MEDICINAL PLANTS SECONDARY METABOLITES USED IN PHARMACEUTICAL IMPORTANCE—AN OVERVIEW

*Ramasubramania Raja, R¹ M. Sreenivasulu²

¹ Asst. Professor, Department of Pharmacognosy, Narayana Pharmacy College, Nellore.
² Principal, Narayana Pharmacy College, Nellore.

ABSTRACT

Medicinal plants are the most important source of life saving drugs for the majority of the world’s population. Plants have formed the basis of sophisticated traditional medicine system that has been in existence thousands of years in countries such as China and India. The compounds which synthesized from the secondary metabolisms are so-called secondary metabolites. Secondary metabolites are formed in only specific organisms, or groups of organisms, are expression of the individuality of species. Secondary metabolites are not necessarily produced under all conditions, and in the vast majority of cases the function of these Compounds and their benefit to the organism are not yet known. It is this area of secondary metabolism that provides most of the pharmacologically active natural products. This review deals with the production of secondary metabolites in valuable medicinal herbs with different pharmacological activity like Anti malarial, anti diabetic, Hepatoprotective, Anti ulcer, anti inflammatory including Antimicrobial activity.

KEYWORDS: Hepatoprotective, Antimicrobial activity, Anti malarial, anti diabetic.

INTRODUCTION

Secondary metabolism is particular environmental conditions or developmental stages. When grown in a nutrient-rich medium, most bacteria employ almost solely basic metabolism in order to grow and reproduce. However, when nutrients are depleted, they start producing an array of secondary metabolites in order to promote survival. Plants produce secondary metabolites as a response to adverse environmental conditions or in particular developmental
stages. For example, exposure to UV radiation induces the biosynthesis of UV-absorbing compounds. Secondary metabolites are those chemical compounds in organisms that are not directly involved in the normal growth, development or reproduction of organisms. Typically, primary metabolites are found across all species within broad phylogenetic groupings, and are produced using the same pathway (or nearly the same pathway) in all these species. Secondary metabolites, by contrast, are often species-specific (or found in only a small set of species in a narrow phylogenetic group), and without these compounds the organism suffers from only a mild impairment, lowered survivability/fecundity, aesthetic differences, or else no change in phenotype at all. The function or importance of these compounds to the organism is usually of an ecological nature as they are used as defenses against predators, parasites and diseases, for interspecies competition, and to facilitate the reproductive processes (coloring agents, attractive smells, etc). Since these compounds are usually restricted to a much more limited group of organisms, they have long been of prime importance in taxonomic research.

**Plant-derived pharmaceutical importance of secondary metabolites**

<table>
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<th>Secondary metabolites</th>
<th>Use</th>
<th>Plant species</th>
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<td>Ajmalicine</td>
<td>Antihypertensive</td>
<td>Cath. roseus</td>
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<td>Artemisinin</td>
<td>Antimalarial</td>
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<td>Ajmaline</td>
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<td>Ra. serpentina</td>
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<td>Acinitone</td>
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<td>Camptothecin</td>
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<td>Capsaicin</td>
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<td>Castanospermine</td>
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<td>Codeine</td>
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<td>Colchicine</td>
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<td>Digoxin</td>
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<td>Diosgenin</td>
<td>Steroidal precursor</td>
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<td>Ellipticine</td>
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<td>Emetine</td>
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<td>Forskolin</td>
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<td>Ginsenosides</td>
<td>Health tonic</td>
<td>Panax ginseng</td>
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<tr>
<td>Morphine</td>
<td>Sedative</td>
<td>P. somniferum</td>
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Podophyllotoxin: Antitumour
Quinine: Antimalarial
Sanguinarine: Antiplaque
Shikonin: Antibacterial
Taxol: Anticancer
Vincristine: Antileukemic
Vinblastine: Antileukemic

Secondary metabolites identification screening methods:

Steroids: 1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Terpenoids: 2 ml of extract was added to 2 ml of acetic anhydride and concentration of sulphuric acid. Formation of blue, green rings indicate the presence of terpenoids.

Fatty Acids: 0.5 ml of extract was mixed with 5 ml of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparency on filter paper indicates the presence of fatty acids.

Tannins: 2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Saponins: 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Anthocyanins: 2 ml of aqueous extract is added to 2 ml of 2N HCl and ammonia. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins.

Leucoanthocyanins: 5 ml of aqueous extract added to 5 ml of isoamyl alcohol. Upper layer appears red in colour indicates for presence of leucoanthocyanins.

Coumarins: 3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins.

Emodins: 2 ml of NH OH and 3 ml of Benzene was added to the extract. Appearance of red colour indicates the presence of emodins.
Biosynthetic pathways of secondary metabolites

Biosynthetic pathway introduction of secondary metabolites in medicinal Plants: During long period evolution, plants struggling to survive gradually gain the ability to synthesize various kinds of secondary metabolites with bioactivities. These compounds played important role in defencing insects, herbivores, microbial pathogens, competing with other plants, and facilitating pollination and reproduction. Based on the structures, the second metabolites can be classified into alkaloids, flavonoids, phenylpropanoids, quinones, terpenoids, steroids, tannins and proteins. These compounds are biosynthesized through series enzyme catalyzed reactions using simple building blocks in different ways. There are several main biosynthetic pathways in plants, including shikimic acid pathway (phenylpropanoids), mavalonic acid pathway (quinones), 2-C-methyl-D-erythritol-4-phosphate pathway (quinones), amino acid pathway (alkaloids), acetate-malonate pathway (fatty acid, phenols and quinones) and combined pathways (flavonoids).

Molecular cloning and characterization of key enzymes involved in specific biosynthetic pathway.[1]

Gene cloning, transformation and regulation have achieved significant progress in biosynthetic pathway of secondary metabolites. The biosynthetic pathway elucidation and gene regulation research of vinblastin and vincristine, anticancer compounds from Catharanthus roseus, is a representative example. Due to the low contents of these two compounds in naturally growing plant, it becomes important to clone and regulate expression of key genes (DXR, SLS, G10H, STR) involved in the pathway, in order to achieve high yield of these two compounds in invitro culture systems, which will be based on well understanding of terpenoid indole alkaloids biosynthesis in C. roseus. As to the approaches applied in the cloning of genes involved in secondary metabolites biosynthetic pathway, there are methods based on Polymerase chain reaction (PCR) and library construction. The former methods include Rapid amplification of cDNA ends PCR (RACE PCR) and RT-PCR. However, these methods need at least partial gene sequence information, or gene sequence from other plant species to synthesize degenerate primers. If there are no reported reference sequences, these methods will not be able to work well. Under this situation, library based method can be selected for gene cloning, however, the library based methods are non specific enough, like cDNA library, BAC library. That’s because of the low ratio of genes involved in secondary metabolism in total expressed mRNAs. As the progress of genomics, functional genomics provided us useful methods in cloning the specific genes involved in the secondary
metabolism, such as Subtractive hybridization ‘Differential screening’ Microarray assay and Serial Analysis of Gene Expression. Functional genomics focused on the selection of treatment responsive genes and to clone genes involved in the biosynthetic pathways, the treatments need to be specific enough to result in the improvement of target secondary metabolites.

**Elicitors and signaling pathways:** Elicitors are substances that can trigger the hypersensitive reaction in treated plant cells. Due to the effective up-regulation of genes expression, and further activation of secondary metabolism, and the improvement effects of secondary metabolites accumulation, elicitors are used widely in medicinal plant cell and tissue culture to maximize the production of target compounds. Elicitors can be divided into biotic and abiotic elicitors. The former one includes fugal polysaccharides, proteins, cell debris and conidium. These kinds of elicitors are made from cultured fungi and some species are specific for specific kinds of secondary metabolites accumulation, for example, Armillaria mellen elicitor for alkaloids accumulation; Verticillium dahlia for phenolic compounds accumulation; Botrytis sp for terpenoids accumulation, oligosaccharides for saponins accumulation and yeast extract for flavonoids accumulation. As to abiotic elicitors, heavy metals ions, rare metal ions, UV lights, osmotic stress and even sonication have all been reported to have positive effects towards improvement of secondary metabolites. Comparing the application potential, biotic elicitor attracted more attention due to their advantages of low cost, little side effects, strong elicitation effects and easy manipulation.

When using elicitors, the main optimizing characters are treatment time, concentration and selection of elicitor kinds. In plant cell/organ culture, it is believed that, treat cells/organs with elicitors at the stabilization stage can increase the accumulation of secondary metabolites significantly. However, there are also reports that treat cells at the beginning of culture, which also improved the final product yield remarkably. In this case, there is possibility identifying key intermediates involved in the biosynthetic pathway. As to the concentration, too high concentration treatment can result in decrease of biomass accumulation and even the content of secondary metabolites can be improved, however, the total yield will still not be maximized. Therefore, to use an elicitor, the conflict between biomass accumulation and secondary metabolite accumulation should be balanced and the optimization should be carried out. Recently, a strategy of repeated elicitation has been developed, and the treatment can result in the increase of target compounds accumulation as
well as related enzyme activity and gene expression more than one time, and therefore, attracts attention in the field of secondary metabolism regulation aiming at maximizing the yield of compound of interest.

**Precursor feeding:** Precursors are compounds existed in upstream of target compounds in biosynthetic pathway. Most intermediates can be used as precursors. Upstream precursors are converted into down-stream compounds after specific enzyme catalysis, the concentration of precursors determines the reaction speed. At higher concentration, the reaction speed is usually higher than that when precursor concentration is lower. To improve the yield of secondary metabolites in plant cell and tissue culture, precursor feeding is an effective approach. However, precursor can inhibit cell growth and enzyme activity if concentration is too high. In some cases, high concentration of intermediates can even be toxic for cells. Originally, in cells, these intermediates are all maintained in very low concentration, and converted to down-stream compounds quickly. Therefore, for one specific plant species, precursor concentration should be carefully adjusted, compared and optimized. The precursor feeding experiment can also be used as a powerful tool for the elucidation of biosynthetic pathways. Isotope labeled precursors can be fed in the media, and as the cell growing and biosynthesis going on, secondary metabolites synthesized can be isotope labeled at different positions. After harvesting the cells, purifying the compounds and elucidating their structures, the biosynthetic origin can be concluded. This method is classic but also most authentic method in elucidating the biosynthetic origin of a specific compound. However, the disadvantages are also obvious, first of all, the precursors are isotope labeled and is dangerous to handle; secondly, the purification of isotope labeled end product requires lots of cells; thirdly, some intermediates are maintained in very low concentration in living plant cells, which will makes the purification of these intermediates very difficult. Therefore, if the isotope labeled precursor feeding combine with elicitor treatment or specific enzyme inhibitors treatment, the problems might be solved.

**Application of specific enzyme inhibitors in regulating the biosynthetic pathway:** In plant cells, all secondary metabolites are derived from glucose that is biosynthesized during photosynthesis. In most medicinal plants, and even model plants, there are several kinds of secondary metabolites accumulated. For example, there are about 15 flavonoids, 50 terpenoids, 15 fatty acid, 20 phenylpropanoids, and 35 glucosinolates in A. thaliana. Different kinds of compounds are competitively related with each other. To improve the
accumulation of one specific kind of compounds or blocking the other main biosynthetic branches, genetic transformation method is ideal for the purpose. However, the transformation method of most medicinal plants has not been established. On the other hand, elicitor treatment is not specific, even the treatment can result in the improvement of secondary metabolites accumulation. Therefore, both methods can’t provide specific evidence for contribution of single gene in plant secondary metabolites biosynthetic pathway. Under these circumstances, how to study the contribution of specific gene(s) involved in the biosynthesis pathway to the production of target compounds? If we treat the cells with enzyme specific inhibitors, the enzyme activity will be specifically inhibited, and result in the corresponding consequences. After content determination of target compounds and enzyme activity assay, correlation analysis between enzyme activity and target compounds accumulation can be carried out, and therefore get to know the contribution of specific enzyme in the biosynthetic pathway of target compounds. This method can also be used to identify key enzymes or rate-limiting steps in the pathway. The application of inhibitors can also be used for other purposes. When treated with specific inhibitors blocking the competitive branch, more carbon flux will be channeled to the biosynthesis of target compounds, and result in the improved accumulation. Furthermore, if the cells are treated with specific inhibitors, the enzyme activity will be inhibited, and the upstream compounds can be consequently accumulated at higher concentration which will facilitate the identification and characterization of key intermediates.

**Application of genetic techniques in regulating the biosynthetic pathway:** With the development of molecular technology, more and more genetic information becomes available online. Until now, there are dozens of plant species whose genome has been sequenced, such like Arabidopsis thaliana, Oryza sativa, Artemisia annua and so on. Besides, genetic techniques (EST, RACE PCR, transformation, T-DNA tagging, siRNA, miRNA etc.) have also been carried out in other medicinal plants, like Panax giseng, Saussurea involucrate and so on. These techniques provided tons of information and possibilities for the manipulation of target compound biosynthesis. Japanese scientists introduced a flavonoid 3’5’ hydroxylase gene into rose plant with red flowers, and the transgenic plant showed blue-purple flowers. In Saussurea involucrate hairy root culture, a chalcone isomerase gene was introduced and over expressed, which resulted in 12 times production of apigenin of that in control hairy root. Tyrosine decarboxylase gene isolated from parsley, was introduced in potato, and in transgenic plant, salidroside was detected. Salidroside is a phenylethanoid compound with
significant adaptive effects and is the main active compound in Rhodiola sachalinensis. This report showed us the potential of producing new pharmaceutically important compounds besides original medicinal herb.

**Combinatorial Biosynthesis in production of useful secondary metabolites:** Combinatory synthesis is a method to combine metabolism pathways of different species genetically. As the development of molecular biology, and the proposal of creative experimental methods and idea, the concept of combinatory will be changed in the future, but there is no doubt about its importance in post genomics. Currently, most research related to combinationary synthesis is using two kinds of microbes in their experiment, Escherichia coli and Saccharomyces cerevisiae. Two microbes are not accidentally selected as model species. Experimental skills related to their mutation, culture, transformation and expression are all well developed. Besides, the whole genome sequence information is also available which provided detailed information for the manipulation and genetic engineering of two microbes. To be noticed is that, full biosynthetic pathways of secondary metabolites are not involved in the microbes or at least partially not involved. Therefore, to produce the specific compound in microbes, whole biosynthetic pathway or part of the pathway must be introduced into the target microbes (through high copy number plasmid or integration to chromosome). This is the main idea of using E. coli and S. cerevisiae as the vehicles of combinatory biosynthesis, and also the bottle-neck limiting the application of this technique, due to the poor understanding of biosynthetic pathway of secondary metabolites in plants. Thus, currently, most researchers are focusing on high-yield intermediate production and use chemical semi-synthesis to produce the final product of interest or precursor feeding in the cultured transformed microbes.

**Pyto-pharmacological evidence**

**Anti-malarial Drugs:** The first drugs to treat malaria came from *Cinchona officinalis* and related Cinchona species (Rubiaceae) which naturally occur in Central and South America. Extracts from Cinchona bark contain quinoline alkaloids, such as quinine, quinidine, cinchonine, and cinchonidine. Quinine served as a lead structure for the synthesis of several antimalarial drugs such as chloroquine, mefloquine, pyrimethamine, proguanil, atovaquone (sold together with proguanil as “Malarone”), or primaquine. Quinine (alone or in combination with doxocycline, tetracycline or clindamycin) is still used today to treat acute cases of severe *P. falciparum* infections. Over the years *Plasmodium* (especially *P.*
falciparum causing tropical malaria) has become resistant against many of the synthetic drugs. A breakthrough for the development of antimalarial drugs was the identification of the sesquiterpene artemisinin from Artemisia annua (Asteraceae), which can even kill multidrug resistant strains of P. falciparum. Several semisynthetic derivatives of artemisinin (e.g., the water soluble artesunate) have been developed which are in clinical practice today. Natural products with antimalarial activity have been identified among widely distributed phenolics (ellagic acid, epigallocatechin gallate, flavonoids, xanthones, coumarins, curcumin), naphthopyrones, quinones, widely distributed terpenoids (iridoids, sesquiterpenes, diterpenes, triterpenes), quassinoids, cucurbitacins (common in Cucurbitaceae), alkaloids (indolizidine, indole, isoquinoline), polyacetylenes.

**Anti diabetic activity:** Costus pictus family Costaceae, a recently introduced plant from Mexico has shown its potential as herbal drug for diabetes. In the present study 24 extracts were prepared from three materials (rhizome, stem and leaf) of two regions (Kerala and Tamilnadu) using different solvents (hexane, ethyl acetate, methanol and water) and they were subjected to phytochemical screening, and checked the hypoglycaemic activity in glucose fed albino mice. The preliminary phytochemical screening indicated very much similarity in the presence of chemical constituents in all 24 extracts of three samples of two regions and the methanol extract (200mg/kg, 500mg/kg b.w.) of leaf were exhibited significant hypoglycaemic activity in glucose fed mice. The study suggests the large scale cultivation of C. pictus at varied geographical locations as the phytochemical profile is quite stable with environmental variables.[2]

**Anti ulcer activity:** Peptic ulcers are a broad term that includes ulcers of digestive tract in the stomach or the duodenum. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti inflammatory drugs (NSAIDs) example, aspirin and Helicobacter pylori infection. Numerous natural products have been evaluated as therapeutics for the treatment of a variety of diseases, including peptic ulcer. There has been considerable pharmacological investigation into the antiulcer activity of some compounds. In this work, we shall review the literature on different medicinal plant and alkaloids with antiulcer activity. This article reviews the antiacid/anti-peptic, gastroprotective and/or antiulcer properties of the most commonly employed herbal medicines and their identified active constituents. The experimental parameters used for
antiulcer activity were cold restraint stress-induced ulcer model, Diclofenac-induced ulcer model in rats, (HCl–ethanol)-induced ulcer in mice and water immersion stress-induced ulcer in rats. The ideal aims of treatment of peptic ulcer disease are to relieve pain, heal the ulcer and delay ulcer recurrence. About 70% of patients with peptic ulcer disease are infected by Helicobacter pylori and eradication of this microorganism seems to be curative for this disease. This article reviews drugs derived from medicinal plant more commonly used in the world for peptic ulcer and, if reported, the antiulcer activity. This article will be concerned only with the antiulcer and gastro-protective effects.[3]

Antimalarial activity: Copaifera langsdorffii Desf. is a medicinal plant found in all regions of Brazil. Copaiba oleoresin is widely trade around the world and well-known for its distinct biological properties. The present work reports the isolation of compounds from leaves, fruits and flowers of Copaifera langsdorffii, as well as in vitro leishmanicidal and antimalarial assays with the crude hydroalcoholic extracts from leaves, fruits and flowers. The hydroalcoholic crude extracts were chromatographed using silica gel by classic chromatography, and purified by preparative TLC. HPLC-DAD was used to purify the polar compounds. The thermally stable compounds were evaluated by GC/MS. NMR and mass spectrometry data were used to establish the chemical structures. Nine compounds were isolated and identified: 1: kaurenoic acid, 2: ent-labd-7,13-dien-15-oic acid, 3: (E)-2-ethoxyvinylphenol, 4: 2-hydroxy-ent-labda-7,13-dien-15-oic acid, 5: caryophyllene oxide, 6: kaurenol, 7: ethyl 4-hydroxyxycinnamate (semisynthetic 4-methylated form of compound 7 was isolated), 8: quercetin-3-O-α-Lrhamnopyranoside (quercitrin), and 9: kaempferol-3-O-α-L-rhamnopyranoside (afzelin), and a new compound (3) from fruits. Compounds 4 and 7 were reported for the first time in the aerial parts of Copaifera genus. Kaurenoic acid and β-caryophyllene were major compounds in all samples studied. Flavonoids 8 and 9 were major phenolics in the leaves hydroalcoholic extract. The Fruits extract displayed antileishmanial activity against Leishmania donovani with IC50 of 40 μg/mL. Leaves and flowers extract displayed antimalarial activity with IC50 of 3.4 and 6.2 μg/mL, respectively for different clones of Plasmodium falciparum. In addition, none of the tested samples displayed cytotoxicity against mammalian Vero cells.[4]

Anti inflammatory activity: The anti-inflammatory activity of two Cat’s claw bark extracts, by comparing a spray-dried hydro alcoholic extract against an aqueous freeze-dried extract, to determine which extract was more effective. We used the carrageenan-induced paw edema
model in mice. In addition, to assess the molecular mechanism of action, we determined the inhibition of NF-kB through the Electrophoretic Mobility Shift Assay (EMSA) and the effects on cyclooxygenase-1 and -2. Results showed that the anti-inflammatory activity was significantly higher using the hydroalcoholic compared with the aqueous extract (PB/0.05). The extracts also showed little inhibitory activity on cyclooxygenase-1 and -2. It cannot be excluded that the slight inhibitory activity on DNA binding of NF-kB is due to cytotoxic effects.\[5\]

**Antimicrobial activity:** The antimicrobial activity of secondary metabolites and lectins, compounds usually associated to defense mechanisms of plants. Secondary metabolites are separated into nitrogen compounds (alkaloids, non-protein amino acids, amines, alcamides, cyanogenic glycosides and glucosinolates) and nonnitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids and coumarins). Lectins are carbohydrate-binding proteins and their biological properties include cell-cell interactions. This chapter reports solvent organic extracts (mixture of secondary metabolites), isolated secondary metabolites and lectins from plants with antimicrobial activity against Gram-negative and Gram-positive bacteria as well as antifungal activity towards human and plant pathogens. Mechanisms proposed for antimicrobial activity of secondary metabolites and lectins against bacteria and fungi are also discussed. The effects of plant secondary metabolites and lectins on deleterious human and plant microorganisms indicates their perspectives of antimicrobial uses.\[6\]

**Hepatoprotective activity:** Isolate the flavonoid from the ethylacetate fraction of corm of Amorphophallus paeoniifolius by coloum chromatography using gradient elution method. The isolated flavonoid was characterized by spectral studies and screened for hepatoprotective activity on ccl4 induced model. The flavonoid (Quercetin) was subjected to various biochemical parameters such as SGOT, SGPT, SALP, bilirubin, total protein and histopathology of rat liver were studied. The results were found to be significant by reducing the elevated enzyme levels, increasing the protein level and attenuating the damaged hepatocytes toward the normal texture. The results were further supported by histopathology of isolated rat liver. Therefore the present study justifies that the isolated flavonoid exhibits significant hepatoprotective activity.\[7\]
CONCLUSION
The secondary plant metabolites are biosynthesized plants. For example, alkaloids, glycosides, tannins, resins etc. These are active constituents at the plant and have marked pharmacological activity. These are very potent in action. Then utility of the plant depends upon the types of secondary plant metabolite present in it.

REFERENCE