: Adaxial epidermis; BS: Bundle sheath; La: Lamina; LM: Leaf Margin; MR: Mid Rib; PM: Palaside Mesophyll; Ph-Phloem; Sm: Spongy Mesophyll; VB: Vascular bundle; X: Xylem.

F. Microscopical authentication of *Cestrum aurantiacum* (stem & leaf)

The transverse section of the stem showed rough circular outline measuring1.7 mm in thickness. The stem showed presence of epidermis, cortex, vascular cylinder and wide pith. The epidermal layer is thin and the cells are small elliptical in shape and thick walled. The sub epidermal layer of cells are radially stretched into vertically oblong. These cells divided by periclinal walls producing the peridermis. The cortical zone is 100 um thick, it is parenchymatous and the cells are circular and thin walled. The vascular system consists of a closed hollow cylinder enclosing wide pith. The cylinder consists of outer phloem and inner (medullary) phloem with xylem cylinder lying in between the phloem cylinders. The outer secondary phloem includes radial fibers of sieve elements and short phloem rays of wide elliptical cells. On the outer border of the phloem occurs thin discontinuous layer of highly thick walled lignified fibers

(Schlerenchyma). The inner medullary phloem is in the form of small discrete masses of sieve elements. Sclerenchyma elements occur in small clusters in the inner zone of the medullary phloem, represented below in the Figure 18.

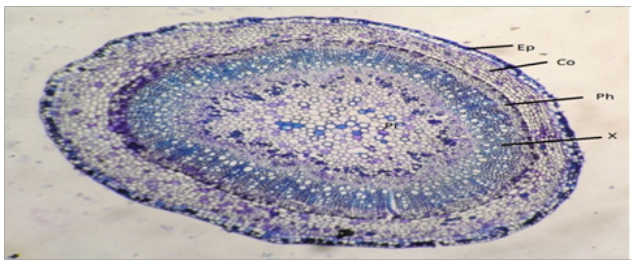


Figure 18: Transverse Section of Stem of *Cestrum aurantiacum.*

Co: Cortex; ep: Epedermis; IPH: Inner phloem; oph: Outer Phloem; pi: Pith; sc: sclerenchyma; Sep: Sub epedermis; x: xylem.

The transverse section of the leaf showed thick mid rib and thin lamina. The midrib consists of short thick adaxial cone and wide semicircular abaxial surface. The midrib is 750 um thick; the adaxial cone is 150 um in height and 250um in width. The abaxial surface is 1 mm wide. The mid rib consists of a thin epidermal layer of small cubical cells. Inner to the epidermis is a narrow zone of two or three layers of collenchymas cells. The remaining region consists of angular thin walled parenchyma cells. The vascular system has single wide bowl shaped strand. The vascular strand is bicollateral and consists of central xylem, adaxial and abaxial phloem strands. The xylem elements occur in short vertical rows of wide, thick walled cells. The phloem strands are in separate large masses distributed in horizontal row, represented below in the Figures 19a&19b.

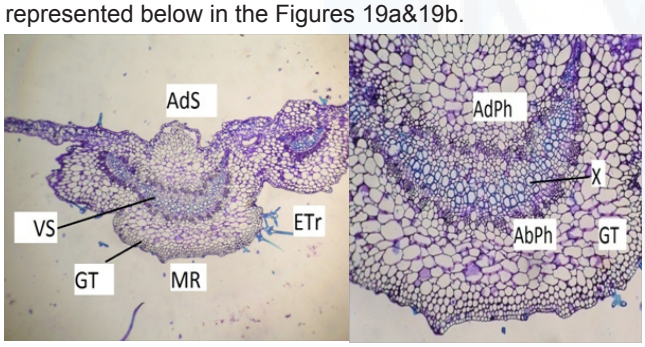


Figure19a: Transverse Section of leaf of *Cestrum aurantiacum.*

Ab ph: Abaxial Phloem; Adph: Adaxial Phloem; Ads: Adaxial Side; Co: Cortex; Etr: Epidermal Trichome; GT: Ground Tissue, MR: Mid Rib, VS: Vascular Strand.

G. Microscopical authentication of *Phytolacca dodecandara* (stem & leaf)

The stem is thick and circular in sectional view. It is about 1.5 mm thick. The stem has three or four thick curved wings. The wing is 200 µm long and 100 µm thick. The epidermal layer of the stem slightly thick walled and squarish in continue collenchymas zone which is 150µm thick. Next inner to the epidermis is a wide collenchymas zone which is 150µm thick. Next inner to the collenchymatous cortex is a narrow zone of 6 layers of parenchymatous cortex. The vascular cylinder is a wide and hollow, consists of outer continuous cylinder of thin secondary phloem. The secondary phloem is unsheathed by thin discontinuous layer of sclerenchyma elements. Secondary xylem is 350µm thick. The vessels are either solitary or short radical multiples. They are circular, thin walled and are up to 40 µm wide. The fibers are thick walled with wide lumen, represented below in the Figure 20.

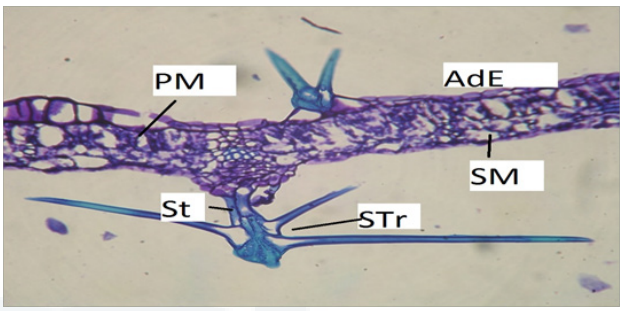


Figure19b: Transverse Section of leaf of *Cestrum aurantiacum.*

AdE: Adaxial epidermis; PM: Palaside Mesophyll; St: Stalk; STr: Stellate Trichome.

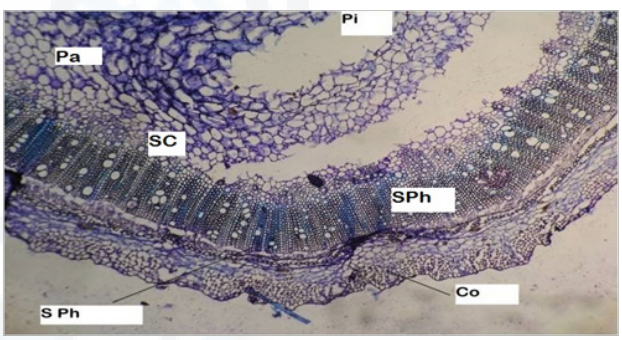


Figure 20: Transverse Section of *Phytolacca dodecandra* Stem.

Pi:Pith, Sc: Sclerenchyma, SPh: Secondary Phloem, Sx: Secondary Xylem.

The leaf consists of thick lamina and prominent midrib, the midrib is slightly grooved on the adaxial side, and thick and semicircular in sectional view. The midrib is 650 µm thick and 950 um wide. The midrib has shallow furrows and ridges. The epidermal layer is fairly thick, comprising thick walled and squarish cells. Inner to the epidermis is a narrow zone of two or three layers of thick walled sclerenchyma cells. Next to the sclerenchyma zone is parenchymatous tissue which includes fairly large, thin walled compact cells. The vascular strand is wide and thick. The structure comprising numerous compact vertical short rows of xylem elements. The xylem elements are narrow, squarish in outline and thick walled. Phloem tissue occurs in thick are on the lower part of the xylem strand. The phloem elements are aggregated in to small clusters and are sheated by a thin sclerenchyma cells, represented below in the Figure 21.

H. Microscopical authentication of *Cnicus wallichi* (stem & leaf)

The stem is regular in sectional view. The surface is deeply ridged and furrowed. There are 2 or 3 long thin wings which are folded along the surface of the stem. It consists of epidermis, cortex, cortical vascular bundles and a ring of wedge shaped stellar bundles and lysigenous pith cavity. The epidermis of the stem is very thin and not very distinct. The wings of the stem have a horizontal row of circular vascular bundles. The outer cortex of the stem has wide collenchymatous tissue. The stellar bundles include thick and radially elongated larger bundles alternating with more or less circular vascular bundles. These two types of bundles occur in a circular outline. The larger vascular bundle has very thick wedge shaped sclerenchymatous cap. The inner part of the vascular bundle includes a large mass of circular thick walled compact xylem elements. On the outer part of the xylem strand occurs a thin tangential segment of phloem elements. The smaller bundles are circular comprising a circular mass of xylem element and a circular bundle of phloem elements. Small sclerenchymatous isolated cells occur on the periphery of the phloem strands. Which are made up of parenchyma cells, represented below in the Figure 22.

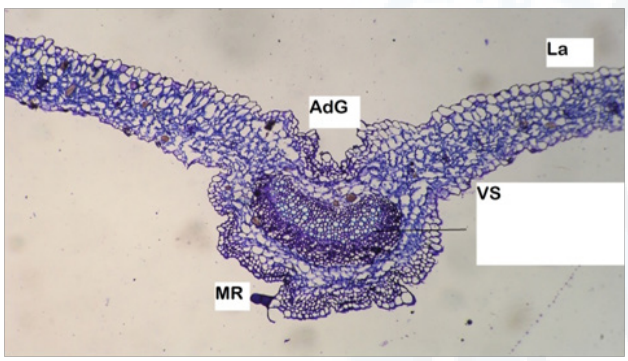


Figure 21: Transverse Section of Phytolacca dodecandra leaf.

Adg: Adaxial Groove; La: Lamina; MR: Midrib; OGC: Outer Ground Cells; Ph: Phloem; Sc: Sclerenchyma; Vs: Vascular Bundle Strand; x: Xylem; C0: Cortex; Col: Collenchyma; Pa: Parenchyma.

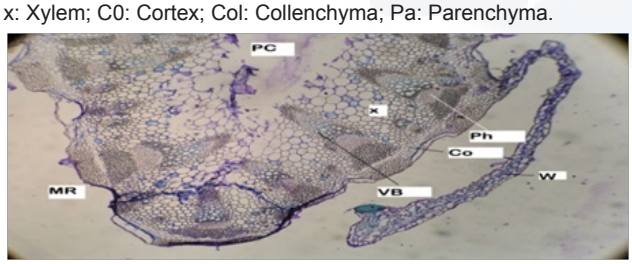


Figure 22: Transverse Section of Cnicus wallichi stem.

Co: Cortex; MR: Midrib; P.C: Pith Cavity; Ph: Phloem; Ri: Ridge; SM: Spongy Mesophyll; VB: Vascular Bundle; X: Xylem; W: Wing.

The leaf has thick abaxially hanging midrib and thin lamina. The midrib is flat on the adaxial side and porojects into thick rectangular abaxial part. The epidermal layer of the midrib consists of cylindrical cells with numerous stomata. The ground parenchyma cells of the midrib consists of a few layers of thin walled compact fairly wide parenchyma cells. The vascular strand is prominent consisting of a thick mass of adaxial xylem elements and abaxial phloem elements. The xylem elements are small and compact. Thick cap of sclerenchyma elements occurs on the phloem part as well as xylem part of the vascular strand, represented below in the Figure 23.

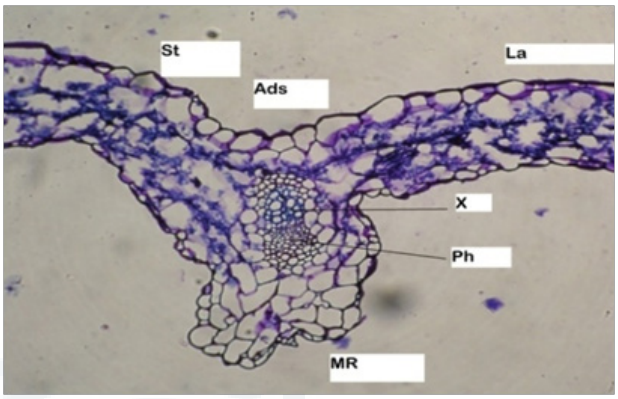


Figure 23: Transverse Section of Cnicus wallichi leaf.

Ads: Adaxial Side; La: lamina; MR: Mid Rib; Ph: Phloem, Sc: Sclerenchyma; St: Stomata; X: Xylem.

I. Ash values

The ash values for the selected weeds were performed, in order to fix the pharmacogno-stical standards and the results were reported in Table 6.

J. Extractive values

The extractive values for the selected weeds were performed, in order to fix the pharmacognostical standards and the results were reported in Table 7.

K. Heavy metal analysis

As many of the weeds being good heavy metal chelators, the selected weeds were subjected to heavy metal analysis in order to know the Lead and Cadmium load. The crude weed drugs were found to be free from the lead and cadmium load and proved that these weeds are not heavy metal chelators and free from the contamination, the results were reported in Table 8 & Figure 24.

L. Extraction and fractionation

a. **Extraction:** The percentage yield of the hydro-alcohol extracts were calculated after subjecting to triple maceration using 70% v/v of hydro-ethanol as menstrum and the calculated percentage yields of each weed extract was represented below in the Table 9.

b. **Liquid-liquid fractionation**: The extracts were subjected to liquid -liquid fractionation using separating funnel and the percentage yield of the obtained fractions were calculated on dry basis. The percentage yield of fractions were represented in the below Table 10.

c. **Qualitative phytochemical screening:** The Qualitative analysis of extracts and fractions were carried out

and the results were given in Tables 11 & 12. All the weed extracts and fractions proved to have important secondary metabolites like alkaloids, flavanoids, Phenolics, Saponins and cardiac glycosides.

Table 6: Ash values of the selected weeds.

Name of the weed	Total Ash (% w/w± SEM)	Acid Insoluble Ash (% w/w± SEM)	Sulphated Ash (% w/w± SEM)
Phytolacca dodecandra	12.77 ± 0.98	6.45± 0.88	2.19± 0.22
Cnicus wallichi	14.16± 0.64	7.32±0.01	2.97±0.88
Erigeron karvinskianus	14.20±0.01	6.93±0.22	2.76±0.64
Cestrum auranticaum	11.71±0.02	5.98± 0.42	1.99±0.54
Cytisus scoparius	2.82±0.13	1.29±0.01	0.59±0.01
Solanum mauritianum	2.73±0.54	1.27±0.02	0.63± 0.23

Table 7: Extractive values of the selected weeds.

Name of the Weed	Pet Ether (%V/V ± SEM)	Chloroform (%V/V± SEM)	Ethyl Acetate (%V/V± SEM)	Hydro Alcohol (70%V/V± SEM)
Phytolacca dodecandra	2.34 ± 0.23	3.5± 0.12	3.9± 0.12	5.96±0.24
Cnicus wallichi	1.52±0.01	2.9±0.13	3.7± 0.13	4.90±0.21
Erigeron karvinskianus	0.53±0.06	1.45±0.09	2.12±0.12	3.24±0.35
Cestrum auranticaum	2.36± 0.21	3.76± 0.09	4.92± 0.13	8.80± 0.49
Cytisus scoparius	2.12± 0.30	7.84± 0.46	8.92± 0.12	17.90± 0.56
Solanum auriculatum	1.13± 0.02	2.43± 0.12	4.43±0.23	14.48± 0.33

Table 8: Heavy Metal Analysis of crude drugs.

Sample Crude Drug	Concentration of Cadmium in ppm (Limit : ≥0.3 ppm)	Concentration of Lead in ppm (Limit : ≥10 ppm)
Phytolacca dodecandra	0.016	0.51
Cnicus wallichi	0.013	0.40
Erigeron karvinskianus	0.022	0.70
Cestrum auranticaum	0.019	0.58
Cytisus scoparius	0.016	0.49
Solanum mauritinum	0.025	0.77

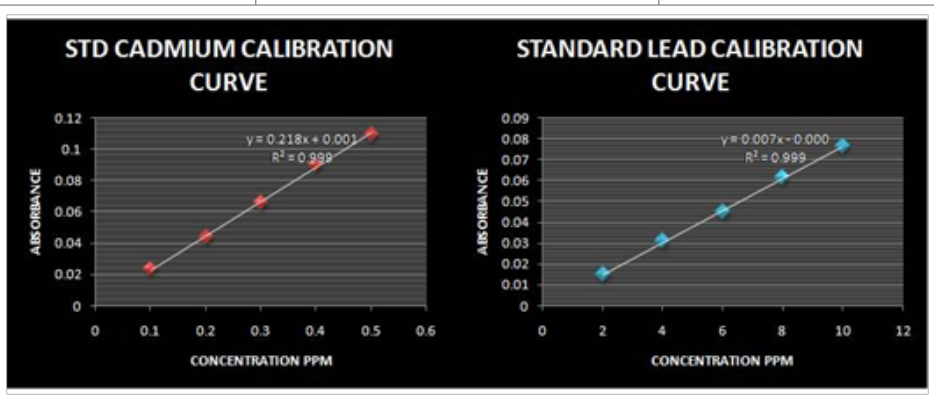


Figure 24: Calibration curves of standard Cadmium & Lead.

d. **Quantitative phytochemical screening:** The hydro-alcoholic extracts were subjected to total phenol content, total alkaloid content , total flavonoid content by using UV-Visible spectroscopy and total saponin content by using gravimetric separation technique and the results proved that these extracts are rich in these secondary metabolites. The results were represented in the following Table 13 and the calibration curves were represented in the Figure 25.

e. **TLC finger printing of secondary metabolites:** The TLC finger printing of various fractions from six different weeds were carried out and the chemical finger prints of the extracts were documented in order to know the nature and number of identified solutes present in various phytochemically positive fractions. The data is represented below in the Figures 26-28

Table 9: Percentage yield of weed hydro alcohol mother extracts.

Name of the Weed	Percentage Yield of Weed Mother Extracts (%w/w)
Phytolacca dodecandra	5.96
Cnicus wallichi	4.9
Erigeron karvinskianus	3.24
Cestrum auranticaum	8.8
Cytisus scoparius	17.9
Solanum auriculatum	14.48

Table 10: Percentage yield of different weed fractions.

S.A (%w/w)				C.S (%w/w)				P.D (%w/w)				C.A (%w/w)				C.W (%w/w)				E.K (%w/w)			
PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF
6.6	16.1	63.9	13.4	12.4	14.3	45.4	27.9	2.6	5.4	37.4	54.6	37.0	23.8	13.8	25.4	2.5	9.3	16.4	71.8	30	4.7	4.1	61.2

P.D: *Phytolacca Dodecandra (Phytolaccaceae); C.W: Cnicus Wallichi (Asteraceae); E.K: Erigeron Karvinskianus (Asteraceae); C.A: Cestrum Auranticaum (Solanaceae); C.S: Cytisus Scoparius (Fabaceae); S.A: Solanum Auriculatum (Solanaceae).* PF: Pet Ether Fraction; CF: Chloroform Fraction; EF: Ethyl Acetate Fraction; AQF: Aqueous fraction.

Table 11: Preliminary Phytochemical Screening of hydro-alcohol extracts.

S. No	Name of the Test	P.D	C.W	E.K	C.A	C.S	S.A
1.	Test for Alkaloids a) Dragendroff test	++	++	++	++	++	++
	b) Mayers test	++	++	++	++	++	++
	c) Hagers test	++	++	++	++	++	++
	d) Wagners test	++	++	++	++	++	++
		++	++	++	++	++	++
2.	Test for Amino acids	-	-	-	-	-	-
	a) Millons test	-	-	-	-	-	-
	b) Ninhydrin test	-	-	-	-	-	-
3.	Test for Carbohydrates	++	++	++	++	++	++
	a) Molischs test	++	++	++	++	++	++
	b) Fehlings test	++	++	++	++	++	++
4.	Test for fixed oils (Spot test)	-	-	-	-	-	-
5.	Test for Flavanoids a) Shinodas test	++	++	++	++	++	++
	b) Alkaline reagent test	++	-	-	-	++	++
		++	++	++	++	++	+
6.	Test for Coumarins	+	+	+	+	+	+
7.	Test for Lignans	+	+	+	+	+	+
8.	Test for Cardiac glycosides	++	++	++	++	-	++
	a) Killerkilani test	++	++	++	++	-	++
	b) Legals test	++	++	++	++	-	++
	c) Baljets test	++	++	++	++	-	++
9.	Test for Coumarin Glycosides	++	++	++	++	++	++
10.	Test for Saponins Foam test	++	+	++	++	+	++
11.	Test for Phenolics & Tannins	++	++	++	++	++	++
	Ferric chloride test	++	++	++	++	++	++
	Tannic acid test	++	++	++	++	++	++
	Lead acetate test	++	++	++	++	++	++
12.	Test for Phytosterols	++	++	++	++	++	++
	a) Libermann-Burchards test	++	++	++	++	++	++
	b) Salkowski test	++	++	++	++	++	++
13.	Test for Triterpenoids	++	++	++	++	++	++
	a)Tschugajiu test	++	++	++	++	++	++
	b)Liberman-burchard test	++	++	++	++	++	++
	c) Salkowski test	++	++	++	++	++	++

PD: *Phytolacca dodecandra (Phytolaccaceae), CW -Cnicus wallichi (Asteraceae), EK: Erigeron karvinskianus (Asteraceae), CA: Cestrum auranticaum (Solanaceae),*
CS: Cytisus scoparius (Fabaceae) and *SA: Solanum auriculatum (Solanaceae).*

f. **High Performance Thin Layer Chromatographic (HPTLC) finger printing of isolated total saponin fractions from different weeds:** The saponins were isolated using gravimetric separation technique, the percentage yield of isolated saponins were discussed in the above Table 13. The isolated saponin fractions were characterized by using HPTLC analysis in order to know

the number of saponins in each total saponin fraction. All saponins fractions found to have more than more than 10 compounds with different RF from 0.17 to 0.79 and the results were represented in the below Figure 28. The phytochemical screening proved that these fractions were of triterpenoidal saponin glycosides nature.

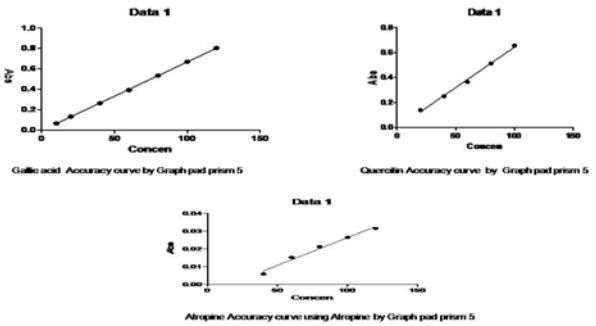


Figure 25: Calibration curves for Quantitative Analysis.

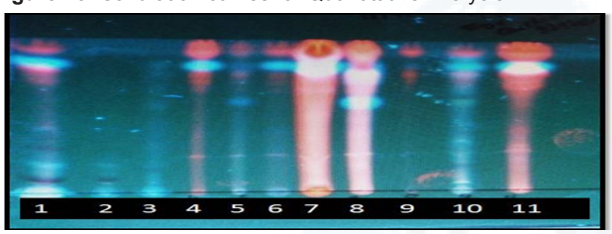


Figure 26: TLC Finger Printing of Cardiac glycosides in various fractions.

1. *Phytolacca dodecandara* ethyl acetate fraction. 2. P*hytolacca dodecandara* aqueous fraction. 3. *Erigeron karvinskianus* chloroform fraction. 4. *Erigeron karvinskianus* ethyl acetate fraction, 5. *Erigeron Karvinskianus* aqueous fraction. 6. *Cestrum aurantiacum* chloroform fraction. 7. C*nicus wallichi* chloroform fraction. 8. C*nicus wallichi* ethyl acetate fraction. 9. C*nicus wallichi* aqueous fraction. 10. *Solanum auriculatum* chloroform fraction & 11. *Sa* ethyl acetate fraction.

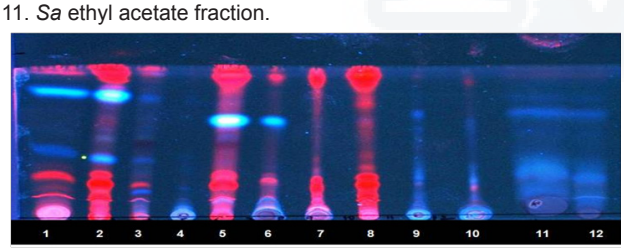


Figure 27: TLC Finger Printing of Flavanoids in various fractions.

1.Phytolacca dodecandara ethyl acetate fraction. 2. Pd aqueous fraction. 3. *Cnicus wallichi* ethyl acetate fraction. 4. Cw aqueous fraction. 5. *Erigeron karvinkianus* ethylacetate fraction. 6. Ek aqueous fraction. 7. *Cestrum aurantiacum* chloroform fraction. 8. Ca ethyl acetate fraction. 9. *Cytisus scoparius* chloroform fraction. 10. Cs ethyl acetate fraction. 11. Cs aqueous fraction & 12. *Solanum auriculatum* ethyl acetate fraction.

g. **LCMS finger printing of total saponin fractions:** The isolated total saponin fractions were subjected to LC-MS finger printing analysis and a total spectrum was run in both positive mode and negative mode. Various known saponins were identified based on mass peaks and chemotaxonomic significance of saponins and the data was represented in the following Table 14 & Figures 29-34.

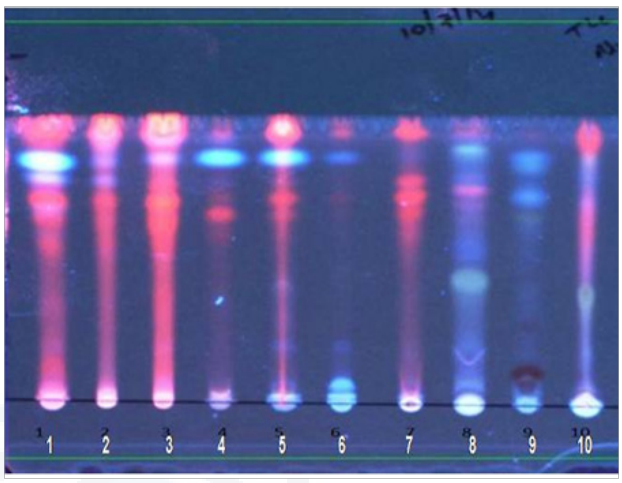


Figure 28a: TLC Finger printing of alkaloids in various fractions. 1.*Phytolacca dodecandra Cloroform Fraction.* 2. *Cnicus wallichi Pet Ether Fraction.* 3. *Cnicus wallichi Chloroform Fraction. 4. Erigeron karvinskianus Pet Ether Fraction*. 5. *Ek* Chloroform Fraction. 6. Ek Ethyl Acetate Fraction. 7. *Ek Aqueous Fraction. 8. Cestrum aurantiacum Chloroform Fraction.* 9. *Cytisus scoparius Chloroform Fraction &* 10. *Solanum auriculatum Chloroform Fraction.*

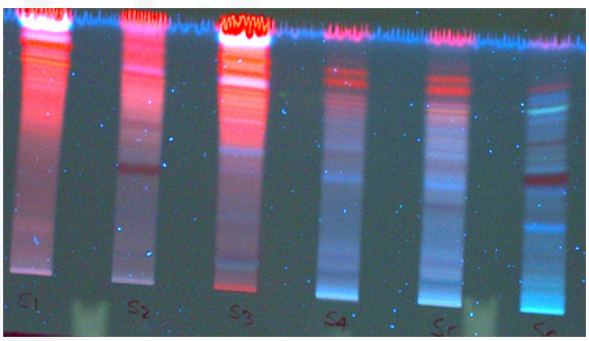


Figure 28b: HPTLC Finger Printing of saponin fractions.

S_1. *Phytolacca dodecandara*, S_2 *Cnicus wallichi*, S_3. *Erigeron karvinkianus*, S_4. *Cytisus scoparius*, S_5. *Solanum mauritianum & S6 Cestrum aurantiacum.*

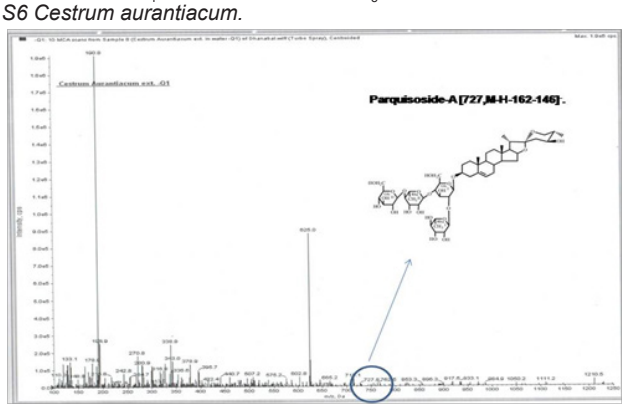


Figure 29: LCMS Characterization of Cestrum aurantiacum Saponin Fraction.

Table 12: Preliminary Phytochemical Screening of different weed fractions.

Name of the Test	P.D				C.W				E.K				C.A				C.S				S.A			
	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF	PF	CLF	ETF	AQF
1. Test for Alkaloids	-	+	-	-	+	+	-	-	+	+	+	+	-	+	-	-	-	+	-	-	-	+	-	-
2. Test for cardiac glycosides	-	-	+	+	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	+	+	-
3. Test for Flavanoids	-	-	+	+	-	-	+	+	-	-	+	+	-	+	+	-	-	+	+	+	-	-	+	-
4. Test for Triterpenoids/ streroids	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	-
5. Test for Saponins	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	+	+	+
6. Test for Napthoquinones	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
7. Test for Phenolics	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	+	-	-	+	+
8. Test for Carbohydrates	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	+

P.D: *Phytolacca dodecandra* (Phytolaccaceae); C.W: *Cnicus wallichi* (Asteraceae); E.K: *Erigeron karvinskianus* (Asteraceae); C.A: *Cestrum auranticaum* (Solanaceae); C.S: *Cytisus scoparius* (Fabaceae) and S.A: *Solanum auriculatum* (Solanaceae). PF: *Pet ether fraction*, CF: *Chloroform fraction*, EF: *Ethyl acetate fraction* and AQF: Aqueous fraction.

Table 13: Quantitative Analysis of hydro alcohol weed extracts.

Name of the Extract	Total alkaloid Content	Total Flavanoid Content	Total phenol Content	Total Saponin Content
Phytolacca dodecandra	154.84 µg/g atropine equivalent	430.6µg/g quercetin equivalent	365.8µg/g gallic acid Equivalent	25 % w/w
Cnicus wallichi	218.97µg/g atropine equivalent	492.4µg/g quercetin equivalent	918.1µg/g gallic acid Equivalent	21.5 % w/w
Erigeron karvinskianus	191.20µg/g atropine equivalent	206.8µg/g quercetin equivalent	919.6µg/g gallic acid Equivalent	33.7 % w/w
Cestrum auranticaum	188.16µg/g atropine equivalent	149.19µg/g quercetin equivalent	993.3µg/g gallic acid Equivalent	13.4 % w/w
Cytisus scoparius	106.27µg/g atropine equivalent	818.5µg/g quercetin equivalent	919.4µg/g gallic acid Equivalent	8.05 % w/w
Solanum mauritinum	163.27µg/g atropine equivalent	594.4µg/g quercetin equivalent	119.94µg/g gallic acid Equivalent	10.42 % w/w

h. **Isolation and characterization of secondary metabolites:** Two compounds Naringenin(Compound-1) and 7 - Methoxy hesperetin (Compound-3) were isolated first time from the ethyl acetate fraction of aerial parts of *Erigeron karvinskianus*. First time a novel compound (compound-2, a saponin glycoside), Tetrahydro-2- (hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)- 2H-Pyran-3, 4, 5-triol was isolated from the aerial parts of *Solanum mauritianum*. First time a novel phenolic compound (Compound -4, (1E-1-hydroxyprop-1-en-2-yl (2E)- 3-(4-hydroxy-3-methoxyphenyl) prop-2-enoate) was isolated from the *Cytisus scoparius* using flash chromatography technique.

The structure of these isolated compounds were characterized and confirmed by using IR, NMR and Mass analysis. The data was represented in the Table 15. The isolated molecules were characterized and the spectras were showed in the following Figures 35-50.

A. ***In-vitro* antioxidant studies**

In-vitro antioxidant studies using DPPH and ABTS assays were carried out. The total extracts, fractions, total saponin

fractions and isolated compounds showed good antioxidant activity in compare to standard quercetin (Table 16 Figures 51&52). Out of all the extracts and fractions, The *Erigeron karvinskianus* fractions showed significant antioxidant activity in compare to other fractions. All the total saponin fractions & isolated compounds showed good or significant activity *in compare* to standard quercetin

Table 14: LCMS characterization of known saponins from total saponin fractions Finger Prints.

Name of the total Saponin Fraction	Identified Saponin Peaks
Cestrum aurantiacum	Parquisoside-A [727,M-H-162-146]$^-$.
Solanum mauritianum	Nocturnoside-B [1014,M-H]$^-$, Protodioscine [1046, M-3H]$^-$, Parquisoside- B [1192, M-H]$^-$, Solasodine [411, M-2H]$^-$, Solasonine [884, M]$^+$
Cnicus wallichi	Lupeol [427, M+H]$^+$
Erigeron karvinskianus	Calenduloside- E [637 ,M+5H]$^+$, Leiocarposide 612 [M-2H]$^-$
Cytisus scoparius	Ruscogenin [431, M+H]$^+$
Phytolacca dodecandra	Phytolaccoside B [663, M-H]$^-$, Phytolaccoside E [823, M-3H].

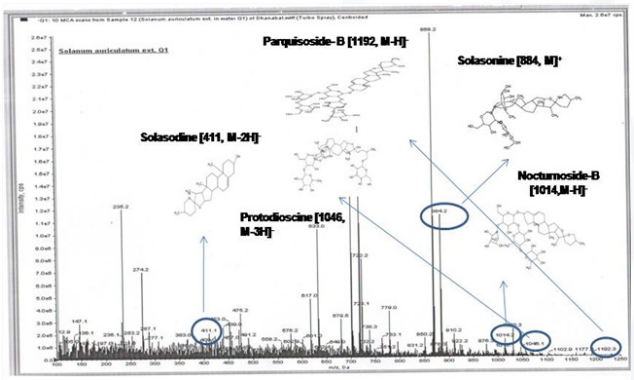


Figure 30: LCMS Characterization of Solanum mauritianum Total Saponin Fraction.

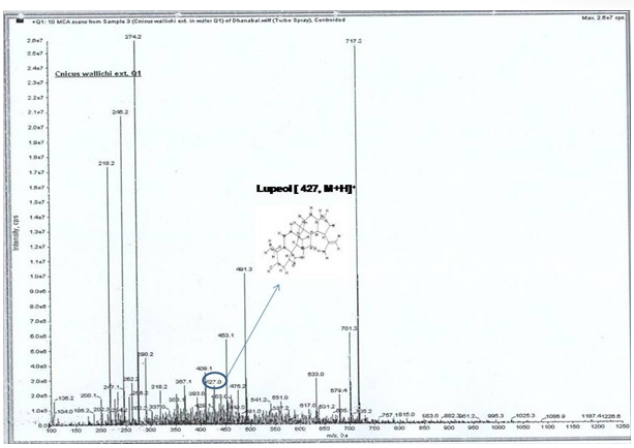


Figure 31: LCMS Characterization of Cnicus wallich Total Saponin Fraction.

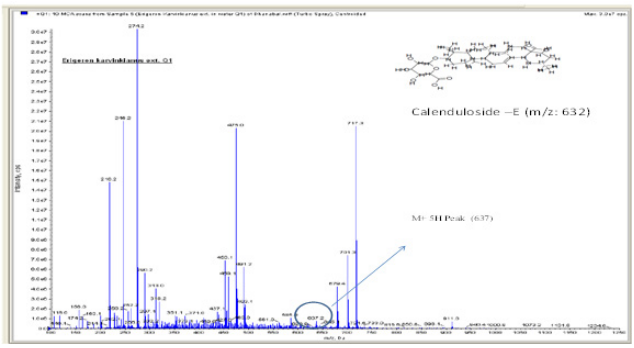


Figure 32a: LCMS Characterization of Erigeron karvinskianus Total Saponin Fraction.

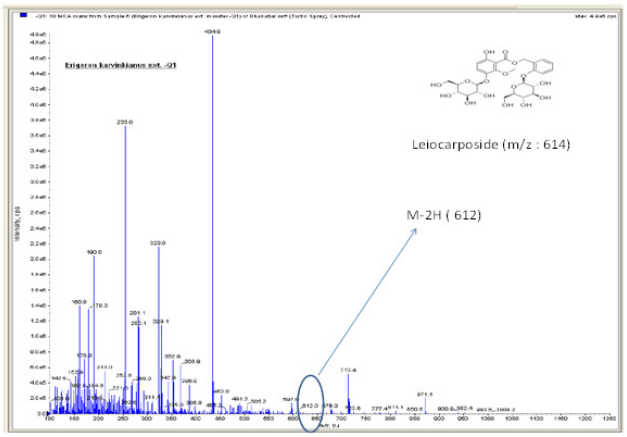


Figure 32b: LCMS Characterization of Erigeron karvinskianus Total Saponin Fraction.

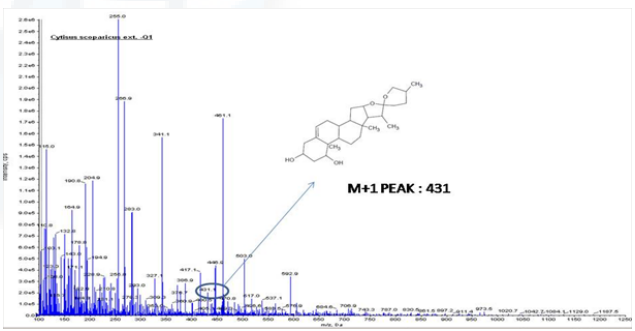


Figure 33: LCMS Characterization of *Cytisus scoparius* Total Saponin Fraction.

B. *In-vitro* cytotoxic studies

The total saponin fractions and isolated compounds showed good anticancer activities. The anticancer activity of these compounds may be due to inhibition of human topo-isomerase enzymes I & II along with other mechanisms, which is supported by molecular docking studies. The results were discussed below in the Tables 17-20 & Figures 53 & 54.

C. Wound healing studies

I. *In-vitro* wound healing studies on human dermal

fibroblast cell lines (HDF): The isolated compounds subjected to cytotoxic studies on HDF Cell lines, *in order* to know the CTC_{50} values of the isolated molecules on human dermal fibroblast cell lines. All the isolated compounds showed good cell migration and showed healing activity on HDF cell lines and the results were discussed below in the Tables 21 & 22, Figures 19 & 55.

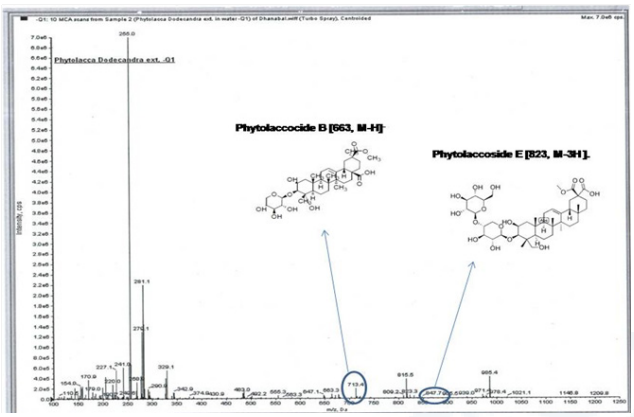


Figure 34: LCMS Characterization of *Phytolacca dodecandara* Total Saponin Fraction.

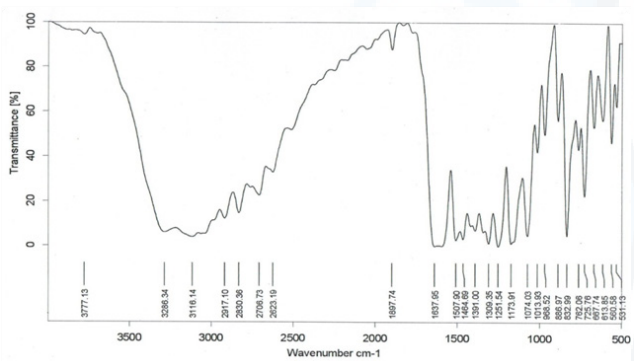


Figure 35: IR Spectra of Isolated Compound-1.

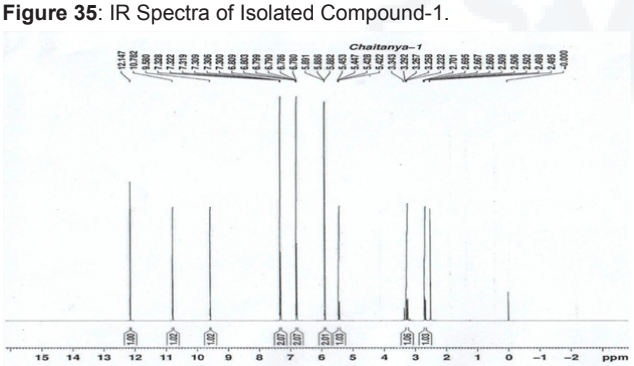


Figure 36: ^{1}H- NMR Spectra of Isolated Compound-1.

II. **TLC based bio-autography studies on *Staphylococcus aureus*:** All the isolated compounds showed good antibacterial zone of inhibition in white colour, proved that these molecules are active against *Staphylococcus aureus* bacteria. The antibacterial properties of isolated compounds were represented below in the Figure 56.

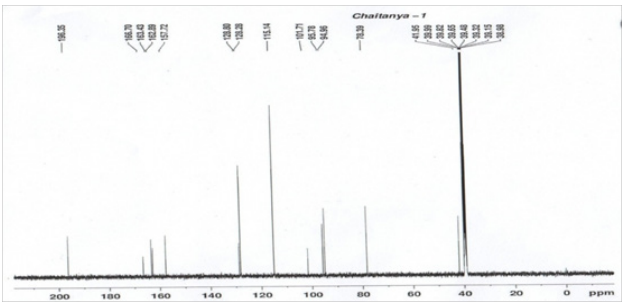


Figure 37: ^{13}C- NMR Spectra of Isolated Compound-1.

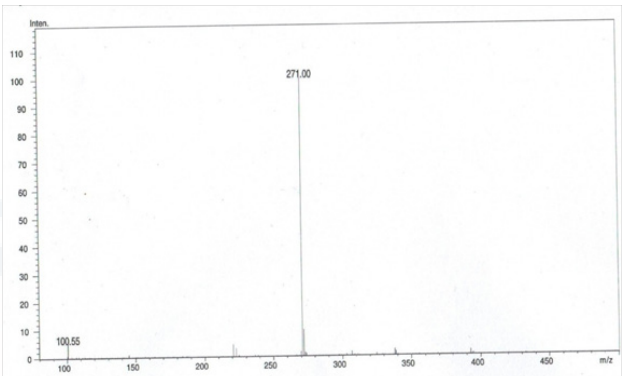


Figure 38: Mass Spectra of Isolated Compound-1.

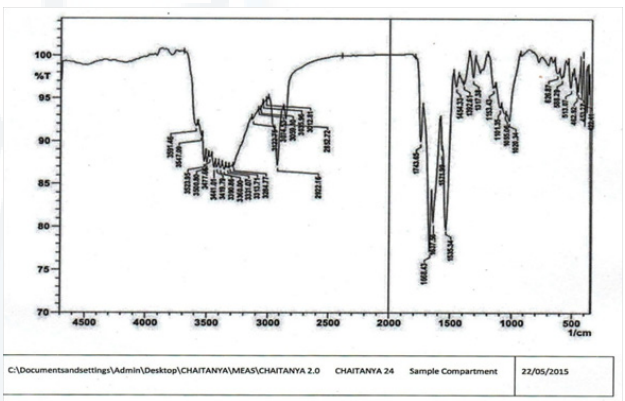


Figure 39: IR Spectra of Isolated Compound-2.

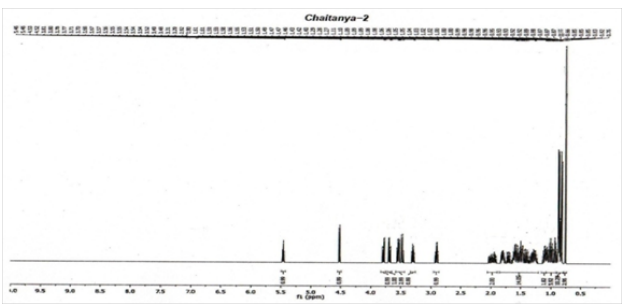


Figure 40: ^{1}H NMR Spectra of Isolated Compound-2.

III. **Molecular docking studies:** *In-silico* studies were carried out for the isolated weed molecules and weed saponins in order to know the mechanism of action

of the isolated compounds as anticancer agents in comparison to standard quericitin. All these compounds showed good g-scores against topo enzymes I & II. All these compounds are having good binding capacity and good ADMET profile. The results were discussed in the below Tables 23-26. Hence these molecules can be good novel lead sources for the discovery of human dual topo poisons

Table 15: Characterization of isolated molecules from different weeds.

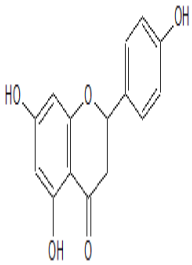
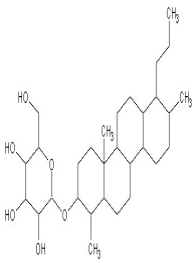
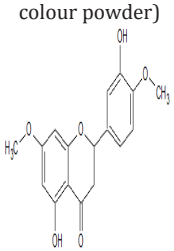
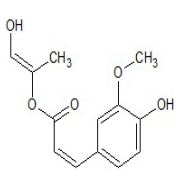
Compound name& Physical State	chemical Test	M.P	R_F (cm)	Characterization			
				IR	^{1}H NMR	^{13}C NMR	LC-MS
Compound- I (6.3% w/w yellow needle crystalline) 	Shinodas test & alkaline reagent test	250°C to 252°C	 (0.54)	3500 (O-H str) 1251(C=O str) 560(Ar C-H str) 2706(C-O str)	□5.43(D) (OH) □6.8(M) (CH) □3.38(T) (CH_2)	□ 163.43 (C) □ 95.78 (CH) □ 94.96 (CH) □ 157.72 (C) □ 196.35 (C) □41.95 (CH_2) □78.39 (CH) □128.80 (CH) □115.14 (CH) □157.72 (C) □196.35(C=O)	(M-1) Peak at –ve mode At 271.
Compound-II Buffy crystalline (1.8%w/w). 	+ Ve For Molisch, legals test and foam test	212°C to 214°C	 (0.72)	3441(O-H str) 2922(C-H str) 2852(C-H str) 1535(C-C str) 1020 (C=0 Str)	□0.96(M) (CH3) □1.06(Q) (CH3) □1.11(D) (CH3) □1.46(T) (CH)	□ 14.06 (CH3) □ 87.15 (CH) □ 74.9 (CH) □ 103 (CH) □143.06 (C) □ 119.28(CH)	M-1) Peak at –ve mode 507.36
Compound- III (2 % w/w Cream colour powder) 	Shinodas test & alkaline reagent test	228°C to 232°C	 (0.73)	1140 (C-O Str) 3407(OH Str) 669(Ar- C-H Bend) 1622 (C=O Str)	□ 6.6(s) (CH) □3.33(s) (CH_2) □4.91(s) (OH)	□56.86 (CH3) □112.0 (CH) □116.7 (C-H)	(M+1) Peak at + ve Mode 317.1
Compound –IV (6%w/w, White fine powder) 	Ferric chloride test	173 to 176 °C	 (0.62)	608 (Ar C-H) 3374 (O-H Str) 1629.38 (C=O Str) 1404 (C-H Str)	□7.1(s) (CH) □6.22 (s)(CH) □3.84 (s)(CH3) □6.19 (s)(CH) □1.96(s) (CH3)	□116.18(C) □128.7(CH2) □156(C)	(M+1) Peak at +ve mode 251.09

Table16: Antioxidant studies of various fractions and Isolated compounds using DPPH & ABTS Assays.

IC50 values (µg/ml) of ABTS assay						
	PD	**CW**	**EK**	**CA**	**CS**	**SA**
Pet. Ether	112.7±1.0	79.8±1.5	138.2±2.4	80.9±1.5	73.1±1.9	56.9±2.3
Chloroform	108.7±2.3	74.9±3.1	58.8±1.6	57.1±2.3	59.2±2.3	62.6±3.4
Ethyl acetate	56.9±1.1	57.0±3.2	56.9±2.3	59.9±2.3	58.6±2.5	66.7±2.1
Aqueous	57.0±3.8	60.8±2.6	67.3±4.2	58.7±1.4	65.3±1.9	56.8±1.2
Saponin fraction	56.7±4.2	55.9±2.2	55.6±2.3	56.2±1.8	55.4±1.8	55.0±1.5
Mother Extract	58.4±2.4	55.4±1.6	66.7±1.2	55.7±1.0	59.1±1.6	55.6±1.9
Compound -1	11.6±2.2*					
Compound -2	12.3±2.1*					
Compound -3	10.8±3.1*					
Compound-4	18.3±1.2*					
Quercetin	7.9±1.8					
IC50 values (µg/ml) of DPPH assay						
	PD	**CW**	**EK**	**CA**	**CS**	**SA**
Pet. Ether	173.8±2.3	510.8±2.6	216.9±3.2	764.1±2.4	129.6±2.3	75.3±1.3
Chloroform	177.7±1.3	29.4±1.6	632.1±2.1	156.3±1.9	249.5±2.6	533.0±2.8
Ethyl acetate	188.5±2.6	110.0±2.6	89.6±2.8	145.4±2.7	119.2±3.8	143.7±2.8
Aqueous	105.3±2.8	151.2±2.3	100.6±2.8	87.4±2.5	161.6±2.3	140.1±1.3
Saponin fraction	118.8±2.7	119.9±1.7	186.0±4.0	85.0±2.6	126.9±3.4	101.7±1.7
Mother Extract	132.6±3.1	122.8±3.5	248.0±3.2	130.0±3.5	272.9±2.9	109.2±2.6
Compound-1	24.1±2.5*					
Compound-2	26.3±2.6*					
Compound-3	22.5 ±2.3*					
Compound-4	25.2±1.1*					
Quercetin	6.94±1.2					

(PD: Phytolacca dodecandra; CW: Cnicus wallichi; EK: Erigeron karvinskianus; CA: Cestrum aurantiacum; CS: Cytisus scoparius; SA: Solanum mauritinum.

Compound -1. Naringenin, Compound -2. Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2,7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol, Compound- 3 . 7-Methoxy Hesperetin & Compound- 4. (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoate). The values are mean ± S.E.M, n=6; * implies P < 0.05 when compared to Standard Quercetin using annova two way.

Table 17: *In- vitro* Cytotoxic Studies of Total Saponin Fractions.

Total Saponin Fractions	Cell Lines (µg/ml)			
	VERO	**A549**	**MCF7**	**HCT116**
Phytolacca Dodecandra	440.08±2.9	185.25±1.6*	68±0.57*	165.833±3.0*
Cytisus Scoparius	>1000	295.833±1.3*	96.333±1.45*	206.917±3.9*
Erigeron Karvinkianus	655.5±0.86	205.917±2.9*	213.583±0.8*	246.75±0.8*
Cnicus wallichi	>1000	311.25±0.72*	97.583±0.93*	208±1.73*
Solanum mauritianum	851.8±1.0	288.917±1.9*	190±1.32*	169.5±1.6*
Cestrum aurantiacum	>1000	170.5±1.7*	144.5±1.8*	310.583±1.3*
Std Quercetin	390.6±0.53	155.3±0	36.25±0.14	20.0± 0.12

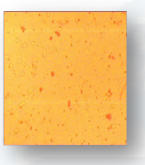
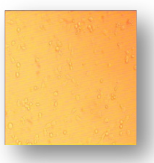
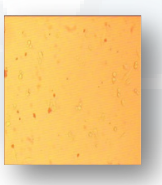
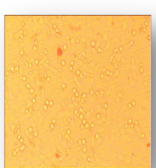
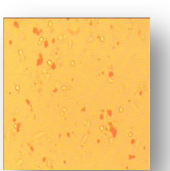
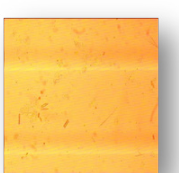
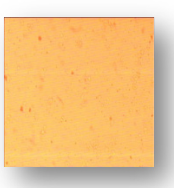
The values are mean ± S.E.M, n=6; * implies P < 0.05 when compared to Standard Quercetin using annova two way.

Table 18: *In- vitro* Cytotoxic Studies of Isolated Compounds.

Name of Compound	Vero	A-549	MCF-7	HCT-116
(Compound-I)	472.8±1.60	92.5±1.32*	42.8±1.36*	86.8±1.013*
(Compound-II)	641.3±0.88	53.7±1.78*	64.6±1.20*	72.8±0.60*
(Compound-III)	353.666±1.85	36.5833±0.93*	81.75±0.80*	152.33±1.45*
(Compound-IV)	383.833±1.8	74.083±1.5*	66.5±1.0*	50.583±1.3*
Std Quercetin	390.6±0.53	155.3±0*	36.25±0.14*	20.0± 0.12*

The values are mean ± S.E.M, n=6; * implies P < 0.05 when compared to Standard Quercetin using annova two way.

Table 19: *In vitro* Cytotoxic Screening of Total Saponin Fractions using SRB assay.

S. No.	Sample	Types	VERO	A549	MCF7	HCT116
1	C.a.S	Normal				
2		50%				
3	SmS	Normal				
4		50%				
5	CwS	Normal				
6		50%				

7	EkS	Normal				
8		50%				
9	CsS	Normal				
10		50%				
11	PdS	Normal				
12		50%				

Cas: Cestrum aurantiacum saponins; SmS: Solanum mauritianum Saponins; CwS: Cnicus wallichi Saponins; EkS: Erigeron karvinskianus Saponins; CsS: Cytisus scoparius Saponins; PdS: Phytolacca dodecandara Saponins.

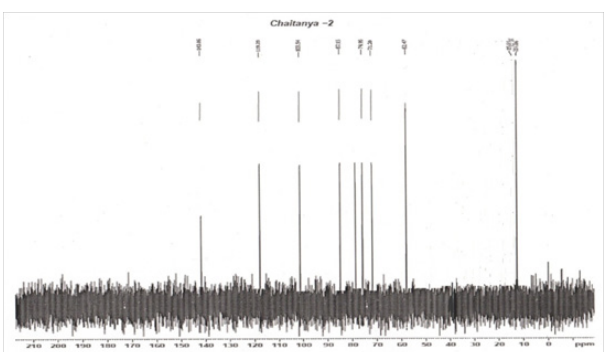


Figure 41: ^{13}C NMR Spectra of Isolated Compound-2.

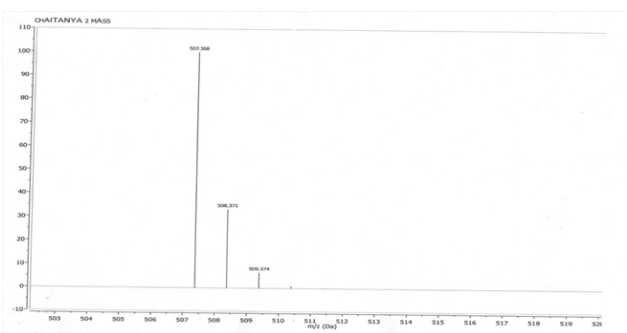
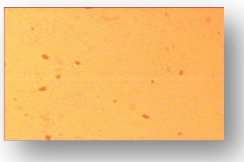
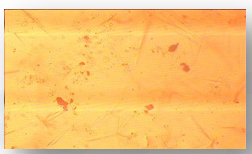
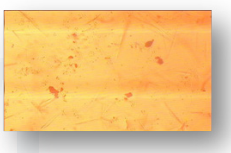
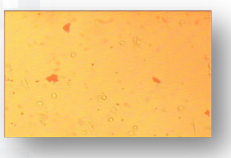
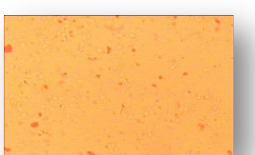
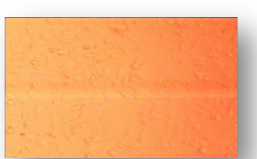
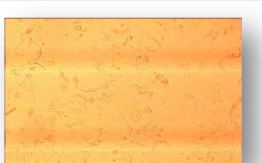
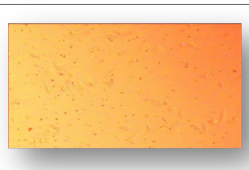
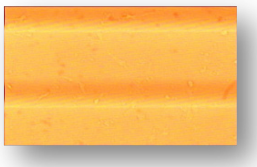
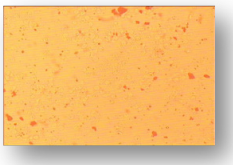
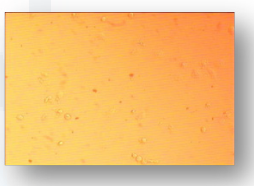
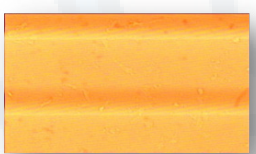
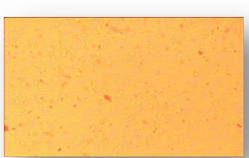
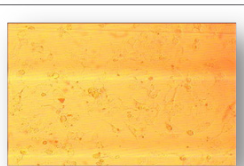
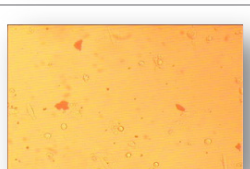


Figure 42: Mass Spectra of Isolated Compound-2.

Table 20: *In vitro* cytotoxic screening of isolated molecules using SRB assay.

S. No.	Compound Name	Cell Line	Normal Cell before Lysis	50% Toxicity
1	Compound-3	VERO		
2		HCT116		
3		MCF7		
4		A549		
5	Compound- 2	VERO		
6		HCT116		
7		MCF7		
8		A549		

9	Compound- 4	VERO		
10		HCT116		
11		MCF7		
12		A549		
13	Compound-1	VERO		
14		HCT116		
15		MCF7		
16		A549		

Compound -1. Naringenin, Compound -2 . Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2,7,10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol, Compound- 3 . 7-Methoxy Hesperetin & Compound- 4. (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate).

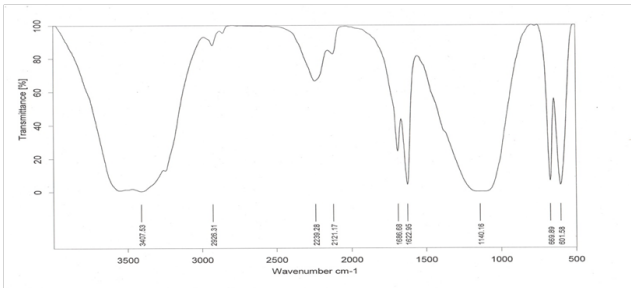


Figure 43: IR Spectra of Isolated Compound- 3.

Table 21: *In-vitro* cytotoxic studies of isolated compounds on HDF Cell lines.

Compound Name	CTC50 (µg/ml)
Compound-1	664
Compound-2	825
Compound-3	635.5
Compound – 4	545

Compound- 1. Narangenin, Compound-2, Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol,Compound – 3, 7-Methoxy Hesperetin, Compound -4, (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate).

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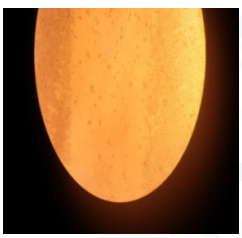
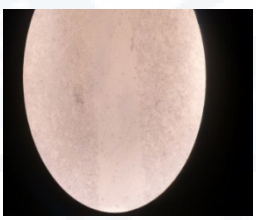
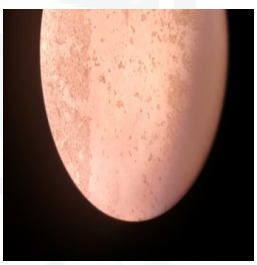
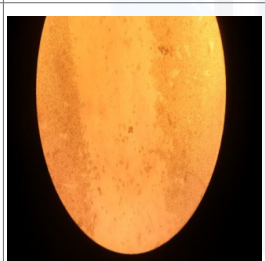
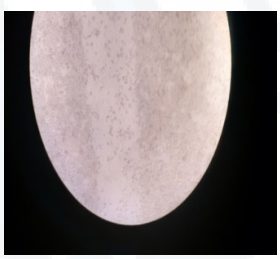
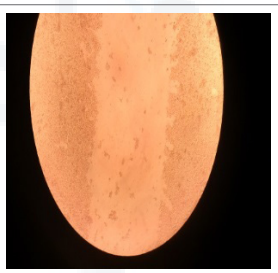
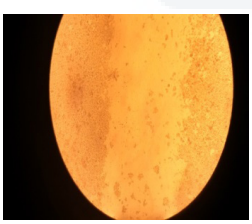
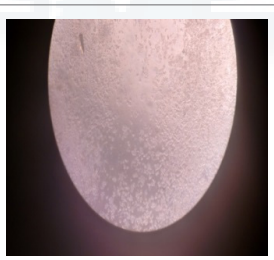
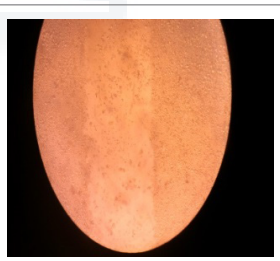
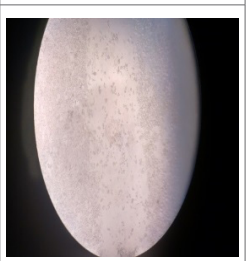
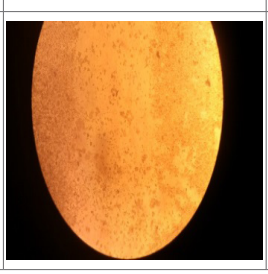
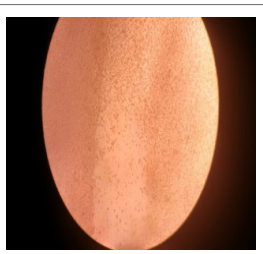
Compound Name	Compound-1	Compound 2	Compound 3	Compound 4
Concentra-tions (µg/ml)	35.1±1.23	46.8±2.36	50.0±1.57	26.5±1.43
Time Intervals / 0 hr				
8 hr				
24 hr				
48 hr				

L. Adoniside Agrimonia eupatoria L. Agrimophol Ammi visnaga (L.) Lamk. Khellin Anabasis aphylla L Anabasine Andrographis paniculata Nees Andrographolide Artemisia annua L. Artemisinin Atropa belladonna L. Atropine Berberis vulgaris L. Berberine Brassica nigra (L.) Allyl isothio

cynate Centella asiatica (L.) Asiaticoside Cissampelos pareira L Cissampeline Colchicum autumnale L Colchicine **C**

S.No	Name of the	1K4T						3AL2					
	Compound	G-Score	H-bond	Penalties	HB Penal	Rot Penal	Epik-stael	G-Score	H-bond	Penalties	HB Penal	Rot Penal	Epik stael
1	PROTODIOSCIN	-8.62	-6.31	0	0	0.03	0	-10.09	-6.92	0	0	0.09	0
2	PARQUISOSIDE-B	-8.6	-5.35	0	0	0.08	0	-9.25	-5.56	0	0	0.03	0
3	CALENDULOSIDE E	-8.29	-1.74	0	0	0.05	0.01	-9.08	-5.67	0	0	0.19	0.15
4	LEIOCARPOSIDE	-8.38	-3.53	0	0	0.19	0.15	-8.57	-5.14	0	0	0.08	0
5	SOLASONINE	-8.04	-4.89	0	0	0	0.01	-8.11	-5.35	0	0	0	0.01
6	NOCTURNOSIDE-B	-7.88	-5.18	0	0	0.08	0	-7.51	-4.88	0	0	0.09	0.01
7	COMPOUND-1	-6.94	-3.03	0	0	0	0.01	-5.15	-4.59	0	0	0.08	0
8	QUERCETIN	-6.7	-2.65	0	0	0.09	0.01	-4.95	-2.44	0	0	0	0.01
9	PARQUISOSIDE-A	-6.59	-4.98	0	0	0.09	0	-4.53	-1.63	0	0	0.09	0
10	COMPOUND-3	-6.51	-2.11	0	0	0.09	0	-4.35	-2.37	0	0	0.48	0
11	COMPOUND-4	-6.14	-2.44	0	0	0.48	0	-4.02	-2.62	0	0	0.05	0.01
12	SALVICINE	-4.94	-1.73	0	0	0.3	0	-3.94	-3.1	2	0	0.09	0.01
13	CAMPTOTHECIN	-4.49	-1.29	1	0	0	0	-3.84	-0.96	0	0	0	0
14	COMPOUND- 2	-4.23	-1.69	1	0	0	0	-2.94	-0.69	0	0	0	0
15	RUSCOGENIN	-3.86	-1.1	0	0	0	0	-2.71	-2.17	1	0	0.14	0
16	LUPEOL	-3.19	-1.29	1	0	0.05	0	-2.49	-0.69	0	0	0.05	0
17	SOLASODINE	-2.2	-0.7	0	0	0	0.01	-1.98	0	1	0	0	0.01

s Roth Monocrotaline Convallaria majalis L Convallatoxin Cytisus scoparius (L.) Sparteine Lobelia inflata L. Lob**e**

S.No	Name of the	1ZXN (TOPO II)						3QX3 (TOPO II)					
	Compound	G-Score	H-bond	Penalties	HB Penal	Rot Penal	Epik-stael	G-Score	H-bond	Penalties	HB Penal	Rot Penal	Epik stael
1	LEIOCARPOSIDE	-10.54	-4.45	0	0	0	0.15	-11.59	-4.81	0	0	0	0.15
2	QUERCETIN	-9.57	-4.65	0	0	0	0.01	-7.24	-2.29	0	0	0	0.01
3	COMPOUND-4	-7.53	-3.11	0	0	0	0	-6.59	-0.48	0	0	0	0
4	COMPOUND- 2	-7.15	-4.29	0	0	0	0	-10.38	-4.87	0	0	0	0
5	SALVICINE	-7.05	-2.31	0	0	0	0	-6.9	-0.48	0	0	0	0
6	COMPOUND-1	-6.8	-2.96	0	0	0	0.01	-7.15	-1.79	0	0	0	0
7	COMPOUND-3	-6.56	-2.1	0	0	0	0	-3.9	-1.66	0	0	0.09	0
8	LEOCARPOSIDE	-5.04	-0.96	0	0	0	0	-11.59	-4.81	0	0	0	0.15
9	RUSCOGENIN	-4.74	-0.66	0	0	0	0	-5.28	-0.35	0	0	0	0
10	CAMPTOTHECIN	-4.34	-0.96	0	0	0	0	-6.39	-0.62	0	0	0	0
11	SOLASODINE	-3.95	-0.48	0	0	0	0.01	-10.96	-4.26	0	0	0	0.01
12	LUPEOL	-3.83	-0.61	0	0	0	0	-4.6	0	0	0	0	0
13	NOCTURNOSIDE B	-3.14	-0.29	0	0	0	0	-13.46	-8.16	0	0	0	0
14	CALENDULOSIDE B	-2.96	-1.44	0	0	0.05	0.01	-7.67	-2.09	0	0	0	0.01

anum (L.) Silymarin Rorippa indica (L.) Rorifone Sophora pachycarpa Schr**e**

S.NO	Name of the Compound	Mol wt	CNS	Donor HB	Acceptor HB	QPLOG HERG	Human oral Absorption	% Oral Absorption	Rule of 5	Rule of 3
1	PROTODIOSCIN	1049.19	-2	13	35.5	-6.394	1	0	3	2
2	PARQUISOSIDE-B	1193.338	-2	14	42.3	-6.818	1	0	3	2
3	CALENDULOSIDE E	632.833	-2	4	11.5	-1.011	1	46.676	1	2
4	LEIOCARPOSIDE	614.583	-2	8	19.3	-5.54	1	0	3	2
5	SOLASONINE	884.069	-2	10	27.25	-6.284	1	0	3	2
6	NOCTURNOSIDE-B	1015.8	-2	10	32.1	-5.949	1	4.311	3	2
7	COMPOUND-1	272.121	-2	2	4.95	-3.318	2	63.198	0	0
8	QUERCETIN	302.24	-2	4	5.25	-4.96	2	52.771	0	1
9	PARQUISOSIDE-A	1046.196	-2	12	35.5	-6.499	1	0	3	2
10	COMPOUND-3	316.283	-2	2	4.75	-4.976	3	75.937	0	0
11	COMPOUND-4	250.251	-2	1	5.5	-4.819	3	76.947	0	0
12	SALVICINE	330.423	-2	2	6.45	-4.802	3	94.964	0	0
13	CAMPTOTHECIN	348.357	-1	1	7.75	-5.156	3	85.865	0	0
14	COMPOUND- 2	508.737	-2	4	10.2	-4.73	3	77.565	1	1
15	RUSCOGENIN	430.626	0	2	4.9	-4.152	1	100	0	1
16	LUPEOL	426.724	1	1	1.7	-3.676	1	100	1	1
17	SOLASODINE	413.642	1	2	3.45	-4.82	3	96.273	1	1

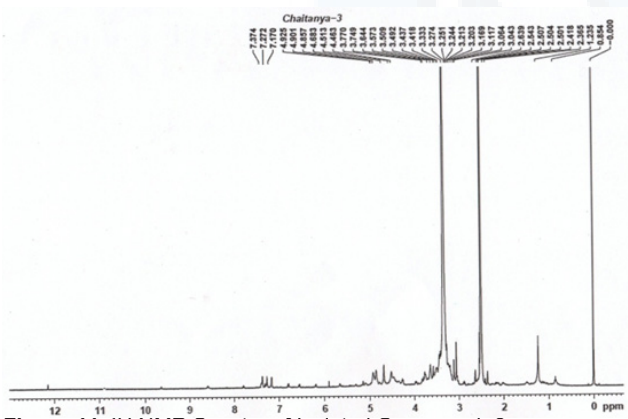


Figure 44: ^{1}H NMR Spectra of Isolated Compound- 3.

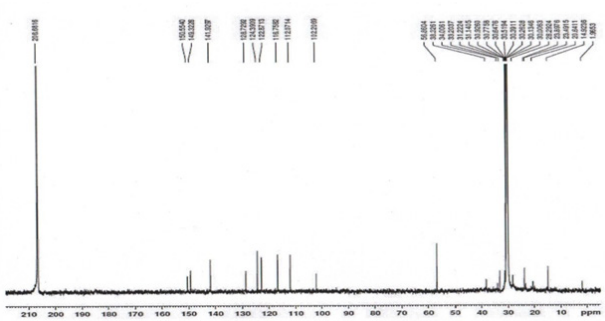


Figure 45: ^{13}C NMR Spectra of Isolated Compound- 3.

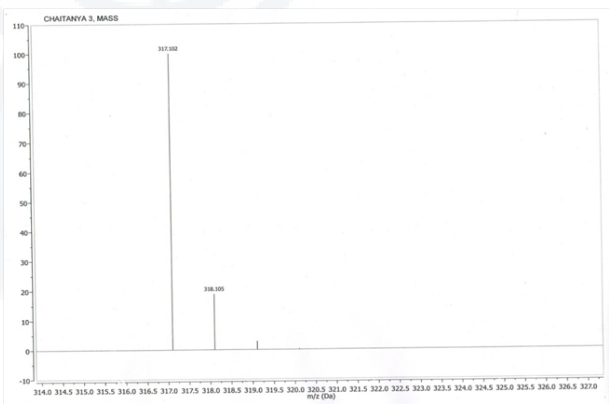


Figure 46: Mass Spectra of Isolated Compound- 3.

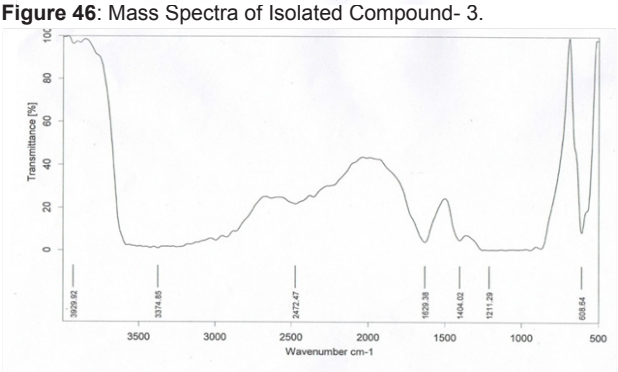


Figure 47: IR Spectra of Isolated Compound- 4.

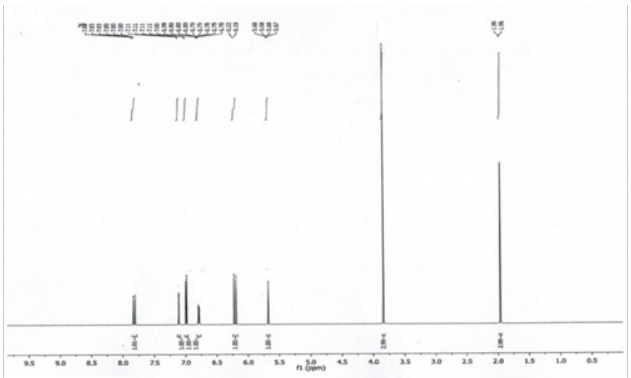


Figure 48: 1H NMR Spectra of Isolated Compound- 4.

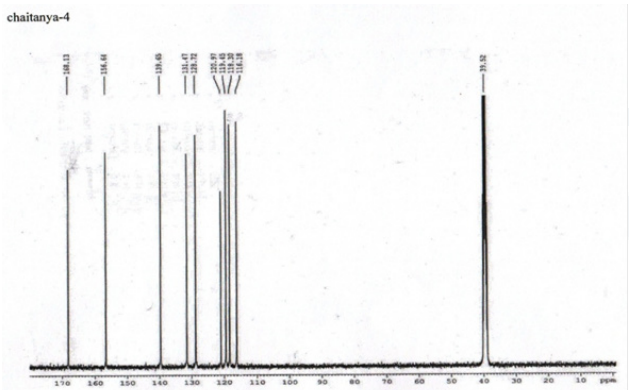


Figure 49: ^{13}C NMR Spectra of Isolated Compound- 4.

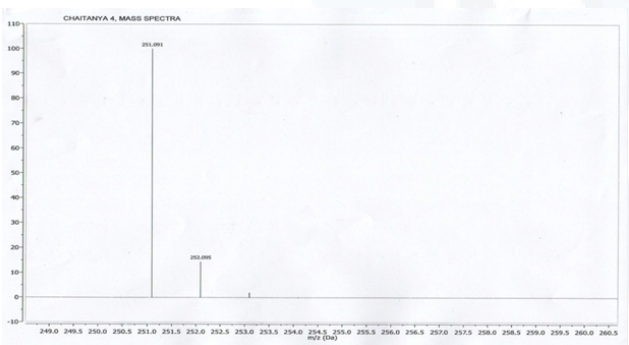


Figure 50: Mass Spectra of Isolated Compound- 4.

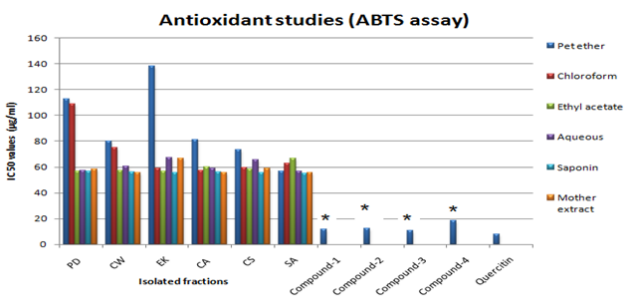


Figure 51: ABTS Assay of Isolated Fractions and Compounds.
The values are mean ± S.E.M, n=6; * implies P < 0.05 when compared to Standard Quercetin using annova two way.

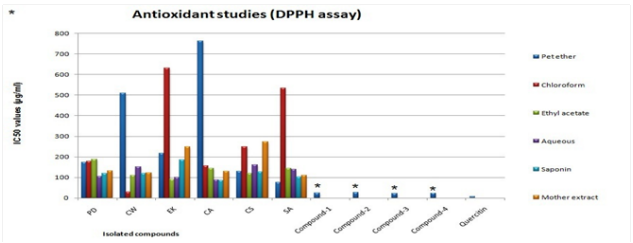


Figure 52: DPPH Assay of Isolated Fractions and Compounds.
(*PD: Phytolacca dodecandra; CW: Cnicus wallichi; EK: Erigeron karvinskianus; CA: Cestrum aurantiacum; CS: Cytisus scoparius; SA: Solanum mauritinum*). The values are mean ± S.E.M, n=6; *implies P<0.05 when compared to Standard Quercetin using annova two way.

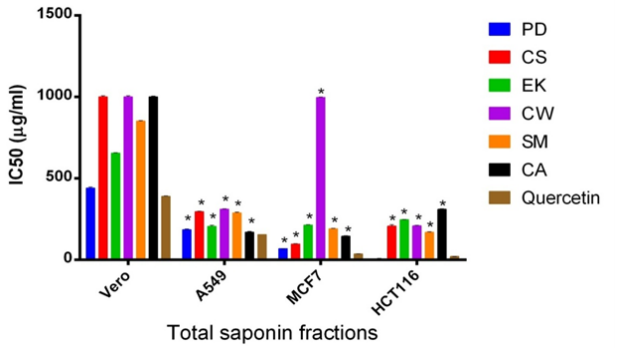


Figure 53: *In vitro* Cytotoxic Studies of Total Saponin Fractions.
(*PD: Phytolacca dodecandra; CW: Cnicus wallichi; EK: Erigeron karvinskianus; CA: Cestrum aurantiacum, CS: Cytisus scoparius; SA: Solanum mauritinum*). The values are mean ± S.E.M, n=6; *implies P< 0.05 when compared to Standard Quercetin using annova two way.

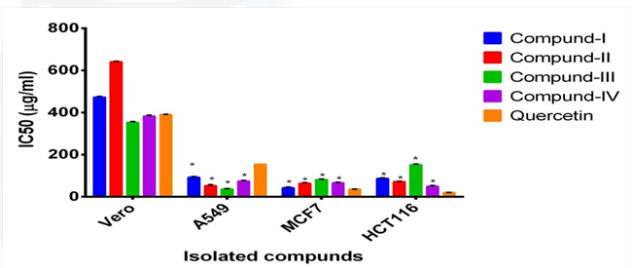


Figure 54: *In vitro* Cytotoxic Studies of Isolated Compounds.

Compound-1. Narangenin, Compound-2, Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol,Compound - 3, 7-Methoxy Hesperetin, Compound -4, (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate). The values are mean ± S.E.M, n=6; * implies P< 0.05 when compared to Standard Quercetin using annova two way.

I. **Topo drugging studies**

a. **Human Topoisomerase I assay:** The isolated molecules were subjected to human topoisomerase I assay. The compounds (I & III) are inactive against topo-isomerase I at low concentration, but the compounds (II & IV) were proved to have topopoison-I activity but at higher concentration of 400µg/ ml in compare to

standard camptothecin which is highly active at 50uM. Hence the results showed the topopoison I activity of the isolated molecules (II & IV), But at high concentrations which proved the clinical insignificant nature of these molecules and was represented in the Figure 57.

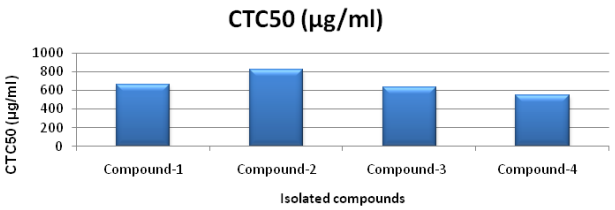


Figure 55: *In-vitro* cytotoxic studies of isolated compounds on HDF Cell lines.

Compound-1. Narangenin, Compound-2, Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol, Compound - 3, 7-Methoxy Hesperetin, Compound -4, (1E)-1-hydroxyprop-1-en-2-yl(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate).

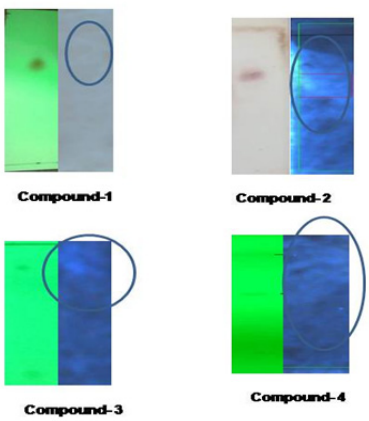


Figure 56: TLC Based Bio-Autography Studies.

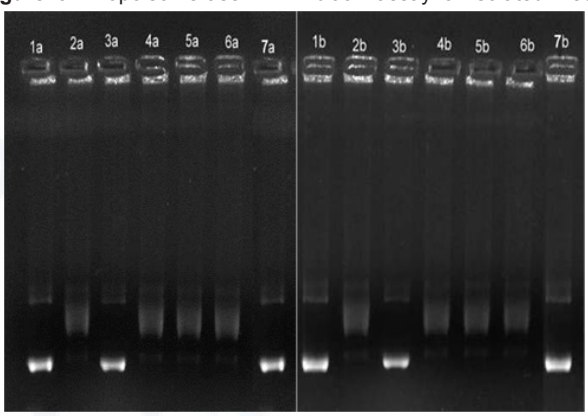
Compound-1. Narangenin, Compound-2, Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3,4,5-triol,Compound - 3, 7-Methoxy Hesperetin, Compound -4, (1E)-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoate).

b. **Human Topoisomerase II assay:** The isolated molecules were subjected to human topoisomerase II assay. The compounds (I & III) are inactive against topo-isomerase II at lower concentrations but at higher concentration of 400µg/ ml in compare to standard Etoposide (600 Um), there is a lysis in the band. Hence the results showed the topopoison II activity of the isolated molecules (II & IV), But at high concentrations which proved the clinical insignificant nature of these molecules.

However these molecules can act as lead sources for the discovery of new topo-poisons where there is very high demand. These molecules have to be taken for further extensive structural activity relationship studies, in order to increase the clinical efficacy of these molecules and were represented in the Figure 58.

c. **Cell cycle analysis:** HELA cells treated with the isolated molecule II (25µg) for 24 hours, when compared to control, in the treated cells there is an increase in the percentage of cells in the S phase showed the cells get arrested in S phase of the cell cycle. When HELA cells treated with the Compound 4 (25µg) for 24 hours, when compared to control, in the treated cells there is an increase in the percentage of cells in the G2/M phase showed that the cells get arrested in G2/M phase of the cell cycle and was represented in the following Figures 59 & 60.

Figure 57: Topoisomerase- 1 inhibition assay of isolated weed



molecules (Compound-2 & Compound-4).

Lane 1 (1a, 1b): Undigested plasmid pBR329

Lane 2 (2a, 2b): pBR 329 + Topoisomerase I (1 Unit)

Lane 3 (3a,3b): pBR329 + Topo I + Camptothecin (50uM) - Positive control Camptothecin inhibits the action of Topo I and so the plasmid is as seen in the native form

Lane 4 (4a, 4b) : pBR329 + Topo I + 50ug/ml of compound 2 & 4

Lane 5 (5a, 5b): pBR329 + Topo I + 100ug/ml of Compound 2 & 4

Lane 6 (6a, 6b): pBR329 + Topo I + 200ug/ml of compound 2 & 4

Lane 7 (7a,7b): pBR329 + Topo I + 400ug/ml of compound 2 & 4

Lane 4- (6a , b) shows the plasmid to be relaxed, as there is no effect of the test compound on the action of Topoisomerase I.

Lane (7a,7b) shows that compounds (2 &4) inhibiting the action of Topo I and so the plasmid is seen in the native form, but in very high concentration in compare to standard camptothecin, hence the compounds have to take for extensive studies for modification of structure. However these compounds can be a lead sources to develop novel topopoisons.

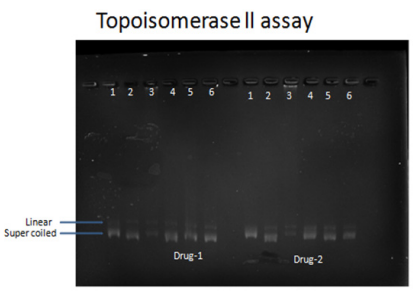


Figure 58: Topoisomerase- II inhibition assay of isolated weed molecules (Compound-2 & Compound-4).

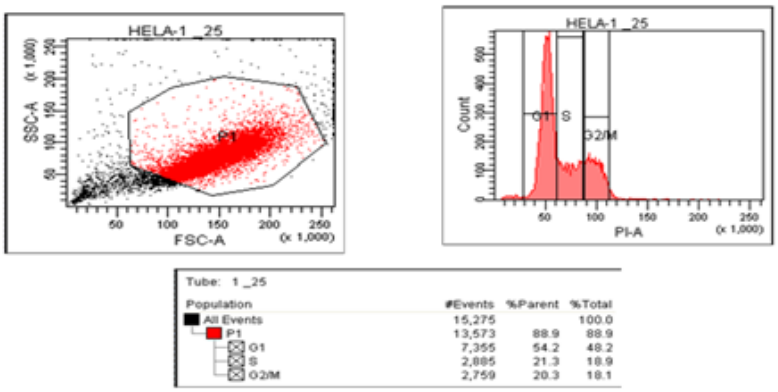


Figure 59: Cell Cycle Analysis of Compound - 2.

Compound II 25 µg

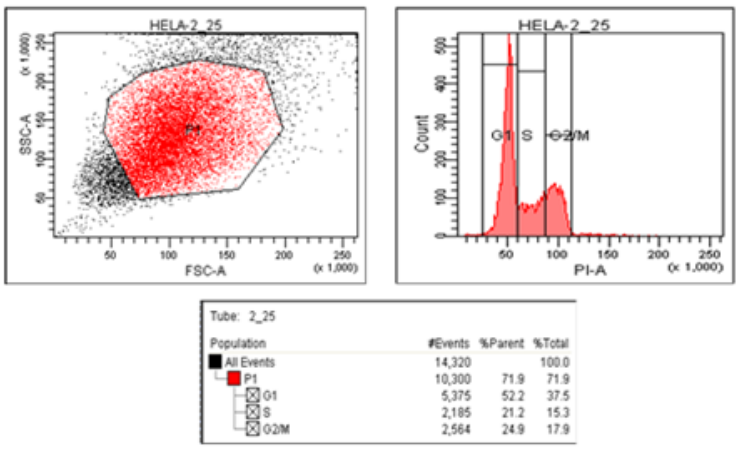


Figure 60: Cell Cycle Analysis of Compound - 4.

Table 26: Mmgbsa Scores of Identified Molecules.

S.No	Name of the Compound	MMGBSA SCORE
1.	PROTODIOSCIN	-107.418
2.	PARQUISOSIDE-B	-125.998
3.	CALENDULOSIDE E	-83.4074
4.	LEIOCARPOSIDE	-134.982
5.	SOLASONINE	-164.248
6.	NOCTURNOSIDE-B	-161.971
7.	COMPOUND-1	-135.629
8.	QUERCETIN	-79.6387
9.	PARQUISOSIDE-A	-73.3722
10.	COMPOUND-3	-59.7579
11.	COMPOUND-4	-41.1026
12.	SALVICINE	-98.8745
13.	CAMPTOTHECIN	-91.5039
14.	COMPOUND- 2	-128.763
15.	RUSCOGENIN	-97.3967
16.	LUPEOL	-55.7387
17.	SOLASODINE	-89.1578

Summary and Conclusion

Summary

i. In the present study, a field survey was carried out and 55 weeds belongs to 26 different families were identified along with their pharmacodynamic uses based on literature survey.

ii. Six Prominent weeds were selected for in depth phytochemical and biological studies.

iii. All the selected weeds were subjected to phytochemical screening, proved that these weeds having interesting secondary metabolites like cardiac glycosides, alkaloids, flavanoids, phenolics, saponins and tannins, which where proved that these metabolites may be responsible for various activities like cancer, wound healing etc.,

iv. The quantitative analysis proved that these weed hydro-alcohol extracts are rich in phenols (119.9 to 993.3µg/g gallic acid equivalents), alkaloids (106.7 to 154.8 µg/g atropine equivalents) and flavanoids (43.0 to 159.6µg/g quercetin equivalents) and saponins (14.2 to 25.0 % w/w), which proved that these weeds are having high amounts of these secondary metabolites which can be a good bio-prospecting tools for drug discovery of new anticancer lead molecules.

v. The LC-MS and HPTLC finger printing of isolated total saponin fractions proved that these fractions having many important saponin molecules of known and many unknown moieties, laid a good platform for discovery of new lead molecules.

vi. Nocturnoside-A (1014, M-H), were identified from *Cestrum aurantiacum*_(Solanaceae) and first time from *Solanum mauritinum* (Solanaceae) total saponin fractions. Lupeol (427, M+H) , Alpha amyrin (429, M+ 2H) were identified first time from saponin fractions of *Cnicus wallichi* (Asteraceae), Calenduloside- E (637 ,M+5H), Leiocarposide 612 (M-2H) were identified first time from saponin fraction of *Erigeron karvinkianus* (Asteraceae), Ruscogenin (431, M+1) was identified first time from the saponin fraction of *Cytisus scoparius*_(Fabaceae) and Phytolaccocide B (663, M Peak), Phytolaccoside A (823, M Peak) were identified from the *Phytolacca dodecandra*_ (Phytolaccaceae) saponin fraction.

vii. Two compounds Naringenin (Compound-1) and 7 - Methoxy hesperetin (Compound-3) were isolated first time from the ethyl acetate fraction of aerial parts of *Erigeron karvinskianus* using flash chromatography.

viii. First time a novel compound (compound-2, a saponin glycoside), Tetrahydro-2-(hydroxymethyl)-6-(octadecahydro- 2, 7, 10a-trimethyl-1-propylchrysen-8-yloxy)-2H-Pyran-3, 4, 5-triol was isolated from the aerial parts of *Solanum mauritianum*.

ix. First time a novel phenolic compound (Compound -4, (1E-1-hydroxyprop-1-en-2-yl (2E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoate) was isolated from the *Cytisus scoparius*_using flash chromatography technique. The structures of these isolated compounds were characterized by using IR, NMR and Mass analysis.

x. The total saponin fractions isolated from the six weeds proved to have good antioxidant activity through DPPH and ABTS assay with IC_{50} values of 55 - 56µg/ml and 85 -126µg/ml and the isolated compounds showed potent IC_{50} values of 11 - 18µg/ml and 22 - 26µg/ml which proved that these weeds having many good antioxidant lead moieties, where there is a current demand.

xi. The cytotoxic studies revealed that these isolated molecules are having potent cytotoxic activity on various cell lines like A 549, MCF-7 and HCT-116 with CTC_{50} values ranging from 20.0 to 92.0 µg/ml and proved to be safe on Vero cell lines with CTC_{50} values 383 to 472 µg/ml in compare to standard quercetin.

xii. All the isolated compounds showed good *in-vitro* wound healing activity on HDF cell lines with active concentration level of 50- 12.5 µg/ml, showed good migration of cells. This showed that these isolated molecules also having good wound healing properties which are important criteria for the molecules having anticancer activity.

xiii. The molecular docking studies proved that these isolated molecules are good dual topo-poisons I & II having good ADMET profiles and binding properties in-compare to standard camptothecin, salvicine and quercetin. The docking studies proved that the cytotoxic induced apoptosis and wound healing properties of the isolated compounds may be due to dual topoisomerase I & II inhibition.

xiv. The isolated compound -2 and compound -4 showed topoisomerase I &II poisoning activity at concentration of 400 µg/ml in compare to standard camptothecin (50 µM) showed that these compounds are active at very high dose and may be clinically insignificant, hence these molecules have to take further for semi synthetic structural modification studies inorder to increase the clinical efficacy properties.

xv. HELA cells treated with the isolated molecule II (25µg) for 24 hours, when compared to control, in the treated cells there is an increase in the percentage of cells in the S phase showed the cells get arrested in S phase of the cell cycle. When HELA cells treated with the Compound 4 (25µg) for 24 hours, when compared to control, in the treated cells there is an increase in the percentage of cells in the G2/M phase showed the cells get arrested in G2/M phase of the cell cycle.

Conclusion

Based on the field survey, the Nilgiris have many rich unexplored weed flora, hence the current research given a platform to expose the phytochemical and biological documentation of these weed flora, proved that these weeds can be an important bio-prospecting tools for drug discovery of novel anticancer leads (Dual Topo-poisons I & II). The phytochemical studies proved that these weeds are reserve houses for many unknown secondary metabolites. The biological screening and molecular docking studies, the isolated compounds proved that they are a good cytotoxic molecules and the mechanism of apoptosis is may be due to dual human topo-poisoning I & II activity. As there is a current demand for these novels dual human topo- poisons, the selected weed may have a richest source of many secondary metabolites which can be a good lead molecules for discovery of dual human topo poisons I & II. The isolated compounds can be good lead molecules in anticancer drug discovery. The topo-poison I and II studies proved that the compounds II & IV only having Dual topo-poisoning activity. However, further in-depth studies have to be carried out on these identified molecules and the plant in order to increase its clinical significance through structure modification of functional groups. The isolated molecules can serve as lead molecules towards anticancer drug discovery. The current research work may give a platform for the discovery of novel dual human topo I & II isomerase poisons, where there is a current demand in the anticancer research. Hence the research work proved that these weeds have good medicinal values and also can be utilized commercially and be good economical sources to farmers as they having many good novel molecules.

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