INTRODUCTION

1. OVERVIEW

This chapter gives a general idea of phytochemistry, pharmacology and endemism. It gives the background for phytochemically exploring the two endemic Tephrosia species. It also states brief objectives of the research tenure.

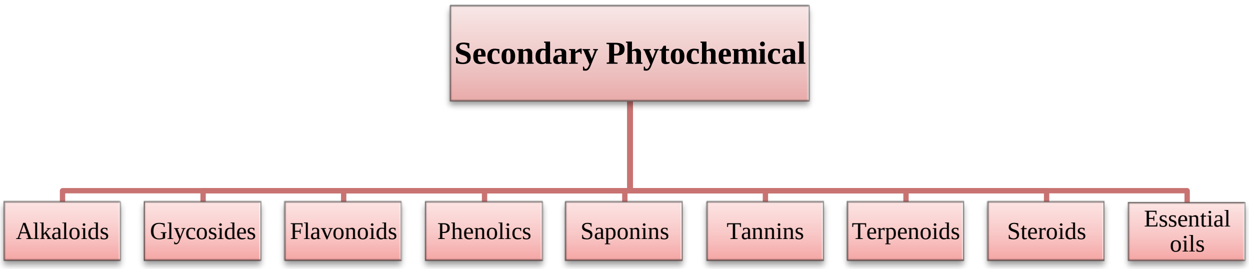
1.1. PHYTOCHEMISTRY

Phytochemistry (Greek words *phyton* - plant; *Chemeia* - chemistry) deals with the knowledge of bioactive natural products or phytochemicals isolated from natural products. It has developed as a distinct discipline, somewhere in between organic chemistry and plant biochemistry. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function (Harbone, 1998).

Phytochemicals are secondary metabolites derived from plants and are not required for functioning of the body, but they are required for maintaining good health and their absence will not result in a deficiency disease. The phytonutrients have much healthy function in the body. For example, they may promote the function of immune system, act directly against microorganisms, reduce inflammation and prevent cancer, cardiovascular diseases or any other malady affecting the health of an individual (Ali, 2011).
The modern pharmacognosy and phytochemistry is especially devoted to the bioactive natural compounds. Modern phytochemistry or pharmaceutical biology is an interdisciplinary science including three primary roles; as

1- Drug discovery
2- Usefulness of plant extract in disease treatment
3- Development of plant products as chemo-preventive agent.

Preparation of herbal drugs involves some techniques like liquid-liquid distribution, supercritical fluids, solid phases and microwave extractions. Natural products are isolated from the extract by using spectroscopic techniques and chemical reactions. Bioactivity of plant products is determined by *Invitro* and *Invivo* bioassays. Proficiency in phytochemistry requires thorough knowledge of the above mentioned techniques in their most sophisticated



forms *e.g.*, Mass Spectrum ionization studies and multidimensional Nuclear magnetic resonance techniques (Ali, 2011).

The range and number of discrete molecular structures produced by plants is huge in comparison to our advance knowledge of them. A major problem in phytochemical research is the correlation of existing data on each particular class of compound. As per few assessments, among the 7.5 lakh species of plants on earth, 5 lakh being classified as higher plants and 2.5 as lower plants, only 5-10% have been studied for their chemical constituents and/or biological activity (Hosler and Mikita, 1987; Chopra, 1987; Hill, 1979; Wagner & Wolff, 1977; Ross & Brain, 1977).

1.1.1 CLASSIFICATIONS OF PHYTOCHEMICALS

Phytochemicals can be classified in a number of different ways based on biosynthetic origin, solubility properties and the presence of certain key function groups. (Harborne 1994) Generally there are two types of metabolites, primary and secondary. Primary metabolites are carbohydrartes, fats and proteins, which are main sources of energy. Plant secondary metabolites like phenolic acids, flavonoids, amines, pigments, vitamins, steroids and alkaloids *etc*. were pharmacologically proved to have antioxidant and other biomedicinal potential (Madlener *et al*., 2009; Catherine *et al*., 1997). They can be classified in the following classes:-

ALKALOIDS

Alkaloid (alkali like substance) forms the largest class of secondary plant substances; at present numbering more than 12,000. These chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen. In fact, one or more nitrogen atoms that are present in an alkaloid, typically as 1°, 2° or 3° amines, contribute to the basicity of the alkaloid (Sarker and Nahar, 2007). They react with acids to form crystalline salts without production of water (Firn, 2010). In general, the free base is soluble in organic solvent and not in water. It can be extracted by classical liquid-liquid extraction under neutral or basic condition (Verpoorte and Schripsema, 2009). The solutions of alkaloids are intensely bitter. These nitrogenous compounds function as protective agents of plants against herbivores and pathogensand are widely exploited as pharmaceuticals, stimulants, narcoticsand poisons due to their potent biological activities. Naturally alkaloids exist in large proportions in seeds and cell sap, cations in various organic acids such as oxalic, succinic, tartaric, malic and other acids. The presence of alkaloids is also recorded from in aerial parts of *Tephrosia* species like *Tephrosia candida, T. coriacea, T. macropoda, T. purpurea* and *T. virginiana* (Willaman *et al.,* 1961).

GLYCOSIDES

Glycosides are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds, in such a manner that the hemi acetal entity of carbohydrate must essentially take part in condensation. They are colourless compounds consisting of carbon, hydrogen and oxygen (some contain nitrogen and sulfur). They are found in cell sap and are water-soluble, bitter taste phytoconsituents. Chemically, glycosides contain a glycone (carbohydrates) and aglycone (non-carbohydrate part) (Kar, 2007; Firn, 2010) which can be alcohol, glycerol or phenol represents aglycones. They are neutral in reaction and can be readily hydrolyzed into its components with ferments or mineral acids. They are classified on the basis of sugar component, chemical nature of aglycone or pharmacological action. The rather older or trivial names of glycosides usually have a suffix 'in' and the names essentially include the source of glycoside, for instance: strophanthidin from *Strophanthus*, digitoxin from *Digitalis*, barbaloin from *Aloes*, salicin from *Salix*, cantharidin from *Cantharides* and prunasin from *Prunus*. However, the systematic names are invariably coined by replacing the 'ose' suffix of the parent sugar with 'oside'. This group of drugs are usually administered in order to promote appetite and aid digestion. In some *Tephrosia* species presence of flavonol glycoside is found for eg. in *Tephrosia purpurea* serratin (Saxena *et al*., 1997) and *T. candida* 6-hydroxykaempferol 4'methyl (Chen *et al*., 2014).

FLAVONOIDS

Flavonoids are an important group of polyphenols widely distributed among plants. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. They are structurally uniform and made of more than one benzene ring in its structure (a range of C_6-C_3-C_6 aromatic parent compounds known as flavans) and numerous reports support their use as antioxidants or free radical scavengers (Kar, *l.c.*). The different classes on these C_6-C_3-C_6 compounds are recognised chiefly by the oxidation pattern C_3 of fragment, additional oxygen heterocyclic rings and glycosylation. The spectra of properties exhibited by this compound include colouring of flowers and fruit. They possess structural stability in dried plant tissue and make up most of the visual pigments in vascular plants (Harborne, 1984). Other group of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, chalcones, catechin and leucoanthocyanidins.

Flavones and Flavonols are the most abundant group of flavonoids. Flavones are the compounds produce by glycosylation at C_7 and the sugars component may be a monosaccaharide or a disaccaharide. Flavonols are 3-hydroxy flavone produce by glycosylation at C_3 these compounds are similar to flavones. The three common members of this group are kaempferol, quercetin and myricetin. Rutin (vit-P) is the 3—O-rutinoside of quercetin. Anthocyanins, a class of flavonoid compounds, are ubiquitous flower and fruit pigments of flowering plants. These pigments are synthesized through a multistep biosynthetic pathway, generally referred as anthocyanin pathway (Coe *et al*., 1988; Baruah and Swain, 1959).

Rotenoids are naturally occurring substances containing a *cis*-fused tetrahydrochromeno [3,4-b]chromene nucleus. Rotenoids are related to the isoflavones. Many plants in the family Fabaceae contain rotenoids. Rotenoids found in. *Tephrosia purpurea* are Rotenone, Deguelin and Tephrosin. Rotenoids can be found in Lonchocarpus sp. Deguelin and tephrosin can be found in *Tephrosia vogelii*. 6'-O-β-D-glucopyranosyl-12a-hydroxydalpanol can be found in the fruits of *Amorpha fruticosa*. Elliptol, 12-deoxo-12alpha-methoxyelliptone, 6-methoxy-6a, 12a-dehydrodeguelin, 6a, 12a-dehydrodeguelin, 6-hydroxy-6a, 12a-dehydrodeguelin, 6-oxo-6a,12a-dehydrodeguelin and 12a-hydroxyelliptone can be isolated from the twigs of *Millettia duchesnei*. Deguelin, dehydrodeguelin, rotenol, rotenone, tephrosin and sumatrol can be found in *Indigofera tinctoria*. 6aα, 12aα-12a-hydroxyelliptone can be found in the stems of *Derris trifoliate*.

PHENOLICS

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits. In plants, they are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL) and are very important to plants. More than 8,000 phenolic compounds identified structurally are broadly distributed in plants and plant based foods. They are classified into (i) non-flavonoid polyphenolies (phenolic acids, lignans and hydrocinnamic derivatives) and (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins). Simplest of the class is phenol (C_6H_5OH). Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Phenolic acids-C_7, C_8, C_9 mostly includes benzoic acids and cinnamic acids. Most well-known common polyphenolics are gallic acid, ellagic acid, catachin, rosmeric acid, chlorogenic acid, ferulic acid, coumaric acid, quinic acid, vanilic acid, syringic acidand catechuic acid (Wu *et al*., 2009). Caffeic acid is regarded as the most common of phenolic compounds distributed in plants followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, *l.c.*).

These compounds are gaining a great deal of attention due to increasing evidence for their role in preventing oxidative diseases such as cancer and other neurological diseases (Catherine *et al*., 1997). Generally food based phenolic compounds (vegetables, cereals, fruits, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.) exhibit multiple biological effects such as antioxidant, anti-inflammatory, anticarcinogenic and protective role against oxidative degenerative disease (Wu *et al*., 2009; Dai and Mumper, 2010). They essentially represent a host of natural antioxidants, used as nutraceuticals and found in apples, green-tea and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree.

SAPONINS

The term saponin is derived from *Saponaria vaccaria* (*Quillaja saponaria*), a plant which abounds in saponins and was once used as soap. Saponins therefore possess ‘soaplike’ behaviour in water, *i.e.* they produce foam. On hydrolysis, an aglycone is produced, which is called sapogenin. There are two types of sapogenin: steroidal and triterpenoidal. Usually, the sugar is attached at C-3 in saponins, because in most sapogenins there is a hydroxyl group at C-3. Saponins are regarded as high molecular weight compounds in which, a sugar molecule is combined with triterpene or steroid aglycone. Saponins are extremely poisonous, as they cause heamolysis of blood and are known to cause cattle poisoning (Kar, 2007). They possess a bitter and acrid taste, besides causing irritation to mucous membranes. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvents like benzene and n-hexane. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides (Harborne, 1984).

TANNINS

Tannins are water soluble polyphenolic compounds with molecular weight ranging from 500 to 4,000 Da. Tannins have a characteristic feature to tan, *i.e.* to convert things into leather. They are classified into two classes: hydrolysable tannins and condensed tannins (Cai *et al*., 2006). Hydrolysable tannins upon hydrolysis produce gallic acid and ellagic acid, depending on the type of acid produced, are known as gallotannins or egallitannins. On heating, they form pyrogallic acid. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Tannins are large class of polyphenolics in dietary plants and medicinal herbs. Many fruits (e.g., strawberries, raspberries, pomegranate, walnuts, peach, blackberry, olive, apple juice and plum), vegetables (e.g., chickpeas, black-eyed peas, lentils and haricot beans) and spices (e.g., clove and cinnamon) contain high levels of proanthocyanidins or ellagitannins (Shan *et al*., 2005; Han *et al*., 2007).

TERPENOIDS

Terpenoids constitute the isoprene molecule [CH_2=C(CH_3)-CH=CH_2] and their carbon skeletons are built up from the union of 2 or more of these C_5 units. Terpenes are among the most widespread and chemically diverse groups of natural products. Terpenoids includes hydrocarbons of plant origin of general formula $(C_5H_8)_n$ and are classified as mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms. Examples of commonly important monoterpenes (C_{10}) include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes (C_{20}) are classically considered to be resins and taxol, the anticancer agent, is the common example. Triterpenes (C_{30}) include steroids, sterols and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Some common triterpenes are amyrins, ursolic acid and oleanic acid. Sesquiterpene (C_{15}) like monoterpenes, are major components of many essential oils (Martinez *et al*., 2008). Sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxic action. The sesquiterpene lactone, palasonin, isolated from *Butea monosperma* has anthelmintic activity. They range from essential oil components, the volatile mono and sesquiterpenes (C_{10} and C_{15}), to the less volatile diterpenes (C_{20}), the involatile terpenes and sterols (C_{30}) and carotenoid pigments (C_{40}) (Harborne, 2007; Mukherjee, 2007).

STEROIDS

Steroids also possess the cyclopentaboperhydrophenanthrene nucleus and 8-carbon side chain at C_{17} but they differ from the tetracyclic by reduction of methyl groups to 2 *i.e.* only at position 10 and 13. These compounds, once believed to be of animal origin, are extensively located in higher plants now, where they occur free as ester or as other derivatives. The typical animal sterols like cholesterol and hormones like estrones and ecdysrones are reported recently from many plants. But their role in plant is still ambiguous. Ergosterol is confined to fungi where as beta-sitosterol, stigmasterol and campesterol are widely distributed in plant kingdom (Kar, *l.c.*).

ESSENTIAL OILS

Volatile oils or essential oil form a group of non-saponifiable lipids produced in schizogenous cavities or in glandular epidermal hair in different plant organs. These compounds volatilized in air even at the ordinary room temperatures. Volatile oils are of two types, containing 1- monoterpenes or 2- sulphur containing compounds, both the groups are mutually exclusive. Essential oils are liquids at ordinary temperatures, though some deposit a little solid matter on standing. They are insoluble in water, but soluble in organic solvent. Most of them are optically active with high refractive index. They are restricted to plant organic like root (Vetiver), stem (Cinnamom), leaves (Basil), flower (Rose), fruit (Ajowan) or seed (Nutmeg). Volatile oils form good solvents. They themselves emit strong fragrance and are used alone as perfumes. These oils are extracted by cold expression, distillation or solvent extraction (Harborne, *l.c.*).

1.2. PHARMACOLOGY

Pharmacology is the science that deals with drugs, their properties, actions and fatality in body. It embraces the sciences of pharmaceutics (preparation of drugs), therapeutics (treatment of diseases by use of drugs) and toxicosis or adverse side-effects that arise from the therapeutic interventions (Magoma, 2014).

Pharmacology can be divided into the following processes:-

i. Pharmaceutical process of drugs deals with chemical synthesis, formulation and distribution of drugs.

ii. Pharmacokinetic process deals with the time course of drug concentration in body. This process can be further subdivided into: absorption, distribution, biotransformation and excretion of the drug.

iii. The pharmacodynamic process deals with the mechanism of drug action *i.e.*, interaction of drugs with molecular structures in body.

iv. Therapeutic process deals with the clinical response arising from the pharmaco-dynamic process.

v. Toxicologic process deals with adverse effects of drugs arising from either over-dosage or interference of biochemical pathways unrelated to the intended drug target. The five processes are related as exemplified in Figure 1.

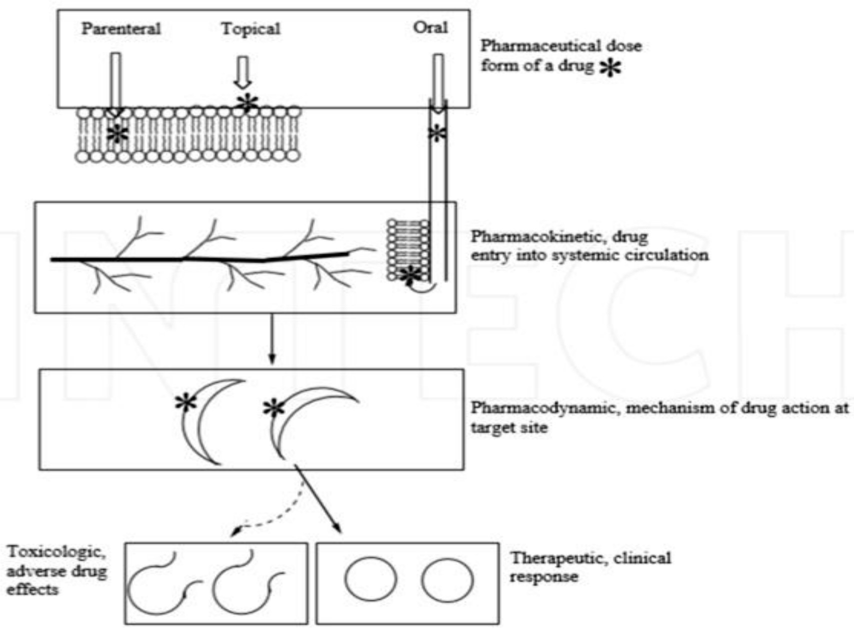


Figure 1. Relationships between the five pharmacological processes starting with the entry of the drug and ending with the clinical response and/or the toxic effects

1.3. ENDEMISM

The word 'endemic' (in Greek *en* means within and *demos* means population) is ascribed to any taxonomic unit or taxon which occurs in a restricted area, usually isolated by geographical or temporal barriers. In broader sense word endemic involves definition of the occurrence of a particular species in a large continental area. The concept of endemism more specifically deals with the ecological state of a species being unique to a defined geographic location, such as an island, nation, country or other defined zone, or habitat type; organisms that are indigenous to a place are not endemic to it if they are also found elsewhere. **Endemic flora elements of a country or geographical region throw light on the biogeography of the area, centres of speciation, area of extinction, vicariance and adaptive evolution of flora occurring in the area**. According to A. P. De Candolle (1855), the concept of *Endemic area* is defined as a taxonomic unit especially species which has a restricted distribution or habitat, isolated from its surrounding region through geographical, ecological or temporal barrier.

1.3.1 TYPES OF ENDEMISM

There are three different types of endemism they are paleoendemic, neoendemic and holoendemic.

Palaeoendemics are ancient endemics, which represent ruminants of older floras usually occurring in geologically old landmass. The main characteristics of paleoendemics are that they do not usually have close connections with other species in the same region, sometimes they are taxonomically isolated, may show disjunction in their distribution or they may have possible fossil evidence provided by homologous taxa or congenera of living taxa (Bramwell, 1972).

Neoendemics are newly evolved endemic taxa of relatively recent origin from an actively evolving genetic stock occurring in a particular ecotone. They have closely related taxa occurring in the same area. Neoendemic also develop through geographical speciation, quantum speciation and sympatric speciation. Polyploidy is also another hallmark of neoendemic.

Holoendemic is the phase of endemic species between its origin, spread and eventually perhaps its loss. According to Richardson (1978), all species start as neoendemics and end up as paleoendemics. It is generally considered that in favourable environmental conditions, neoendemic tend to behave as holoendemics and may lead to the formation of palaeoendemics through the following steps: origin, expansion, stabilization, diversification, migration, fragmentation, contraction and later extinction, due to selection pressures and environmental stress some relict species can become active epibiotics (Nayar, 1981).

1.3.2. NEED OF NEW ALTERNATIVE PLANT RESOURCES

There are many reasons for exploring wild, exploring endemic plants. They are specified with pharmaceutical view as stated below

The major one is the usage of plants for medical purposes by man due to their therapeutic properties has been in vogue since from pre-historic times. The advancement in bioassay techniques along with scientific and sophisticated technology encouraged the researchers to rediscover the benefits of herbs and spices that were so well known to our forefathers. These benefits are directly connected to the increasing awareness of the impact of food on the well-being and health of the person. According to the literature more than 139 drugs of conventional system of medicines owe their origin to herbs and herbal active principles. In India and worldwide, use of herbal ingredients has a long tradition. The indigenous medicinal systems like Ayurveda, Siddha and Unani are followed even today as a part of curing many ailments without any side effects (Sharma, 2008).

The second reason is the natural products, which are being obtained from plants are safe against the synthetics which might later potentially cause harmful side effects. Herbal products perceive utmost care for the environment, which includes biodegradable and ecofriendly products and even avoid testing on animals (Sharma, 2008).

The third reason is the awareness of natural ingredients and demand for herbal product, worldwide. In recent years the drugs of great medicinal value have been extracted from herbs are morphine, cocaine, strychnine, digitalis, ephedrine, mandragorine, aconitine, ergotarmine, rauvolfine, atropine and quinine, responsible for curing many diseases. This awareness about the natural ingredients from medicinal plants and their gain in economic terms has attracted many farmers to go for their cultivation. The trade of medicinal plants in India is estimated to be around Rs. 1000 cores per annum and the world trade to be over US $ 60 billion. It is found that each year an estimated 10% of the UK population received a CAM therapy (Thomas and Coleman, 2004). In 2003-04, $5bn was spent on herbal treatments alone in Western Europe (Anonymous, 2008). A study in UK founded that 20% of cancer patients had used herbal medicines (Damery *et al.*, 2011).

The forth reason can consider as lack of raw medicinal plant material as per supply (Sharma, 2008). About 95 per cent of the medicinal plant species used by the Indian industry at present are collected from the wild forests. Less than 20 species of plants are under commercial cultivation of which about 400 species is used in production various herbal drugs by the industry. The seventy per cent medicinal plant collections involved destructive harvesting because of the use of parts like root, bark, wood, stem and the whole plant which lead to the threat of genetic stocks and diversity of medicinal plants. As per the guidelines of IUCN (International Union for Conservation of Nature and Natural Resources) – The World Conservation Union, in India has already listed around 60 of plant species as Critically Endangered and 141 as Endangered of which 132 are in redlist (Sudhi, 2012).

In addition to these reason today large proportions of natural drugs are mostly made from the temperate plant. Only small amount of drugs are made from tropical plants. This so, as the plants from the region were not vigorously examined for its phytochemistry and biological activity, as compared with plants of temperate region. Further out of 0.3 million plants species available on the plant only 15 % have been examined phytochemically. A quote from Atharvaveda "The boar knows the plants, the mangoose know the remedical herb. What the serpents, the gandharvas know, I call to aid", suggested that from the animals and birds we can learn alot about the healing properties of herbs and may develop new medicines (Sharma, 2008).

From these reasons it can said that discovery and development of new therapeutic agents is a continuing process. In spite of the fact that at present we have at our command a formidable array of modern drugs, the need to discover and invent new agents from plant is genuine and urgent. It has been estimated that satisfactory therapy is available only for about one third of all presently known human ailments and several diseases, such as cancer, AIDS, senile dementia, autoimmune diseases, to mention just a few continue to evade reasonable solution. It is also predicted that due to several other reason including global warming etc., infectious diseases may become one of the main scourge of mankind in the near future. Thus fight against disease must be carried on relentlessly by drugs isolated from plants. With these details, I decided to work on the unexplored, species of medicinal important genus *Tephrosia.*

1.3.3 THE GENUS *TEPHROSIA*

The genus *Tephrosia* belongs to the family Leguminosae and subfamily Papilionaceae. The genus name *Tephrosia* derives from the Greek word "tephr (o)" meaning "ashes, ash coloured and gray" as the colour of the stems, leaves and fruit of all the species are gray in colour. It is a large genus of herbs and shrubs. Centres of species diversity include tropical and subtropical regions of the world with highest concentration in Africa–Madagascar (c. 170 spp.), Australia (c. 90 spp.) and Central and tropical North America (c. 45 spp.) (Lewis *et al*. 2005). The base chromosome number of this genus is X=11 and are placed in the tribe Galegeae of the family Leguminosae (Agarwal and Gupta, 1983; Atchison, 1951).

Phytochemical investigations have revealed the presence of glucosides, rotenoids, isoflavones, chalcones, flavanones, flavanols and prenylated flavonoids of chemotaxonomic importance in the genus (Waterman and Khalid, 1980). Moreover, bioactivity has been studied extensively, indicating that chemical constituents and extracts of the genus *Tephrosia* exhibited diverse bioactivities, such as insecticidal (Kole *et al*., 1992), antiviral (Sanchez *et al.*, 2000), antiprotozoal (Ganapaty *et al*., 2008), antiplasmodial (Muiva *et al*., 2009) and cytotoxic (Sinha *et al*., 1982) activities. Many plants from this genus have been used traditionally for the treatment of diseases like rheumatic pains, syphilis, dropsy, stomach ache, diarrhoea, asthma, abortifacient, respiratory disorder, laxative, diuretic and inflammation (Qureshi *et al*., 2010; Dzenda *et al*., 2007).

In South Asia, the genus is represented with 29 species, two subspecies and one variety (Kumar and Sane, 2003). In India the genus is represented by 27 species and one variety (Sanjappa, 1992), of these 6 are endemic species (Ahmedullah and Nayar, 1986). In semi arid region of India, there are 13 species of which two are endemic species i.e. *Tephrosia jamnagarensis* Sant. and *Tephrosia collina* Sharma (Nayar, 1988; Shah, 1978). They are also enlisted as threatened and rare endangered plants (Rao *et al.*, 2003; Walter *et al.*, 1997). The other commonly occurring species are *T. purpurea*, *T. villosa*, *T. senticosa* and *T. strigosa*.

T. jamnagarensis is an endemic species distributed in narrow pocket of Saurashtra peninsula, Gujarat, India. It was first discovered by Santapau (1958) and was identified as new species based on the fruit characters (Santapau, 1958 and 1962). The first detailed morphological description of this plant with flower was given by Ahluwalia and Smith. (1967); stating it as a new species *Tephrosia axillaris*. Later, this error was correted by Raghavan *et al. (*1968) and restated that *T. axillaris* is a synonym of *T. jamnagarensis.* Thereafter Nagar *et al.*, (2003), rediscovered this species from the Khadkhambaliya vidi of Jamnagar district of Gujarat (Nagar, 2003).

T. collina is an endangered plant, possessing similar pattern of restricted distribution (Nagar, 2007). This species of *Tephrosia* is restricted to semi arid region of Gujarat and Rajasthan, India (Bole and Pathak, 1988; Shah 1983). In both these geographical locations, the plant is confined to the hillocks dominated by grasses in small pocket. This species was first discovered by Sharma (1963) from Ajmer district Rajasthan. Later this species was reported from the Rajasthan by Yadav (2000), Rajpipla, southern Gujarat by Shah (1978) and Motividi, Jamjodhpur Taluka from western Gujarat by Nagar (2007).

Based on the above literature both the endemic species of *Tephrosia* were studied for phytochemical constituents and biological activities. As a basis for phytochemical correlation the extensively worked out *Tephrosia* species i.e., *T. purpurea, T. toxicaria, T. candida, T. elata* and *T. villosa*, were taken. The main classes of compounds present in this genus include flavonoids, rotenoids, terpenoids and sterols (Sharma *et al.*, 2013). It should be noted that flavonoids are the most abundantly isolated and identified compounds in the *Tephrosia* genus (Touqeer *et al.*, 2013). Overall the present research work deals with exploration the phytochemical and pharmacological activity as hepatoprotective agent.

1.4 OBJECTIVE

1. Macromorphological studies (Phenetic character).
2. Molecular characterization using *matK* gene.
3. Distribution pattern and ecological parameters.
4. *Ex-situ* conservation by Seed germination and Phenology.
5. Micromorphology and Pharmacognosy
6. Phytochemistry
    - Physicochemical parameters
    - Preliminary phytochemical
    - Phytochemical marker
7. Pharmacology *In vitro* and *Invivo* analysis as hepatoprotective agent.