

Chapter 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 IMPORTANCE OF ETHNOMEDICINAL SURVEY

Ethnomedicinal surveys provide information about traditional uses of medicinal plants by indigenous communities of a particular area (Ford *et al.*, 1994; Verpoorte *et al.*, 2005). These surveys also focus on the development of drugs using plant products. In recent years, the studies on medicinal plants have gained immense attention among the scientific communities (Tripathi *et al.*, 2017) because they serve as an alternative for high cost synthetic drugs used for personal health care (Hoareau and DaSilva, 1999). Recently WHO (World Health Organization) has also recognized the significance of traditional medicine in the healthcare sector (Ekor, 2014 and Yigezu, 2014). Ethnomedicinal plants when scientifically evaluated, provides evidence-based alternative medicines which establish the basis of herbal drug industry along with the discovery of drug targets in the pharmaceutical industry (Patwardhan, 2005). Traditional medicine is regarded as one of the important parts of the primary health care system in most of the developing countries (Akerle, 1998). It is therefore necessary to understand the concept of traditional medicine to meet our health needs now and in the future (Omosehinde, 2006).

There are several advantages of traditional medicine for the people of rural areas as the healers are usually found within a close proximity to their homes. They are also familiar with the patient's culture and environment and the costs of such treatments are very less (Cheikhoussef *et al.* 2011). However, indigenous knowledge of medicinal plants used for healing human ailments is in risk of gradually becoming perished, because it is generally passed from one generation to another orally without any written records (Kaido *et al.*, 1997). Most of the ethnic cultures possess a vast store of undocumented traditional knowledge of herbal remedies for the treatment of various ailments (Offiah *et al.*, 2011). Thus it has now become more important than ever to document and conserves the traditional herbal knowledge, in order to support the development of new drugs and possibly to find enhanced applications of traditional medicine (Kone and Atindehou, 2008). Documentation of traditional medicine may also

help to protect a vital part of an indigenous people's cultural heritage of a particular area for future generations. Ethnomedical study is regarded as the most useful method of identification of new medicinal plants or refocusing on those plants documented earlier for the extraction of beneficial bioactive compounds (Thirumalai *et al.*, 2009). Modern medicinal facilities are now taking high interest in plants' herbal medicines. Such activities may lead to disappearance of traditional use and knowledge of herbal wealth. It is therefore important to record and conserve the valuable knowledge of the medicinal uses of locally available plants to treat different diseases.

2.2 ETHNOMEDICINE IN SIKKIM WITH EMPHASIS ON WEST DISTRICT

Sikkim is a small hilly state in the Eastern Himalayas of India. Hooker (1871-97) was the first to study the flora of Sikkim, which was later followed by Biswas (1956) with a comprehensive study on medicinal plants of Darjeeling and Sikkim. A varied topography of Sikkim has resulted in harboring a large biodiversity and is known to be a biodiversity hotspot (Hajra and Verma, 1996). The geographical area of the state has about 80% of forest which consists of an estimated of over 4500 species of flowering plants including many important medicinal plants. The Himalayas have attracted many researchers with its enormous ethno-medicinal wealth which have been used by the ethnic people for thousands of years. Jha *et al.* (2004) recorded medicinal plants commonly used by the Lepcha people of Sikkim. About 118 plants used by the Lepcha tribe of Sikkim inhabiting in the Dzongu valley was documented (Badola and Pradhan, 2008). They also documented traditionally useful medicinal plants used by Limboos residing in southwest of Khangchendzonga Biosphere Reserve (Badola and Pradhan, 2013). Similarly, Panda (2012) has reported 26 medicinal plants used in the primary healthcare sector of Sikkim. Das *et al.* (2012) also enumerated the use of 79 plant species by the rural people of Sikkim.

Medicinal plants against gastrointestinal tract disorders used by the herbal healers of Sikkim were recorded by Chanda *et al.*, 2007 which included 36 plants. It was reported that the indigenous people of Rangit valley, South Sikkim use around 45 plant species distributed across 36 families curing about 20 ailments (Idrisi *et al.*, 2010). Dash

(2009) discussed the uses of 167 plants species for dealing with 27 major ailments among ten rural communities of Sikkim.

Some recent work was done by Jha and Jha (2016) on Sherpas of Sikkim who exploited ten medicinal plants which were also used in traditional Tibetan medicines along with modern medication systems.

West district of Sikkim is the second largest district situated between 27°00'46" -28°07'48" N latitude and 88°00'58- 88°55'25" longitude covering an area of 1166 Sq. Km. The altitude ranges from 300m to 8,568m above sea level. The landscape of West Sikkim is a hilly region covered mostly by forest and large population settling in the forest fringe areas. The district comprises of two sub-division viz., Soreng and Gyalshing and nine blocks. The major ethnic communities of district are Lepcha, Bhutia and Nepali. Despite being the second largest district it was noted that the work regarding ethnomedicinal survey in this region was negligible. Only few reports were found including the 36 anti-hypoglycemic plants of west district of Sikkim by Gurung *et al.*, (2014). Another ethnobotanical survey was done on the threatened medicinal plants of West Sikkim (Tamang *et al.*, 2017). The medicinal plants in Sikkim only exist in the wild and are not cultivated in private fields. In current scenario, the collection of medicinal plants has been highly harvested not only for personal use but to sell to local markets as well as in bigger markets. The problem is the people who use these herbs are not taking any action to regenerate them. This gradually will result in loss of precious and valuable plants. Considering the seriousness of the situation, the Forest Department of the Government of Sikkim has restricted the collection of some important medicinal plants such as *Swertia chirata*, *Heracleum wallichii*, *Picrorhiza kurrooa*, *Nardostachys jatamansi*, *Orchis latifolia* etc. The Forest Department has also taken initiative of planting some of the medicinal plant species in their natural habitat. The G.B. Pant Institute of Himalayan Environment and Development, Sikkim Unit, is also carrying out research on conservation and development of these endangered plant species (Singh *et al.*, 2002).

2.3 NECESSITY OF PHARMACOGNOSTIC STUDIES

Recently there is a huge interest growing in drugs originated from natural sources as they are considered to be safer than the synthetic ones. The main advantages of natural drugs are their easy availability, low cost with less or no side effects. However the disadvantage is that they are having high risk of adulteration. If a natural drug is effective, its demand will increase which in turn increases the chances of non-availability. Thus to meet the growing demand, the plants used for making these drugs are collected randomly as well as the drugs can easily be adulterated with low grade materials. Therefore it is necessary to maintain reproducible efficacy and safety of these natural drugs if they have to be considered as rational drug for which they should be standardized and their pharmaceutical quality must be approved (Bauer *et al.*, 1993).

Adulteration is replacing original plant with another plant or presence of foreign substances which could have been done either intentionally to gain more profit or due to careless handling during the preparation time. Therapeutic value of medicinal plants depends upon the presence of chemical constituents in it. Herbal medicine or natural products can be misused due to wrong identification of wild plants. One of the major reasons is one common vernacular name given to two or more totally different species (Kumar, 2007). Reports are there about poisoning due to confusion in herbal drugs and have raised international concern. These problems can be overcome by pharmacognostic studies of medicinal plants. It is very essential to carry out pharmacognostic studies of medicinal plants which are used for preparing various drugs. Unlike taxonomic identification, pharmacognostic study helps in identifying plants even in dry powder form when it has lost its morphological identity as well as to identify the adulterants present in it. Such studies can be useful in authentication of the plants ensuring the quality of herbal products leading to safety and efficacy of natural products.

At present, plenty of methods are available to authenticate crude drugs, from simple morphological investigation to physical and chemical assessment. However, microscopic analysis is still the most easy and useful method for primary authentication

(Zhao, 2010). It is the most commonly used technique to authenticate herbal medicines in India and many other countries because it is fast, requires low cost and small amount of sample (Singh *et al.*, 2018). Industry also put emphasis on powder microscopy as these tests are approved in pharmacopoeial monographs. Moreover, minimum expertise and equipment is required and the botanical identity of the plant can be established within seconds (Narayana 2010; Dobriyal and Ananthanarayana, 2009). Besides, herbal medicines are known for its low cost so the price should not be increased because of unnecessary highly sophisticated methods used for authentication. Although powder microscopy has been provided in various pharmacopoeias, but description of characters is not extensive in nature and very small amount of information is provided about microscopic characterization of herbal drugs in powder form.

Some recent pharmacognostic studies were done on *Eranthemum nigrum* stem (Prasanth and Rao, 2018), *Limonium stocksii* (Kanakiya *et al.*, 2018), *Trianthema portulacastrum* L. (Pande *et al.*, 2018), *Andrographis echioides* Nees. and *Tridax procumbens* L. leaf and stem (Pande *et al.*, 2018), leaves of *Wedelia trilobata* L. (Karthika and Manivannan, 2018). The parameters they have used and the standards they have established for these plants can be used to compare and authenticate the drugs prepared from them as future reference.

2.4 FREE RADICALS AND OXIDATIVE STRESS

A free radical is a chemical species either an atom, a molecule or an ion containing unpaired electron in an atomic orbital. There are mainly three sources of free radicals: internal, external and physiological. Internal sources include mitochondria, enzyme oxidases, xanthine, phagocytes, reactions involving iron and other transition metals, peroxisomes, inflammation etc. Some external sources of free radicals generation are smoking, environmental pollutant, ultraviolet light, radiations, ozone, pesticides, certain drugs, anesthetics and industrial solvents. The physiological sources are mental status like emotion, stress etc. and disease conditions (Halliwell *et al.*, 2006). However one for the most common sources is the mitochondria using 90% of the O₂ in human body (Wickens, 2001). Many free radicals are unstable and reactive in nature.

They donate or accept an electron from other molecules, thus acting as oxidants or reductants (Cheeseman and Slater 1993). These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates and lipids (Young and Woodside, 2001). The majority of free radicals that damage biological systems are oxygen-free radicals, and these are known as “reactive oxygen species” (ROS). The most important oxygen free radicals are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical etc. ROS can cause various damages to biological macromolecules such as nucleic acids, proteins and lipids. Oxidation of the nucleic acids (DNA and RNA) causes mutations in mitochondrial as well as nuclear genomes (Hekimi, 2011). Almost all amino acids of a protein or an enzyme can be oxidized by ROS. This oxidation can cause modifications which lead to alteration or elimination in their function (Ugarte, 2010). The term “oxidative stress” represents a shift towards the pro-oxidants in the pro-oxidant/antioxidant balance which have occurred due to an increase in oxidative metabolism. ROS not only cause lipid peroxidation and oxidative DNA damage, but also cause regulation of intracellular signal transduction and physiologic adaptation phenomena. Oxidative stress causes the highest risk in central nervous system of the body. Confirming the function for ROS in nervous system especially in brain aging (Poon *et al.*, 2004), many studies point out ROS level and neurodegenerative diseases of neural cells specifically causing death of astrocytes (Rouach, 2004) and neurons (Sonee *et al.*, 2003) by both necrosis and apoptosis. There are many more evidences of ROS involved in degenerative disorders and neuronal aging. Some studies also show the role of oxidative stress in degeneration of dopaminergic cells in Parkinson’s disease (Jenner, 2003). Oxidative stress was found to be one of the first observed processes in Alzheimer’s disease (Honda *et al.*, 2004). Considering the studies carried out up to this time, we can say that there is a strong relationship between free radical induced oxidative stress and lifestyle-related diseases.

2.5 PLANTS AS NATURAL ANTIOXIDANTS

An antioxidant is “any substance that slows down, prevents or removes any damage caused by oxidative stress to a target molecule” (Halliwell, 2007). Humans

have evolved a highly sophisticated and complex antioxidant protection system to protect the cells and organ systems of the body against ROS. Antioxidants inhibit the oxidation process even at small concentration thus contributing in various physiological systems in the body. Their function is neutralizing or deactivating free radicals before ROS could attack any cells or sub-cellular compartments (Jacob, 1995). Free radicals and other ROS are produced in human body by various biochemical and physiological processes (Halliwell, 1994); however, their overproduction could lead to the development of numerous diseases. Antioxidants act at different levels by (i) prevention of chain initiation caused by scavenging initiating radicals, (ii) decreasing localized oxygen concentration, (iii) decomposition of lipid peroxides to peroxy and alkoxy radicals, (iv) decomposition of peroxides by converting them to non radical products, and (v) breaking chain to prevent continued hydrogen abstraction (Miguel, 2010). Sometimes antioxidant system present in our body is not enough to decrease the free radicals produced in our body. This problem can be overcome by taking supplementary antioxidants. Synthetic antioxidants such as BHT and BHA have been widely used (Lalas and Tsaknis, 2002; Gunstone, 2004). However, recent studies have provided proof for their role as carcinogens (Ardabili *et al.*, 2010). Natural antioxidants are considered to be more potent, efficient and safer than synthetic ones (Tavasalkar *et al.*, 2012). Thus, the search for natural, effective, and non-toxic compounds having antioxidative activity has been increased in recent years (Gupta and Sharma, 2006). The basic source of natural antioxidants is plants and plant-derived products. Phenolics present in plants are multifunctional and can act as free radical terminators, reducing agents, singlet oxygen quenchers and metal chelators (Mathew and Abraham, 2006). In addition, they have antimicrobial activity (Rauha *et al.*, 2000). Some aromatic plants have showed anti-inflammatory, antimutagenic, antithrombotic, antiallergic, antiviral and vasodilatory actions (Hollman *et al.*, 1996). Antioxidant constituents of plant are capable of scavenging radical and convert them to less reactive species (Devi and Ganasoundari, 1999). A variety of antioxidants are found in dietary sources like fruits, vegetables, spices, herbs, tea etc. Regular consumption of vegetables and fruits with antioxidants reduces the risk of chronic diseases (Dembinska-Kiec *et al.*, 2008). Studies revealed that antioxidant rich diet improves the health in the long run (Sin *et al.* 2013

and Willis *et al.*, 2009). Medicinal plants are one of the important sources of natural antioxidants. It reduces the risk of certain diseases such as cancer, heart diseases etc. Phenolics and flavonoids are the secondary metabolites present in plants which have free radical scavenging property. These secondary metabolites are found in all parts of plants such as fruits, seeds, leaves, roots and bark. Nature has a wide variety of antioxidants which are different in their physical and chemical properties, composition, mechanism and site of action. Some of the recent works in the antioxidant activity were carried out on 64 organic extracts from 37 species, belonging to 23 botanical families of plants used in the folk medicine of southern Ecuador by DPPH and ABTS⁺ method (Armijos *et al.*, 2018). Antioxidant activity with DPPH, ferric reducing power and β -carotene bleaching assay were evaluated on the ethanolic extract of *Calendula suffruticosa* ssp. *suffruticosa* Vahl (Sofiane *et al.*, 2018). Similar studies were done on water and methanolic extracts of *Phyllanthus* species (Zain and Omar, 2018).

2.6 PHYTOCHEMICAL STUDIES IN PLANTS

Recently herbal medicines have gained considerable attention as an alternative source to compensate for the deficiencies in pharmacotherapy worldwide (Arika *et al.*, 2015). The therapeutic property of these medicinal plants can be attributed to the phytochemicals present in them especially the alkaloids, flavonoids, terpenoids, sterols, phenolics, tannins, lignins, and saponins (Nyamai *et al.*, 2016). Phytochemicals are considered as non-nutritive, chemical compounds which occur naturally in plants during metabolic processes and they are believed to have diverse positive properties or disease defensive action. Plants are known to produce phytochemicals to protect themselves. Recent studies demonstrate that they can also play a vital role in fighting human diseases. Some of these plants have been in use in traditional medicine for centuries now (Minakshi *et al.*, 2016). Phytochemical constituents are credited to be useful for healing as well as for curing of human diseases (Nostro *et al.*, 2000). Studies have shown that many of these phytochemicals have useful properties as being antioxidant, anti-inflammatory, antitumor, antiatherosclerotic, anticarcinogenic, antimutagenic, antibacterial, and antiviral (Sala *et al.*, 2002; Rice-Evans *et al.*, 1998 action. More than 4,000 phytochemicals have been recorded and are classified according to their defensive

function, physical and chemical characteristics (Meagher and Thomson, 1999). Phytochemicals are neither essential nutrient nor required by the human body for sustaining life, but they have important properties which might help to prevent or to fight some common diseases (Holst and Williamson, 2008). Phytochemicals are categorized as primary and secondary compounds. Primary compounds include chlorophyll, proteins and common sugars while secondary compounds are terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.*, 2007). Secondary compounds are the chemicals that work with nutrients and fibers to form an integrated part of defense system to fight against various diseases and stress conditions (Thilagavathi *et al.*, 2015). These chemical compounds are called secondary metabolites. Relationship between the phytoconstituent and the bioactivity of plant can be studied to understand the synthesis of compounds with particular activities to treat various common as well as chronic diseases (Pandey *et al.*, 2013). No wonder plant products have been part of phytomedicines since a long time. It is important to study about the chemical constituents of plants because such information facilitates the synthesis of complex chemical substances (Mojab *et al.*, 2003; Parekh and Chanda, 2007 and 2008). Thus there is a widespread interest in evaluating drugs obtained from plant sources. This interest has grown from the belief that green medicine is safe and dependable as compared to costly and harmful synthetic drugs. A knowledge of the phytochemicals of plants is advantageous not only for the discovery of therapeutic agents but also because of its usefulness in disclosing new sources of such economic materials such as tannins, flavonoids, gums, essential oils, precursors for the synthesis of complex chemical substances etc. Phytochemical substances vary from species to species and even from plant to plant, depending on the age and various ecological and climatic factors (Baquar 1989).

2.7 PHARMACOLOGICAL ACTIVITIES OF HERBAL FORMULATIONS

2.7.1 Anti-inflammatory activity

Inflammation is a defensive mechanism of the body to the host and it's an essential immune response that helps the body to survive during injury or infection and

maintains tissue homeostasis in toxic conditions. The characteristic symptoms of inflammation are pain, redness, heat, swelling and loss of function resulted from dilation of the blood vessels which is further leading to an increased blood circulation and increased intercellular spaces predominantly due to the movement of leukocytes, protein and fluids towards the inflamed regions (Medzhitov, 2008; Parham, 2000). A series of biochemical events propagate the inflammatory response involving the immune system, local vascular system and various cells within the injured tissue. Chronic inflammation causes a progressive shift in the cell types present at the inflammation region and is distinguished by simultaneous destruction along with healing of the tissue inflammation (Yogesh *et al.*, 2013). Inflammation is also considered as a primary physiological defense mechanism of the body to protect itself against injury, infection, toxic chemicals, burn and allergens. However an uncontrolled and consistent inflammation may act as a sign of much chronic illness (Kumar *et al.*, 2004).

During inflammation, the reactive oxygen species produced from the action of free radicals increase abnormally. It causes an imbalance in the oxidizing molecules with antioxidant system of the body. This oxidative stress in turn causes inflammatory mechanism that damages the cellular components (Seril *et al.*, 2003). The cellular and humoral mechanisms of inflammation are complex and numerous. Gene regulatory factors such as the nuclear factor-kappa B (NF- κ B) are involved and signaling substances are synthesized by cells of immune system such as prostaglandins and cytokines (Ahmed, 2011). Cyclooxygenase (COX) is the vital enzyme for the synthesis of prostaglandins, prostacyclins and thromboxanes involved in pain, inflammation and platelet aggregation (Pilotto *et al.*, 2010). Steroidal anti-inflammatory drugs (SAIDS) and Non-Steroidal Anti-inflammatory Drugs (NSAIDS) are used during inflammation. These drugs work by blocking COX-1 and COX-2 enzyme activity. Steroids are highly effective in the treatment of inflammatory diseases, but their adverse effects cannot be ignored due to which it cannot be used for a long period of time. Prolonged use of NSAID may have reverse side effects (Miller, 1983). Their use is often associated with adverse effects such as gastrointestinal bleeding, capillary fragility, renal damage, peptic ulcers, hypertension and hyperglycemia (Corley *et al.*, 2003). Besides these side-

effects, the greatest demerit of these drugs is reappearance of symptoms after discontinuation of drugs. Therefore it is extremely necessary to develop new sources of anti-inflammatory agents with smallest amount of side effects. In this regard, there are evidences of secondary metabolites present in various medicinal plants to be useful in the treatment of inflammation and pain (Sheir *et al.*, 2001). During the last decade, researchers are using *in vitro* and *in vivo* models of inflammation which have led to the identification of numerous natural extracts with anti-inflammatory activity (Sultana & Saify 2012; Orlikova *et al.* 2014). Several reports are there where the different plant parts as fruits, galls and oil have pharmacological effects including anti-inflammatory activities (Bahmani *et al.*, 2015; Heidarian *et al.*, 2017; Minaiyan *et al.*, 2015; Tanideh *et al.*, 2014).

2.7.2 Antimicrobial activity

The use of antibiotics is well known in the fields of clinical medicine, agriculture, and veterinary research. The widespread use of antibiotics may promote the development of microbial strains with antibiotic resistances which could be infectious in nature. This creates a very serious trouble in the treatment of pathogenic microbes (Kapil, 2005). Infectious diseases are regarded as one the major reasons for the morbidity and mortality in the general population, mainly in developing countries. In a developing country like India, farmers and people of small villages and native communities particularly with low income use folk medicine to treat common infections in their day to day life (Fabricant and Farnsworth, 2001). Patients from rural areas have a lesser risk of getting infected from resistant pathogens than the people from urban areas who have been treated with modern antibiotics. So, instead of depending only on synthetic antimicrobial agents, people of urban areas should also explore natural resources which are non-synthetic in nature for the treatment of pathogenic infections. Natural extracts of some medicinal plants are considered to be more efficient than synthetic antibiotics to treat infectious diseases as claimed by traditional practitioners (Mathur *et al.*, 2010). Medicinal plant extracts are beneficial for treating infections as they are abundant source of antimicrobial molecules. Natural products such as secondary metabolites produced by plants are widely used in the pharmaceutical

industry for their extraordinary structural diversity and variety of pharmacological activities (Ernst, 2005).

Apparently, bacterial species have developed genetic ability to acquire as well as transmit resistance against antibacterial drugs currently available in the market. There are many reports on the isolation of bacteria which are sensitive to regularly used drugs and became multi-resistant to other drugs available in the market (Nascimento *et al.*, 2000; Sakagami and Kajimura, 2002). Thus, pharmaceutical companies have begun to look for new antimicrobial drugs in recent years, due to the continuous appearance of microorganisms resistant to commonly used antimicrobials. Some of the strategies adopted by pharmaceutical companies to deal with this problem include change in the molecular structure of the existing medicines so as to make them more efficient and to recover the activity vanished due to bacterial resistance mechanisms (Chartone-Souza, 1998).

It is known that plants have been used for a long time to treat infections and are considered as an important source of antimicrobial agents (Cowan, 1999). Several works have been reported regarding the antimicrobial property of plants extracts from roots, stem, leaves or flowers (Abu-Shanab *et al.*, 2004; Abbassi and Hani, 2012). Today, various antimicrobial agents from plant sources are known to treat various infectious diseases (Kingston, 2008). Since there is a rapid global spread of resistant clinical isolates, it is highly necessary to find new antimicrobial agents. However, there are evidences that even new range of antimicrobial agents have a short life expectancy (Coates *et al.*, 2002). Hence researchers are giving more attention to herbal products in search of new leads to develop alternative drugs against MDR (multiple drug resistance) microbe strains (Braga *et al.*, 2005). The antimicrobial efficiency of some plants in treating common diseases has been beyond belief. However it is estimated that about 10% of all flowering plants used by local communities to treat various infections, only 1% have been recognized by modern scientists (Kafaru, 1994). A wide variety of secondary metabolites produced by plants such as tannins, alkaloids and flavonoids have found to possess antimicrobial properties (Lewis and Ausubel, 2006). Many phytotherapy manuals have mentioned a number of medicinal plants for treating

infectious diseases because of their availability, less side effects and toxicity (Lee *et al.*, 2007). There are numerous reports on the antimicrobial activity of different plant extracts (Bonjar, 2004; Islam, 2008; Boer *et al.*, 2005). Ethanolic extracts of five plants showed antimicrobial activities against MDR strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans* (Khan *et al.*, 2009). Some plants were recorded which were known to cure gastrointestinal disorders, respiratory diseases, urinary tract infections and cutaneous infections (Brantner and Grein, 1994; Somchit *et al.*, 2003). Crude extracts of *Arisaema flavum*, *Debregeasia salicifolia*, *Carissa opaca*, *Pistacia integerrima*, *Aesculus indica*, and *Toona ciliata* were tested against three Gram positive and two Gram negative ATCC bacterial species. Among them, *P. integerrima* and *A. indica* were active against all tested bacterial strains while other four plant extracts were active against different bacterial strains (Bibi *et al.*, 2011). Extracts of root barks of *Berberis vulgaris*, *Cinnamomum cassia* peels, *Cistus monspeliensis* aerial parts, *Punica granatum* fruit peels, *Rhus tripartita* aerial parts and *Withania frutescens* leaves have potential antibacterial effects on bacterial strains tested, especially *S. aureus* (Bereksi *et al.*, 2018). Antimicrobial activity of some Indian medicinal plants against the soil bacteria was also recorded (Jangra *et al.*, 2018).

2.7.3 Hepatoprotective activity

Liver is the largest and one of the vital internal organs in the human body. It is the most important site for enhanced metabolism and excretion. It plays important role in maintenance, and regulation of homeostasis of the body. It is involved in almost all biochemical pathways for growth, nutrient supply and to fight against diseases, reproduction and energy production (Ward and Daly, 1999). It is connected to interactive metabolism of fats, proteins and carbohydrates. It also plays vital role in synthesis of number of plasma proteins such as fibrinogen, albumin and various other enzymes along with formation and excretion of bile (De *et al.*, 2017). The liver regulates many important metabolic functions and hepatic injury is mainly related with alteration of these metabolic functions. The toxins absorbed in the intestine first reaches to liver, causing a variety of liver ailments. Thus, liver diseases are still one of the serious existing health issues. About 20,000 people die every year suffering from liver

disorders (Bhardwaj *et al.*, 2011). It is a global concern affecting people of almost every age group (Dhiman *et al.*, 2012). There are numerous disorders associated with liver (Wolf *et al.*, 1999). Jaundice and hepatitis are two major liver disorders which have caused high death rate (Pang *et al.*, 1992). The most common reason for withdrawal of a drug from the market is because it is reported that more than 900 drugs are responsible for hepatic injury (Friedman *et al.*, 2003). Several chemicals are there which induce hepatotoxicity. Carbon tetrachloride (CCl₄), thioacetamide, galactosamine, d-galactosamine/lipopolysachharide, paracetamol, arsenic, antitubercular drugs etc., are used to induce hepatotoxicity in laboratory animals for experiments. Drug-induced liver injury creates problem not only in health care profession but also the people working in drug regulatory agencies and pharmaceutical industry are affected adversely. According to the United States Acute Liver Failure study group, more than 50% of acute liver failure is caused by overdose of acetaminophen (39%) along with idiosyncratic liver injury caused by other drugs (13%) are due to drug-induced liver injury (Holt and Cynthia, 2006).

Improvement in hepatic disease is less with modern medicines. Approximately 160 phytochemicals and other phytoconstituents produced from plants have been claimed to acquire hepatoprotective activity (Jannu *et al.*, 2012). In India, about 40 polyherbal commercial formulations are known to have hepatoprotective property. These herbal drugs contain a variety of chemical compounds like phenols, carotenoids, glycosides, coumarins, lignans, essential oil, monoterpenoids and xanthines. Plant extracts of several crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders (Sharma *et al.*, 2002). Hepatoprotective potential of the methanolic shoot extract of *Bambusa bambos* was also reported (Patil *et al.*, 2018). *Stachys pilifera* extract could provide considerable protection against CCl₄ hepatotoxicity in rats (Kokhdan *et al.*, 2017). The crude extract of *Origanum majorana* produced significant hepatoprotective activity (Kamble and Rao, 2018).

2.7.4. Antidiabetic activity

Diabetes mellitus is an assembly of metabolic changes characterized by hyperglycemia which have resulted from the defects in secretion and action of insulin. It is of two type viz. Type I and Type II. Type I diabetes is insulin dependent and have affected only 5% of the diabetic population. Type II is non-insulin dependent which usually develops in adults from the age of 40. Diabetes mellitus is the third greatest cause of death all over the world. If it is not treated, it may lead to numerous complications affecting various organs in the body (El-Hilaly *et al.*, 2007). Chronic diabetes is associated with serious damage, dysfunction and failure of various organs (Lyra *et al.*, 2006). It may lead to long term complications such as neuropathy, retinopathy and angiopathy (Kristova *et al.*, 2008). It is currently affecting around 143 million people (Mentreddy *et al.*, 2005) and the number of those affected is increasing day by day, by 2030 it is predicted to reach 366 million population worldwide (Ponnusamy *et al.*, 2011). It is projected to become one of the world's main disablers and killers within the next 25 years.

Many factors are responsible for the on-set of diabetes such as environmental factors whether it is diet, obesity and inactive life style. Other risk factors include old age, insulin resistance, nutritional status, and lifestyle change due to urbanization (Deepashree and Prakash, 2007). The complete management of diabetes is a global issue and successful treatment is not yet developed (Malviya *et al.*, 2010). Currently available treatment for diabetes includes insulin injection and other common oral hypoglycemic agents such as metformin, sulfonylureas, glucosidase inhibitors, troglitazone, etc. However, these therapies are known to produce adverse side effects such as liver problems, diarrhoea and lactic acidosis (Rajalakshmi *et al.*, 2009). The side effects of such drug treatment are sometimes not satisfactory in maintaining blood glucose levels. Many medicinal plants used for the treatment of diabetes mellitus in various traditional system of medicine worldwide. It has provided a safe and potential source of antidiabetic principle many of which are known to be effective against diabetes.

Blood glucose level in the body is controlled by pancreatic endocrine hormones, insulin and glucagon. Insulin is secreted by the β -cells in response to high blood sugar level and it also potentiates the ability of muscle, fat cells and red blood cells to absorb sugar from blood and using it in metabolic processes making the sugar levels normal. On the other hand, glucagon is secreted by α - cells present in pancreas. It stimulates the liver and other muscles cells to release stored glucose from the body (Gupta and De. 2012). Postprandial hyperglycemia plays a key role in the onset of type 2 diabetes with its complications. Decreasing of blood glucose rise after a meal is a chief therapeutic approach so that the absorption glucose could be delayed by inhibiting the carbohydrate hydrolyzing enzymes, α -glucosidase and α -amylase (DeFronzo, 1999; Chiasson *et al*, 2002). Alpha-glucosidase inhibitors like miglitol, acarbose and voglibose are oral drugs commercially used for lowering blood glucose. They lower postprandial hyperglycemia without inducing insulin secretion. Although these compounds do not induce hypoglycemia but side effects such as gastrointestinal trouble may limit long-term fulfillment to therapy (Neuser, 2005).

The research for natural products especially from traditional medicines has become an attractive approach for the treatment of postprandial hyperglycemia. Published research suggests that there is a direct relationship between of phenolic compound, flavonoids, and condensed tannin in the plant extract and the ability to inhibit α -glucosidase and α -amylase activities (Tadera *et al.*, 2006; Disakwattana and Chanathong, 2011). Many phenolic compounds such as flavonoids and anthocyanin have positive effects on diabetes, by inhibiting these enzymes (Lo Piparo *et al.*, 2008; Jo *et al.*, 2010; Rubilar *et al.*, 2011; Sales 2012; Wongsa *et al.*, 2012). From the literature available, more than 800 plants species have been reported to possess hypoglycaemic activity (Rajagopal and Sasikala, 2008). In Sikkim and Darjeeling Himalayan region, about 37 plant species belonging to 28 families were used for treating diabetes in the folk medicinal practices (Chhetri *et al.*, 2005). Of these, 30 species of plants (81%) have not been reported as hypoglycemic agents in the Dictionary of Indian Folk Medicine and Ethnobotany (Jain, 1991). *Calamus erectus*, a common wild fruit found in Sikkim and Darjeeling Himalayas, was reported to be a good source of antidiabetic agent by experimenting in streptozotocin induced diabetic

rats (Ghosal and Mandal, 2013). Some recent works reported antidiabetic activity of polyherbal formulations. Combination of alcoholic extract of *Tribulus terrestris* fruits, *Boerhavia diffusa* roots and *Azadirachta indica* leaves showed potential antidiabetic property in Wister albino rats (Saxena and Argal, 2018). Similarly, formulation prepared from seven plants (*Curcuma caesia*, *Evolvulus alsinoide*, *Citrullus lanatus*, *Gymnema sylvestre*, *Tinospora cordifolia*, *Withania coagulans* and *Caesalpenia bonduc*) proved to be a promising antidiabetic agent (Mahajan *et al.*, 2018).

2.7.5 Cytotoxic activity

Cancer is an uncontrolled production of the cells in any part of the body causing a bulge of organ or tumor resulting into a risk to the body (Neerugatti *et al.*, 2016). It is the second leading disease which is growing largely causing deaths around the world after cardiovascular disease. It mainly affects the parts of the body such as lungs, ovary, liver, oral, breast, stomach, etc (WHO, 2014). Medicinal plants have various advantages over chemical products to deal with cancer because plant derived compounds are more tolerant and less harmful to normal human cells. Already available anticancer therapies for the treatment of cancer are radiotherapy and chemotherapy which comes with harmful effects as neurological, cardiac, renal and pulmonary toxicity, seriously damaging the health of the person. At present, chemotherapy is mostly used for treatment of cancer which uses alkylating agents, antimetabolites, antitumor antibiotics, platinum analogs, all of which may indirectly leads skin or lung cancer with undesirable side effects in a long-term use. Therefore, an alternative treatment needs to be developed which include less toxic and more effective anticancer drug as compared to the drugs available in the market. So, identifying new molecules for the treatment of cancer could be helpful in many ways by providing safe, low cost and highly efficient treatment. The natural medicinal plants have been using for the treatment of many diseases including cancer since ancient time (Cragg and Newman, 2005). The International Agency for Research on Cancer estimates that 184 countries of the world revealed 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. By 2030, it is estimated to have 26 million new cancer cases and 17 million deaths caused by cancer

per year (Thun *et al.*, 2009). Today, despite extensive efforts, cancer still remains an aggressive killer worldwide. Furthermore, during the last decade, novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development. Approximately 35,000 plant species are screened by National Cancer Institute with anticancer activity and among them, 3000 plant species were found to have potential anticancer activity (Desai *et al.*, 2008). There are several reports on the anticancer activity of numerous plants. The extracts of *Urtica membranacea*, *Artemesia monosperma* and *Origanum dayi* exhibited dose and time dependent killing capacity in human derived tumor cell lines and primary cultures collected from patients' biopsies. The killing capability was particularly towards tumor cells and these extracts had no effect on healthy human cells (Solowey *et al.*, 2014). Ethyl acetate extract of *Aerva lanata* showed a strong cytotoxic activity against MCF-7 breast cancer cell line (Krishnamoorthi and Elumalai, 2018). Aporphine alkaloid isolated from medicinal plant *Alphonsea sclerocarpa* showed remarkable cytotoxic activity against K562 (CML-chronic myeloid Leukaemia blood cancer) cells (Joshi *et al.*, 2018).

2.7.6 Anti-hypertensive activity

Hypertension, commonly called high blood pressure is also known as arterial hypertension. It is a chronic medical condition characterized by increase of blood pressure in the arteries. It is one of the most common cardiovascular diseases which have become a major health issue in public. According to WHO, 1.5 billion people will suffer from hypertension by 2025 and that more than 7 million deaths yearly are likely to be caused by hypertension (Guilbert, 2003). Recent studies have reported that the prevalence of hypertension in Indian subcontinent is elevating in high rate. In the last three decades, this elevation was found to be about 30% and 10% in urban and rural population respectively (Gilbert, 2014). Hypertension leads to major risk for cardiovascular diseases such as atherosclerosis, renal insufficiency, heart failure, coronary artery disease and stroke (Alamgeer *et al.*, 2013). It is one of the leading causes of death in developed countries due to lifestyle (Rout *et al.*, 2010). The worldwide impact of hypertension has led to the investigation of antihypertensive

medications and more new therapeutic substitutes (Shi *et al.*, 2017; Pal and Shukla, 2003). Antihypertensive medications include angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers and beta-blockers as blood pressure controls many physiological mechanisms such as cardiac output, peripheral vascular resistance, and circulating blood volume (Niaz *et al.*, 2017; Page, 1982). These antihypertensive drugs available for the treatment of hypertension are reported to have adverse side effects as well. ACE inhibitor, angiotensin receptor blockers, and calcium channel blockers cause abstraction and angioedema in upper respiratory track in adults and children (Hom *et al.* (2012). Calcium channel blockers were found to be involved in development and progression of cancer due to the inhibition of vascular cell growth and angiogenic growth factors stimulated by the increase of apoptosis (Shapovalov, 2013). Other side effects these drugs are dyspnea, hair loss, cough, headache, edema and flushes (Kumbhare *et al.*, 2014; Ozkaya and Yazganoglu, 2014). Thus, an alternative therapy especially from herbal products is preferred as medicinal plants are considered to provide more safety. Recent attention has been inclined towards these natural products which have been traditionally used for prevention and management of cardiovascular diseases (Bhatt *et al.*, 1998). Medicinal plants have been reported to be effective against hypertension since a long time (Oh *et al.*, 2008; Prahalathan *et al.*, 2012). It is believed that antihypertensive activity of plants is credited to their antioxidant property as oxidative stress is a major risk factor in hypertension and cardiovascular diseases (Adefegha *et al.*, 2015; Schiffrin, 2010). Various ethnomedicinal surveys have reported treatments of cardiovascular disorders. Plants like *Syzygium guineense*, *Passiflora nepalensis* Wall, *Ginko biloba*, *Stephania tetandra* and *Uncaria rhynchophylla* have been used in hypertension to lower high blood pressure (Mahady, 2002; Yohannes *et al.*, 2010). Supplements of potassium, magnesium, amino acids like arginine and taurine, vitamins C and E and omega-3 fatty acids have found to be effective in the treatment of cardiovascular diseases, including hypertension (Agrawal *et al.*, 2010). Previous studies have also claimed that alkaloids in plants can reduce the blood pressure through vasorelaxation caused by production of increased prostacyclin and nitric oxide (Hussain *et al.*, 2012). Similar study was done by Alamgeer *et al.*, (2013) on *Berberis orthobotrys* where it was explained that the presence of alkaloid berbenine is

responsible for antihypertensive and vasorelaxation effects. Some researchers have also reported improvement of vascular functions by lipid lowering and thus exhibiting the hypotensive property (Aikawa *et al.*, 2002; Bilanda *et al.*, 2018). Quercetin present in plants was also considered as one of the reasons of antihypertensive effect as the experiment being carried out with *Centella asiatica* (Intharachatorn and Srisawat, 2013). High K^+ is another factor that may decrease the risk of stroke, reduces blood pressure, inhibits formation of free radical, arterial thrombosis and proliferation of vascular smooth muscle. Haji *et al.*, (2016) reported that leaf extract of *Thymus schimperi* contains higher concentration of K^+ which might be responsible for induction of hypertensive activity. Numerous plants have been reported to possess antihypertensive property in traditional medicine, some of which have been even proven scientifically through standard methods. These documents could be used for the development of newer molecules to obtain safer and efficient drugs.

2.8 INFLUENCE OF EXTRACTION METHODS ON EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANTS

Natural products particularly from plant extracts are a major source for the development of new therapeutic agents (Cosa *et al.*, 2006). Approximately 20% of plants used in pharmaceutical studies have helped the healthcare system by treating harmful diseases such as cancer (Naczka and Shahidi, 2006). The therapeutic ability of plants is mainly due to the presence of a wide range of chemical compounds which can treat various diseases (Duraipandiyani *et al.*, 2006). For obtaining these biological components from plants, extraction method is a key step (Jadhav *et al.*, 2009). Better quality and high efficiency of extraction can be obtained after optimizing the methods with better efficiency. Extraction efficiency can vary significantly when different extraction methods are applied on same plant material with same solvent. Mode of extraction plays an important role in the amount and type of biomolecules present in the extract (Das *et al.*, 2010). Thus it is necessary to select the most appropriate extraction method for obtaining desirable compounds (Gupta *et al.*, 2012). In addition, the selected method also needs to be standardized properly to achieve acceptable degree of

reproducibility as it contributes significantly to the final quality of the herbal drug (Dhanani *et al.*, 2017).

Researchers are making effort to develop best extraction methods in order to get high efficiency and efficacy. Efficiency means the yield of extraction while efficacy refers to the potency of the extract. The selection of extraction method to obtain active components with best yield and purity from plants is dependent mainly on the nature of compounds and raw material that is going to be used (Kothari *et al.*, 2009). Phytochemical processing of plant materials is necessary to optimize the concentration of known constituents and also to maintain their activities (Aziz *et al.*, 2003). Dietary phytochemicals are available in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Saxena *et al.*, 2013). They have antioxidant, anti-inflammatory, anti-cancer, anti-bacterial properties etc (Majekodunmi, 2015).

There are various kinds of plant extraction methods; some are traditional or conventional while some are advanced. Maceration, percolation, decoction, soxhlet etc are some of the conventional extraction methods. The advanced methods are supercritical fluid extraction, microwave assisted, ultrasound-assisted extraction and accelerated solvent extraction etc (Azwanida, 2015; Raaman, 2006).

Conventional methods are simple, standard and easy to use, but these methods have some demerits being insufficient and slow, cause damage of heat labile constituents and consume large quantities of organic solvents. It also has quality related problems such as lack of consistency, safety and efficacy. The benefits of modern processes are elimination of additional sample clean-up and concentration steps before chromatographic analysis; improvement in extraction efficiency and selectivity are some of them (Kothari *et al.*, 2010). Modern extraction methods have better than conventional methods in terms of less solvent consumption and in minimizing sample degradation. The undesirable and insoluble components from the extract can also be eliminated in these methods (Gupta *et al.*, 2012). The best extraction technique should be able to obtain high yield extracts with minimal changes to the quality in terms of functional properties of the extract required (Quispe *et al.*, 2008).

Numerous studies have reported variations in the biological activities of extracts prepared using different extraction techniques. Antioxidant activity of thyme, cinnamon and cumin extracts obtained through heating method was higher than cold method probably due to the increase of the phenolic content in the hot extract (Abdelfadel *et al.*, 2016). Thermal treatment is believed to break the cell wall and the subcellular compartments of plants to liberate more components which could be potent radical-scavengers (Maeda *et al.*, 1992). Variation in extraction methods can also affect the physical properties of the extract, particularly its solubility in solvents. It was also observed that heat based methods yield more extracts than that of cold based extraction methods. When antimicrobial activity was studied, the extracts obtained from cold method exhibited better activity than Soxhlet extracts (Abah and Egwari, 2011). Mechanical agitation, a conventional extraction method uses high energy, time, and huge solvent-consumption (Patil and Shettigar, 2010). However, microwave assisted extraction (MAE) is a method of extraction which can overcome these demerits (Ali *et al.*, (2013). Nana *et al.*, (2015) reported that MAE gave the best yield with shortest extraction time and highest antioxidant activity but lower antiplasmodial activity. Thus, MAE was suggested to be the best choice for the extraction of compounds from *Trichilia roka*. During evaluation of antioxidant activity of *Quercus infectoria* galls, supercritical CO₂ method showed much better activity when compared with soxhlet method but the extraction yield was better in the latter (Hasmida *et al.*, 2014). There are many more reports on the effect of extraction method of plants on biological activity which makes it important to look for a better extraction procedure for particular plant extracts.

2.9 ISOLATION OF BIOACTIVE PHYTOCONSTITUENTS FROM PLANTS AND BIOASSAY GUIDED PURIFICATION

In ancient times, plant and its products were the only source of medication in crude or partially purified form for the treatment of human and animal diseases. This generated an idea that the effect of a drug in human body could be credited to an interaction of drug with biological molecules. This was the beginning of using pure, isolated chemicals, instead of extracts for treatment of diseases. At present, there is

countless number of bioactive compounds which has been isolated from crude extracts, the chemical structures of which were elucidated (Bajpai and Kang, 2011). Moreover, plants have always produced secondary metabolites with potential pharmacological properties (Russell and Duthie, 2011). Plants contain polyphenolic compounds which have many beneficial health effects due to their pharmacological activity such as antioxidant, anti-inflammatory, anticancer activity etc (Li *et al.*, 2014). The phytochemicals such as alkaloids, saponins, steroids, flavonoids, lipids, carbohydrates, tannins, proteins and vitamins etc, have many positive effects on human and animal system. Discovery of new drugs from natural products can play a key role in conservation and sustainable economic development especially in non industrialized countries that have poor economy but are rich in natural resources (David and Emma, 2011). Natural products are mainly categorized into three groups as primary and secondary metabolites and high molecular weight polymeric materials (Hanson, 2003). The technique of purification and isolation of bioactive compounds from plants has undergone new development in recent years (Altemimi *et al.*, 2015; Altemimi *et al.*, 2015). Some modern techniques are useful for the development and availability of many advanced bioassays which can provide precise techniques of isolation, separation and purification. The main objective while searching for bioactive compounds is to develop an appropriate method that can check the source material for bioactivity such as antioxidant, antibacterial activity or cytotoxicity with simplicity, specificity, reproducibility and speed (Mulinacci *et al.*, 2004). Selection and collection of plant materials are primary but key step during this process. The next step is the recovery of ethno-botanical information to distinguish possible bioactive molecules. Extracts can be prepared with various solvents to isolate and purify the active compounds that are responsible for the bioactivity. Column chromatographic technique is preferred for isolation and purification of the bioactive compounds. Sophisticated instruments such as High Pressure Liquid Chromatography (HPLC) accelerate the process of purification of the bioactive molecules.

2.10 IDENTIFICATION OF BIOACTIVE COMPOUND OF INTEREST

Over the last decade, the interest in nutraceuticals and the consumption of nutraceutical products have largely increased owing to their proven health benefits (Daliu *et al.*, 2019). This has brought the natural products into the attention of public, researchers and pharmaceutical industries. The demands of new compounds as well as the information on the sources of their bioactivity, advanced techniques are required to investigate it (Valentino *et al.*, 2020).

Different varieties of spectroscopic techniques like UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy can identify the purified compounds (Popova *et al.*, 2010).

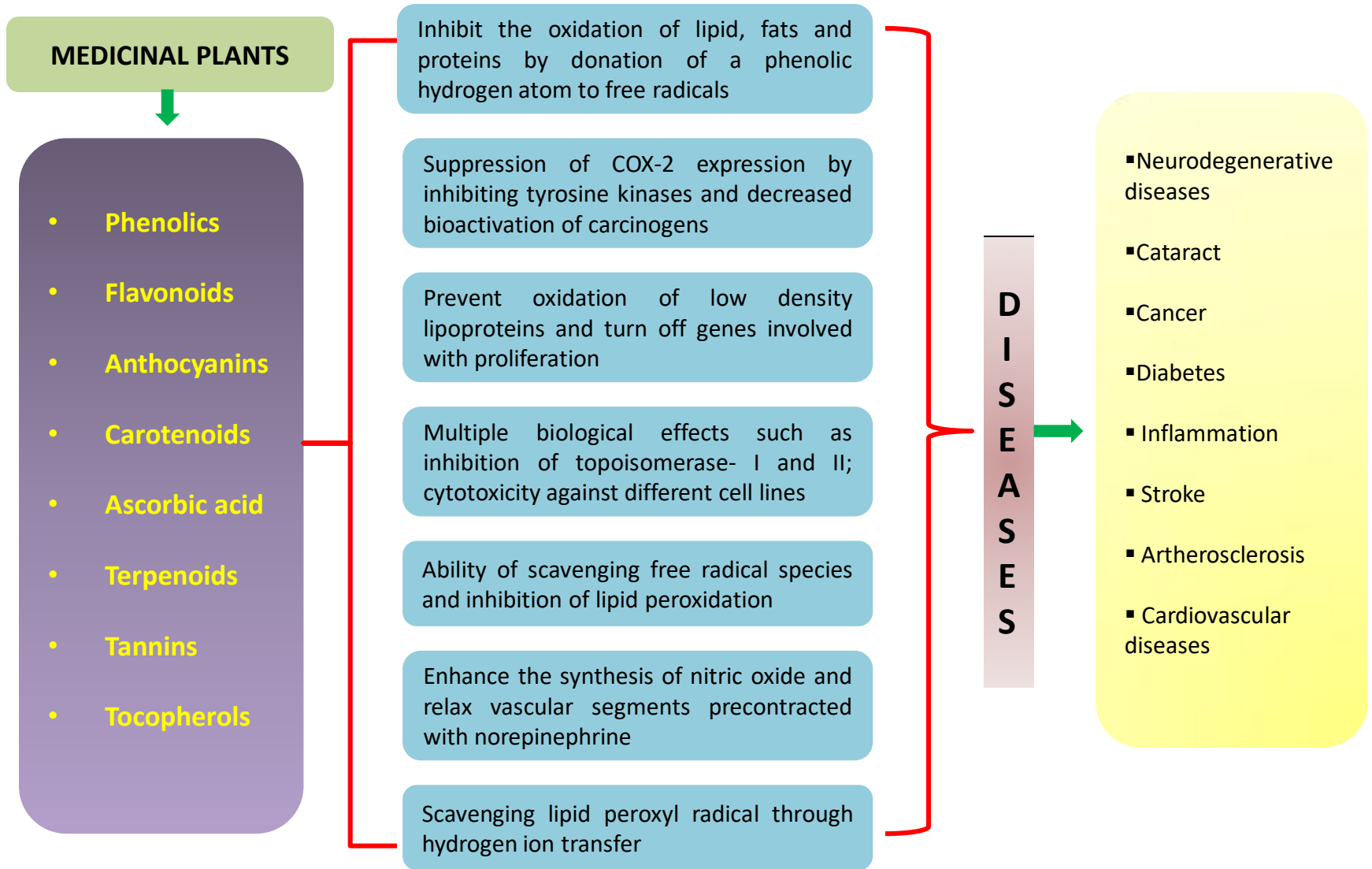


Figure 2.1: Antioxidant phytochemicals and their mechanism of action to prevent different diseases

Bhatt *et al.*, 2013

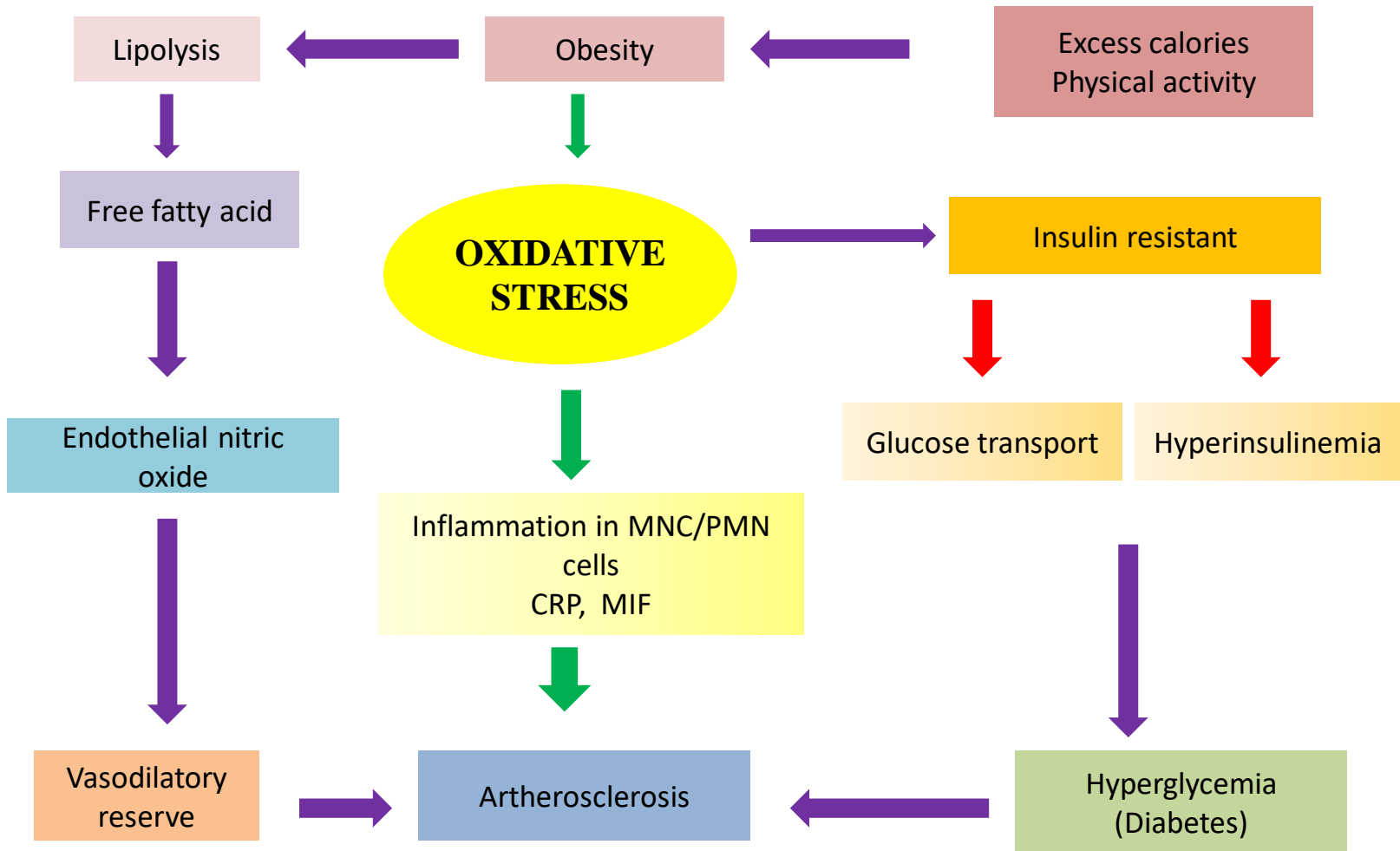


Figure 2.2: Relation between life style, oxidative stress, insulin resistance, inflammation and atherosclerosis. MNC: Mononuclear cells; PMN: Polymorpho nuclear cells; CRP: C-reactive protein; MIF: Migration inhibitory factor.

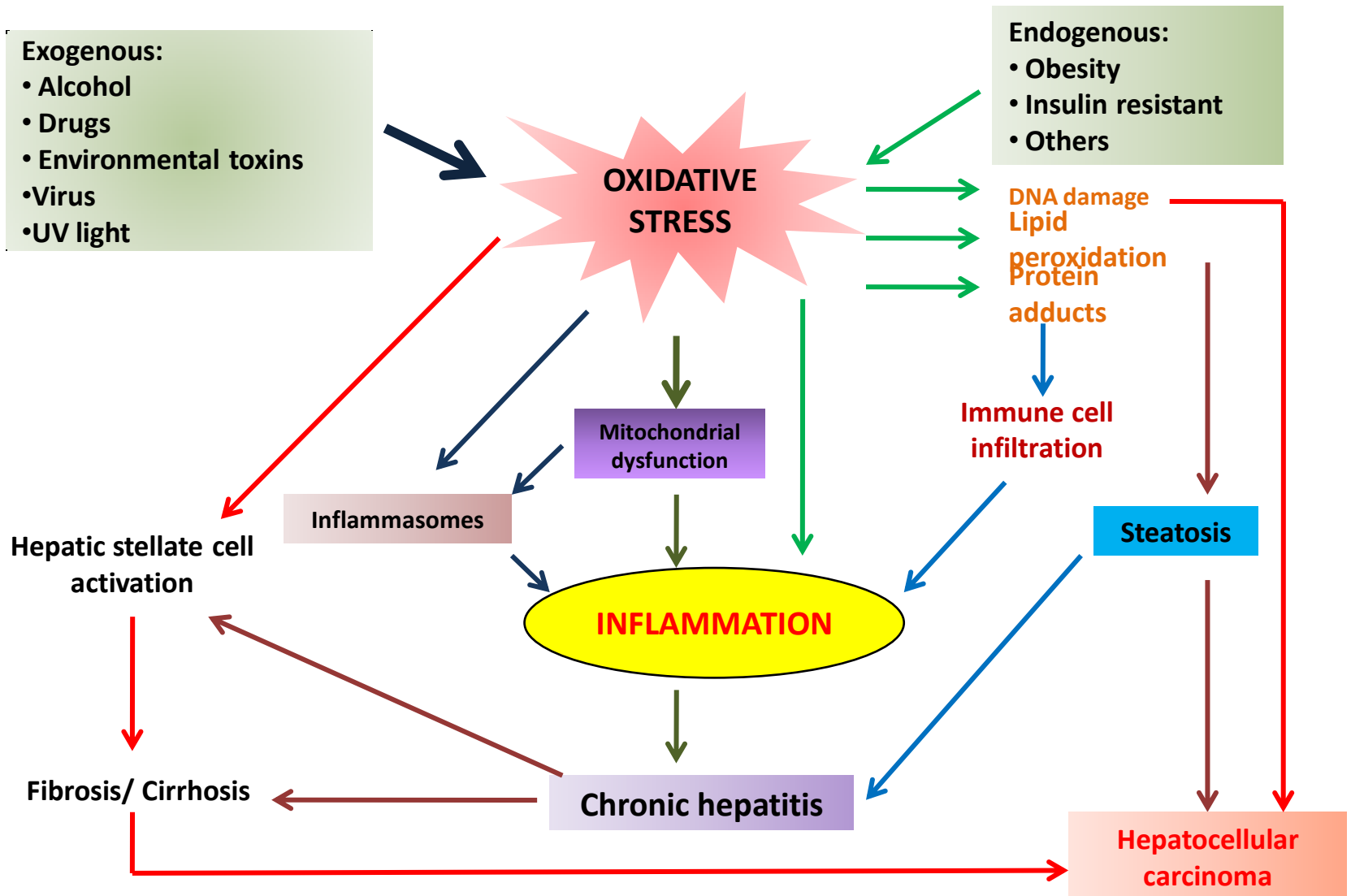


Figure 2.3: The general mechanism of oxidative stress induced by various factors on liver diseases

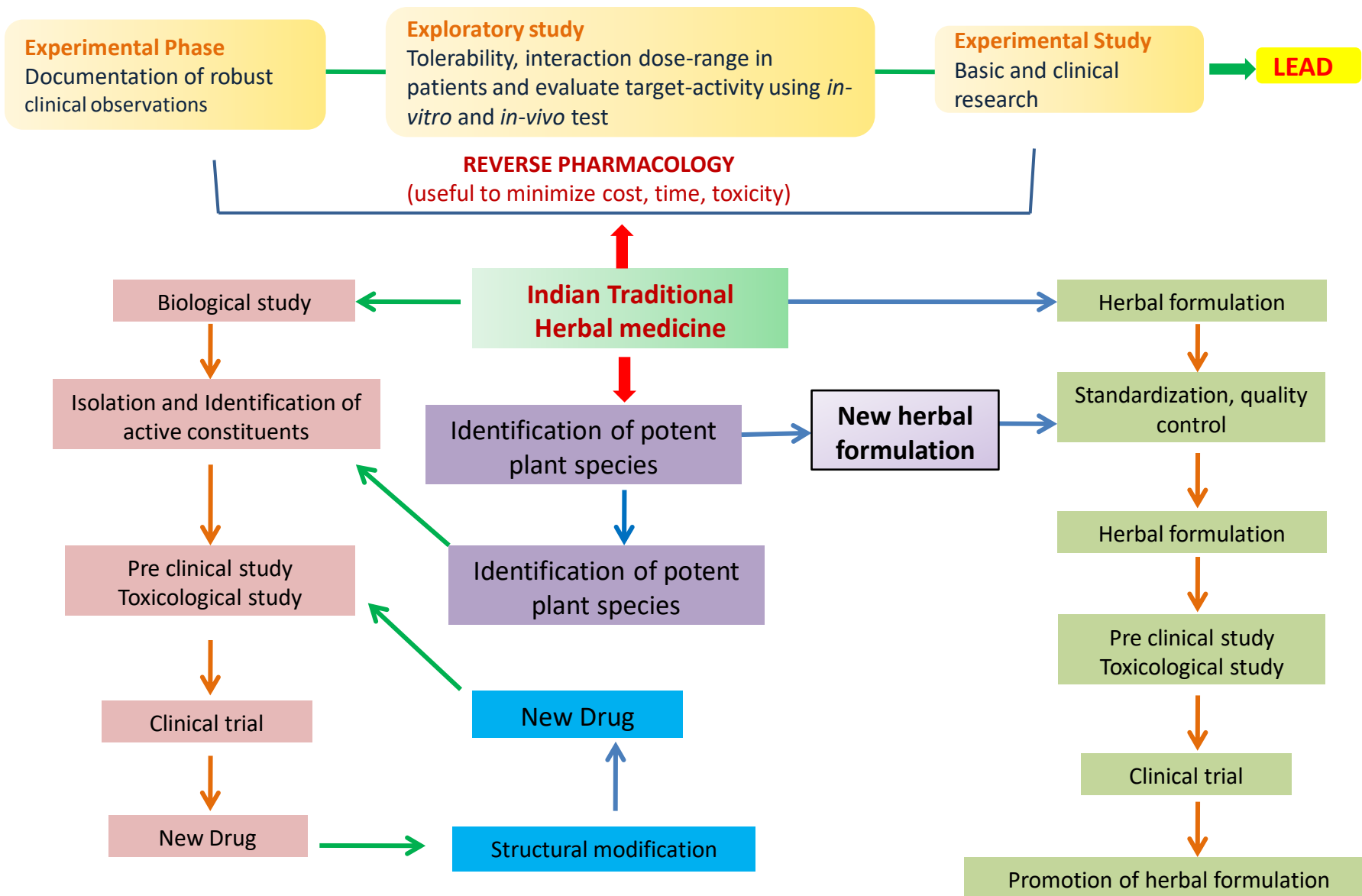


Figure 2.4: Opportunity of drug discovery from Indian Traditional Herbal medicine