CHAPTER-2
LITERATURE REVIEW
2. LITERATURE REVIEW

2.1. Itrifal formulations

In Unani system of medicine polyherbal formulations available with a wide range of indications like protective to liver, appetite and growth promoters, gastrointestinal and hepatic regulator, as treatment for hepatic dysfunction, for hepatic regeneration as well as liver stimulant and tonic. According to the Unani system, Itrifal or Trifaloon is actually the name of semi solid herbal preparation of three different types of fruits i.e. Halela (*Terminalia chebula*), Balela (*Terminalia belerica*) and Aamla (*Emblica officinalis*). Itrifals are proven for its anti-oxidant activities; most generally it is used as an overall body tonic, thought to be effective in cleansing and detoxifying the system. Further, ingredients may be added to the base formulation Itrifal and are named accordingly like Itrifal-e-Aftimoon (IA) and Itrifal-e-Badiyan (IB), which we selected for the present study. The major antioxidants and free phenolic acids like ascorbic acid, gallic acid, ellagic acid, chebulinic acid and tannic acid as well as free flavonoids like rutin and quercetin forms the basis of bioactivity of these Itrifal formulations. Itrifal should be used continuously for not more than two month. Its continuous use may weaken the gastric secretion. The Itrifals are mainly prescribed in Unani system of medicine as brain tonic (Koneru *et al.*, 2011).

2.1.1. General method of preparations of Itrifals

For making Itrifals or any of its allied preparations base of different consistencies is generally made, depending on the nature of ingredient drugs to be used in a particular formula. The ingredient drugs in a base may be used either in the powder or liquid form. Qiwm is generally made by adding water, distillate or fruit juice. In many of the bases of purified honey with sugar, candy or jaggery etc. is boiled over a low flame till it acquires a required consistency. The base generally purified by adding lemon juice, lemon extract or almum etc before making the qiwm (basic solution of particular consistency). Qiwm is generally made, depending on the nature of ingredient drugs to be used in a particular formula. The ingredient of drugs may be mixed in Qiwm either in powder or liquid form. It is generally made by adding Aab (Water), Arq (distillate) or Aab-e-Samar (fruit juice) etc. in any of the bases of purified Asl (Honey), Sugar, Turanjabeen (*Tamarix indica* gum), Sheerkhisht (*Fraxinus ornus* exudate) etc. boiled over low fire till it acquires a required particular
consistency. The bases are generally purified by adding Aab Leemun (Lemon juice), Sat Leemun (Lemon extract) or Shibb-e-Yamani (Alum) etc, before making the Qiwam. Afterwards, the ingredient drugs are mixed in Qiwam to prepare Itrifal. For making Itrifals or any of its preparations, the consistency of Qiwam is three tars. For mixing of the ingredient drugs of different origin (plant, animals and mineral) in the qiwam following precautions should always be taken: plant origin drugs like Haleela (*Terminelia chebula* fruit), Balela (*Terminelia belerica* fruit) and Amla (*Emblica officinalis* fruit) before powdering should always be charb (crisped) with Roghan-e-Badam (Almond oil) or Roghan Zard (Ghee). The dried fruits are be first cleaned and washed with water to remove the impurities and dust, etc, thereafter they are soaked in water or cow’s milk for 12 hours to remove the Kasela (acrid) taste of the fruit (Hifzul, 2003).

### 2.2. Habb & Qurs Formulations

Habb is spoken in many other means also. This word is also used in term of seed, but in relation to form of drug, it is such solid form, which is made in round. The constituents of pill may be either one or more than one. Volume or size of pill varies. Some are very small like size of Sarson (*Brassica nigra* seed), Masoor (*Lens culinaris* seed) or about to one cm or more. Pills of one cm diameter are called Bundqa. The term Habb, in Unani system of medicine is used for indicating pills. It is a solid dosage form which is made in round shape. The diameter of pill may vary from several mm to cm. The constituents of pill may be either one or more than one.

Qurs is singular of Aqras. It is an Arabic word, which means Tikya (Tablet). Hakeem Indrumakhas is said to be its inventor. It is a form of pill, as pill is round but Qurs is round/triangular and flat. Their names are according to their constituents, inventor actions or shapes. These are flat and round/triangular/quadrangular biconvex. These are made mechanically also. Pill and tablet are differentiated by only shape. Their aims are somehow similar, dose determination, coating sugar or using agents to mask taste, easy to swallow.

#### 2.2.1. Method of Preparation

Two types of methods are used for the preparation of Habb and Qurs according to National Formulary of Unani Medicine.
2.2.1.1. Manual Process

Crude drugs are grounded into fine powder and passed through No.100 mesh sieve. The powder is mixed with any adhesive like water, honey, rose water etc. After making a mass by mixing, it is rolled into sticks of required size and thickness and cut into pieces with a knife. These cut pieces are rounded between the fingers to shape of pill with required size and weight.

2.2.1.2. Mechanical Process

Crude drugs are grounded into fine powder and passed through No.100 mesh sieve. The powder is then mixed with water or a specified adhesive to make a semi solid mass and granulated by passing through No.20 mesh sieves. The granules thus obtained are dried and kept in cooling panel and revolved. To make the pills, little water is sprinkled over the granules to keep them moist. Later on, these granules in the pan are coated with fine powder of crude drugs by rotating the pan with an interval of one minute to ensure the uniform and smooth coating of the granules and lastly passed through different size of sieve. The process is repeated till the pills of required size are obtained (Anonymous, 2006).
2.3. Analytical and pharmacological reviews of bioactive constituents present in the formulations

2.3.1. Gallic acid

Gallic acid is trihydroxybenzoic acid, a type of phenolic acid. It is found both free and as a part of tannins which are astringent in nature. Foods such as blueberries, walnuts, apples, flax seed, oak bark etc. are rich source of gallic acid.

IUPAC Name: 3, 4, 5-trihydroxybenzoic acid

![Fig. 2: Chemical structure of Gallic acid](image)

2.3.1.1. Analytical Reviews

A simple validated HPLC method for the separation and quantitative determination of gallic acid, tannic acid, syringic acid and epicatechin along with ascorbic acid using an acidic mobile phase within a 20 min analysis (Singh et al., 2008).

The free radical-scavenging activity of individual compounds from *Emblica officinalis* extract based on the combination of HPTLC with a diode array detector (DAD) and post chromatographic DPPH radical derivatization. It was established that the DPPH scavenging activity of emblicanins A and B was more than that of ascorbic acid and gallic acid (Pozharitskaya et al., 2007).

HPLC identification and quantification of isolated compounds from *Phyllanthus emblica* were also performed. Gallic acid was found to be a major compound in the ethyl acetate extract and geraniin showed highest nitric oxide scavenging activity among the isolated compounds (Kumaran and Karunakaran, 2006).

Study on analysis of phenolics and flavanoids of leaves of *Eucalyptus globulus* were found a rich source of rutin, *Moringa oleifera* for kaempferol, aerial parts of *Centella asiatica* for quercetin, fruits of *T. bellerica* and *T. chebula* for gallic acid, and bark of
T. arjuna, leaves and fruits of T. bellerica and bark, leaves and fruits of T. muelleri for ellagic acid (Bajpai et al., 2005).

2.3.1.2. Pharmacological Reviews

Gallic acid and its derivatives are a group of naturally occurring polyphenol antioxidants shown to have potential healthy effects. These polyphenol antioxidants exhibited different hydrophobicity and could cross through the liposome membrane to react with 1, 1-diphenyl-2-picryl-hydrazyl free radical in a time and dose-dependent manner. The structure–antioxidant activity relationship of gallic acid derivatives on scavenging DPPH free radical in the liposome was analyzed based on theoretical investigations (Lu et al., 2006).

The preventive effect of gallic acid on lysosomal enzymes in isoproterenol treated myocardial infarcted rats was studied and found that the levels of lipid peroxidation products were significantly increased whereas the level of reduced glutathione was significantly decreased in the plasma and heart of isoproterenol induced cardiotoxic rats. The activities of lysosomal enzymes (β-glucuronidase, β-N-acetylglucosaminidase, β-galactosidase, cathepsin-B and D) were increased significantly in the serum and heart of isoproterenol induced cardiotoxic rats (Stanely et al., 2009).

The activities of the antioxidant enzymes catalase and glutathione peroxidase in the blood and liver of the aging model induced by injection of different doses of D-galactosamine into normal mice, and in senescence accelerated mice of different ages, were determined. When gallic acid purified from rose flowers was used to treat the 9-month-old male senescence accelerated mice, it not only reinstated the activities of catalase and glutathione peroxidase but also significantly reduced the amount of malondialdehyde in the liver, brain and kidney (Li et al., 2005).

2.3.2. Ellagic acid

Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables including blackberries, raspberries, strawberries, cranberries, walnuts, pecans, pomegranates, wolfberry and other plant foods.

IUPAC Name: 2, 3, 7, 8-Tetrahydroxy-chromeno [5, 4, 3-cde] chromene-5, 10-dione
2.3.2.1. Analytical Reviews

Phenolic compounds and antioxidant capacity of acidified methanolic extract of *Mangifera pajang* peel cultivated were analyzed. Gallic acid, p-coumaric acid, ellagic acid, protocatechuic acid and mangiferin were the major compounds among the 16 phenolics that have been identified and quantified in *M. pajang* peels with 20.9, 12.7, 7.3, 5.4, and 4.8 mg/g BPP, respectively. The 16 phenolic compounds identified in *M. pajang* K. using HPLC-DAD and TSQ-ESI-MS are reported here for the first time (Hassan et al., 2011).

The whole plant of *Phyllanthus wightianus* extracts were subjected to isolation of their compounds: isomeric sterol mixture [stigmasterol, compesterol and β-sitosterol], fredilin, lupeol, gallic acid, bergenin, geraniin, corilagin and ellagic acid were established through the use of column chromatographic methods and spectral data. The percentage of tannins was also determined and estimated using the HPLC method (Priya et al., 2011).

LC-DAD method has been developed for quantification of analytes in four Drosera species used in medicine (*D. anglica*, *D. intermedia*, *D. madagascariensis*, and *D. rotundifolia*). During elaboration of the method 13 compounds, including three substances not previously described for Drosera species, were detected and unambiguously identified by means of extensive LC-MS and LC-NMR experiments and by off-line heteronuclear 2D NMR after targeted isolation (Zehl et al., 2011).

HPLC analysis was developed and validated for the quantification of ellagic acid as an analytical method can be useful for the standardisation of the extracts of *Phyllanthus amarus* to allow further biological and pharmacological investigations.
Ellagic acid showed a linear relationship in the range of 1.74-20.91 µg/mL and a single-point calibration was allowed. The method was shown to be precise with respect to time (RSD of 1.84%, 3 days, n = 6) and concentration (RSD of 2.54%, 3 levels, n = 6). The overall mean content of ellagic acid was 2.06%. (Dhooghe et al., 2011).

HPLC-DAD-ESI-MS method, followed by fractionation by chromatography on a Sephadex LH-20 column has been developed to determine the phenolic composition of fruit of *Eucalyptus globulus* growing in Algeria. The presence of 18 gallotannins, 26 ellagitannins, and 2 flavonols was established. Tentative identification is provided for these compounds on the basis of UV-visible spectra and mass spectrometry data. Quantitatively, ellagic acid and its derivatives, including ellagitannins, are largely predominant (Boulekbache et al., 2010).

### 2.3.2.2. Pharmacological Reviews

A study was conducted for understanding of the effects of ellagic acid, in an experimental murine model of Crohn's disease by intra-colonic administration of TNBS in rats. Results shows ellagic acid reduces the damage in a rat model of Crohn's disease, alleviates the oxidative events and returns pro-inflammatory proteins expression to basal levels probably through MAPKs and NF-κB signalling pathways (Rosillo et al., 2011).

The role of ellagic acid in the modulation of protein kinase Cα (PKCα) activity and expression and its correlation with the oncogene, c-Myc, and tumor suppressor gene, transforming growth factor-β (TGF-β1), in lymphoma bearing mice was evaluated. Results show that ellagic acid leads to down-regulation of the expression and activity of PKCα via decreasing the oxidative stress, measured in terms of lipid peroxidation and protein carbonylation (Mishra and Vinayak, 2011).

The antiulcer activities of ellagic acid were evaluated in acute and chronic ulcer models in Wistar rats. Its gastroprotective mechanism in ethanol-induced ulcer were partly due to intensification in the endogenous production of nitric oxide, an antioxidant effect by replenishing depletion of endogenous nonprotein sulphydrys and attenuation of tumor necrosis factor-α increase, whereas in indomethacin ulcer, it is partly due to a reduction in the plasma level of leukotriene B(4) (Beserra et al., 2011).
2.3.3. Tannic acid

Tannic acid is a specific commercial form of tannin, a type of polyphenol. Tannic acid is odourless but has a very astringent taste. Pure tannic acid is a light yellowish and amorphous powder. Tea, nettle, wood, berries, Chinese galls, Oak wood is very rich in tannic acid.

IUPAC Name: [3,5-dihydroxy-2-(3,4,5-trihydroxybenzoyl)oxy-6-[(3,4,5 trihydroxybenzoyl)oxymethyl]oxan-4-yl] 3,4,5-trihydroxybenzoate.

![Chemical structure of tannic acid]

Fig. 4: Chemical structure of tannic acid

2.3.3.1. Analytical Review

Normal-phase HPLC-fluorescence-mass spectroscopy separation and quantification of tannic acid in cranberry extracts containing varying processing aids of production has been established. Cranberry extracts were best extracted using an acetone/water technique versus an acid/alkaline extraction. Characterization and quantification of procyanidins up to octamers and higher molecular weight compounds, including separation of the A- and B-type dimers to tetramers was achieved (Wallace and Giusti, 2010).

UPLC-MS method for determine tannic acid in different biological tissues, such as liver, brain, the aorta vein and adipose tissue has been developed. The extracts were analyzed by UPLC-MS, using a triple quadrupole as the analyzer. The optimum extraction solution was water/methanol/phosphoric acid 4% (94/4.5/1.5, v/v/v). The extraction recoveries were higher than 81% for all the studied compounds in all the
tissues, except the anthocyanins, which were between 50 and 65% in the liver and brain (Serra et al., 2011).

Tannic acid content in the seeds and juice of boysenberry were quantitatively analyzed by HPLC-MS. The study revealed that the seeds contained a 72-fold higher amount of proanthocyanidins than the juice. These results indicate that boysenberry fruits contain short oligomeric proanthocyanidins along with flavanol monomers and the seeds represent a good source of short oligomeric proanthocyanidins (Furuuchi et al., 2011).

2.3.3.2. Pharmacological Reviews

The antioxidant activity and contents of various polyphenol classes in the seeds of seven soybean varieties of different seed color and one yellow seed cultivar, representing a reference genotype, were evaluated. The highest antioxidant activity was observed in the extracts of black and brown varieties, which also showed high levels of all polyphenol classes examined. Yellow seed had the highest total isoflavone content (Malencic et al., 2012).

Effect of active molluscicidal components of Sapindus mukorossi and Terminalia chebula on the acetylcholinesterase (AChE), acid and alkaline phosphatase (ACP/ALP) activity in the nervous tissue of freshwater snail Lymnaea acuminata were studied. In vivo and in vitro exposure of saponin and tannic acid significantly inhibited the AChE, ACP and ALP activity in the nervous tissue of L. acuminata (Krishnamoorthy et al., 2011).

The effects of supplementation of tannic acid were compared with the effects of clofibrate supplementation in apo E-deficient mice fed AIN-76 semi-synthetic diet over 20 weeks. Both clofibrate and tannic acid supplementation resulted in significant decreases in hepatic HMGR mRNA levels in association with its enzyme activity. The results suggest that the overall effect of tannic acid is more desirable than clofibrate supplementation for the alleviation of hepatic lipogenesis and atherogenesis in apo E-/- mice (Do et al., 2011).
2.3.4. Ascorbic acid

Ascorbic acid (vitamin C) is a water-soluble vitamin. It occurs as a white or slightly yellow crystal or powder with a slight acidic taste. Ascorbic acid is freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform, in ether, and in benzene.

(IUPAC) name: 2-Oxo-L-threo-hexono-1,4-lactone-2,3-enediol

![Chemical structure of Ascorbic acid]

Fig. 5: Chemical structure of Ascorbic acid

2.3.4.1. Analytical Reviews

Ion-pairing reversed-phase HPLC/electrochemical detection method for simultaneous determination of ascorbic acid, aminothiols, and methionine in biological matrices was developed. The present method was specific for the analysis of these analytes and demonstrated acceptable values for linearity, recovery, precision and sensitivity indicating that the proposed method could be efficiently used for determination of these analytes in the context of clinical research (Khan et al., 2011a).

A HPLC with UV detection method for the simultaneous determination of ascorbic acid and uric acid in human seminal plasma has been developed. Analytical performance of this method is satisfactory for both ascorbic acid and uric acid: the intra-assay and inter-assay coefficients of variation were below 10%. Quantitative recoveries from spiked seminal plasma were between 92.1 and 102.1%. This assay is a simple and reproducible HPLC method for the simultaneous measurement of ascorbic acid and uric acid in human seminal plasma (Kanar et al., 2011).

A HPLC linked with electrochemical detector method has been developed and optimized for different experimental parameters to analyze the most common monothiols and disulfide and ascorbic acid present in human plasma and erythrocytes using dopamine as internal standard. Complete separation of all the targets analytes and IS at 35°C on Discovery HS C18 RP column (250 mm x 4.6mm, 5 μm) was achieved using 0.05% TFA: methanol (97:3, v/v) as a mobile phase pumped at the
rate of 0.6 ml/min using electrochemical detector in DC mode at the detector potential of 900 mV (Khan et al., 2011b).

RP-HPLC method for the simultaneous determination of vitamin C in honey has been developed. The method provides low detection and quantification limits, very good linearity in a large concentration interval, very good precision, and the absence of any bias. It has been successfully applied to 28 honey samples (mainly from Sardinia, Italy) of 12 different botanical origins (Ciulu et al., 2011).

2.3.4.2. Pharmacological Reviews

The daily oral intake of vitamin C inhibits antimultiple myeloma activities of bortezomib was studied. The results for the first time show that vitamin C can significantly reduce the activity of bortezomib treatment in vivo; and importantly, suggest that patients receiving treatment with bortezomib should avoid taking vitamin C dietary supplements (Perrone et al., 2009).

The oxygen-uptake kinetics and computational methods were combined to study the reaction of peroxyl radicals with ascorbyl palmitate and 5, 6-isopropylidene-L-ascorbic acid in non-aqueous solvents were evaluated. Gas-phase calculations for the neutral/anionic forms were in good agreement yielding 80.1/69.0 kcal mol$^{-1}$ using B3LYP/6-31+g(d,p) and 79.0/67.8 kcal mol$^{-1}$ at CBS-QB3 level (Amorati et al., 2011).

2.3.5. Chebulinic acid

Chebulinic acid is an ellagitannin found in the seeds of Euphoria longana and in the fruits of Terminalia chebula.

IUPAC name: 1, 3, 6-Tri-O-galloyl-2, 4-chebuloyl-β-D-glucopyranoside
2.3.5.1. Analytical Reviews

RP-HPLC method is developed for analysis of Triphala Churna using gallic acid, chebulagic acid and chebulinic acid as markers. HPLC method validation data suggest that this HPLC method is accurate, precise, specific (match factor > 90), with low limit of detection and quantification for all three markers, with high recovery values and highly robust (Pawar et al., 2009).

RP-HPLC method for determining fourteen components (gallic acid, chebulic acid, 1,6-di-O-galloyl-D-glucose, punicalagin, 3,4,6-tri-O-galloyl-D-glucose, casuarinin, chebulanin, corilagin, neochebulinic acid, terchebulin, ellagic acid, chebulagic acid, chebulinic acid, and 1,2,3,4,6-penta-O-galloyl-D-glucose) in the fruit of Terminalia chebula Retz. has been developed. The HPLC methods developed were both successfully applied to the assay of tannins in commercial samples of Chebulae fructus (Juang et al., 2004).

High-speed counter-current chromatography system was equipped with a preparative HPLC which enabled the successful isolation of hydrolysable tannins from the fruits of Terminalia chebula, a traditional Chinese medicine. The two-phase solvent system was composed of n-hexane-ethyl acetate-methanol-water (1:20:1:20 v/v). As a result, 33.2 mg chebulagic and 15.8 mg chebulinic acids were obtained in one step from 300 mg of crude extract. Their purities were determined by HPLC to be 95.3 and 96.1%, respectively (Han et al., 2006).
The chemical changes occurring during fermentation in Abhayarishta (Ayurvedic formulation) have been studied for the purpose of its standardization. An HPLC-DAD method for quantitative estimation of selected marker constituents in the formulation has been developed and validated. A comparison of decoction and final processed formulation revealed that major polyphenolics of T. chebula were hydrolyzed to their respective monomers and, consequently, there was an increase in the amount of chebulic acid, gallic acid, ellagic acid and ethyl gallate after fermentation (Lal et al., 2010).

2.3.5.2. Pharmacological Reviews

The effects of chebulinic acid on erythroid and megakaryocytic differentiation in K562 cells have been studied. The study confirms that chebulinic acid had inhibitory effect on erythroid differentiation likely through changing transcriptional activation of differentiation relative genes, which suggests that chebulinic acid or other tannins might influence the efficiency of some anti-tumor drugs-induced differentiation or the hematopoiesis processes (Yi et al., 2004).

Methanolic extract of Terminalia chebula fruit, was studied for its effects on growth in several malignant cell lines including a human (MCF-7) and mouse (S115) breast cancer cell line, a human osteosarcoma cell line (HOS-1), a human prostate cancer cell line (PC-3) and a non-tumorigenic, immortalized human prostate cell line (PNT1A) using assays for proliferation ([3]H-thymidine incorporation and coulter counting), cell viability and cell death (Saleem et al., 2002).

2.3.6. Sennoside A&B

![Fig. 7: Chemical structure of Sennosides](image-url)
2.3.6.1. Analytical Reviews

HPLC method for determination of sennoside A and B simultaneously in health food has been developed. Samples were extracted by ultrasound extraction and determined by HPLC with a UV detector. The average recovery rates of sennoside A and sennoside B were 85.2% - 97.2% and 86.1% - 96.2%. The RSD was 7.5% and 6.8%, the limit of detection was 0.8 mg/kg and 0.6 mg/kg, and the limit of quantification was 2.1 mg/kg and 2.0 mg/kg for sennoside A and B respectively (Xiao et al., 2011).

A HPLC method for the determination of sennoside A in incubation mixture of DKT with mouse feces was established. The retention time of sennoside A was 9.26±0.02 min with a TSKgel ODS-80TsQA column by linear gradient elution using a mobile phase containing aqueous phosphoric acid and acetonitrile and detection at 265 nm. It was found that the activity of sennoside A metabolism in intestinal bacteria was significantly accelerated when glycyrrhiza, liquiritin or liquiritin apioside coexisted with sennoside A, whereas that of glycyrrhizin was not altered (Takayama et al., 2011).

The sennoside A and sennoside B contents of various samples of crude drugs were determined using solid-phase extraction and HPLC. The samples examined were crude drugs, conventional crude drug products, and Kampo formulations. Sennoside A and B were eluted with methanol-water-formic acid (70:30:2, v/v), and the eluate was used as the sample solution for HPLC analysis (Yamasaki et al., 2010).

2.3.6.2. Pharmacological Reviews

The effect of oral treatment with sennosides on the time-course of net H2O and electrolyte transport rates was studied in 1-hour incubation experiments in the rat colon in vivo. The study concluded that there may be regional differences in the absorptive behavior of the colon induced by sennosides. Slow transit and increased absorption in some parts of the colon may overcome secretion in other parts (Leng, 1993).

The cytochemical effects of sennosides on rat colonic epithelial cells were studied. Most striking and consistent changes were found in the rectum including total acidic mucin content which significantly increased, with sulfomucin decreased and sialomucin increased in the three treatment groups. Cytokeratin AE1 expression
increased on picosulfate and sennosides. Soybean agglutinin total binding increased on bisacodyl and picosulfate (Yang et al., 1993).

2.3.7. Rutin

Rutin belongs to a group of plant compounds called bioflavonoids that also include the important catechins of tea and the polyphenols of red wine.


![Chemical structure of Rutin](image)

**Fig. 8:** Chemical structure of Rutin

2.3.7.1. Analytical Reviews

HPLC method was developed for analyzing marker compound of *Wrightia tinctoria* in 777 Oil for routine standardization purpose. The chromatography was performed on Phenomenex C$_{18}$ (250 x 4.6mm, 5.0 µm) column using methanol-water; adjusted to pH 3.0 by orthophosphoric acid (60:40, v/v) as mobile phase. The developed HPLC method is useful for the qualitative and quantitative estimation of rutin in 777 Oil and other products of traditional systems of medicine (Musthaba et al., 2011).

An optimized extraction procedure and modified method of HPLC by coupling to an ultraviolet detector to simultaneously analyze protodioscin and rutin in asparagus extracts has been developed. White asparagus spears and the crown of the plants were revealed to be rich sources of protodioscin and contained 2.59 to 10.4 mg/g dry weight. Green asparagus spears, particularly the upper portion, were rich in rutin and contained between 1.51 and 7.29 mg/g dry weight (Lee et al., 2010).
HPLC method for simultaneous determination of rutin, isoquercitrin and chlorogenic acid in *Farfarae flos* has been developed. The analysis was carried out on a Phenomenex Synergi POLAR-RP 80A column (4.6 mm x 250 mm, 4µm) with gradient elution using methanol-acetonitrile-water (adjusted to pH 2.5 with formic acid) as mobile phase (Wu et al., 2010).

### 2.3.7.2. Pharmacological activity

The effects of rutin, quercetin and their copper complexes on the catalytic activity of the protein were investigated. Rutin shown an enhancement in the ribonucleolytic activity whereas the copper complexes and quercetin behave as non-competitive type inhibitors with K(i) values in the μM range (Tripathy et al., 2011).

The ability of rutin to inhibit the activity of serine proteases trypsin, thrombin, elastase and urokinase has been investigated. Potent protease inhibition in micromolar range was displayed by rutin and rutin derivatives esterified with medium and long chain, mono- and polyunsaturated fatty acids (1e-m), followed by phloridzin and esculin esters with medium and long fatty acid chain length (2a-d, 3a-d), while unmodified compounds showed only little or no effect (Viskupicova et al., 2011).

Antioxidant activities of four flavonoids (rutin, quercetin, luteolin, and kaempferol) and two non-flavonoids (chlorogenic acid and pyrocatechol) against four reactive oxygen species have been measured with a myoglobin method was developed. The four flavonoids shown a very similar pattern in the 5-axe cobweb charts, while the patterns of two non-flavonoids are quite different from that of the flavonoids (Terashima et al., 2012).

### 2.3.8. Quercitrin

Quercetin is a flavonoid and, to be more specific, a flavonol. It is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions.

IUPAC name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one
2.3.8.1. Analytical Reviews

Soxhlet extraction, ultrasound extraction, and accelerated solvent extraction, followed by RP-HPLC with a photodiode array detector has been developed for the determination of flavonoids in fruits of *Peucedanum alsaticum*. The study confirms the presence of three compounds, a kaempherol derivative and two quercetin derivatives (Skalicka *et al.*, 2011).

A UHPLC method has been developed and validated for simultaneous identification and analysis of the isoflavones genistein, daidzein, glycitin, puvarin, and biochanin A, and the flavonoids (+)-catechin, (-)-epicatechin, rutin, hesperidin, neohesperidin, quercitrin, and hesperetin in human urine. UHPLC was performed with a Hypersil Gold (50 x 2.1 mm, 1.9 µm) analytical column. Elution was with a gradient prepared from aqueous trifluoroacetic acid (0.05%) and acetonitrile. UV detection was performed at 254 and 280 nm (Baranowska and Magiera, 2011).

HPLC method for determination the contents of quercitrin in *Polygonum capitatum* and *Relining granules* has been established. The samples were analyzed on a Diamonsil C18 column (4.6 mm x 250 mm, 5 µm) eluted with the mobile phase consisted of methanol-1% HAc-THF (Xie *et al.*, 2009).

RP-HPLC method with photodiode array detection was established for the determination of major constituents (rutin, hyperoside, isoiruercitrin, quercitrin, quercetin, pseudohypericin, hyperforin and hypericin) in St. John's Wort dietary supplements. The samples were extracted with methanol by means of sonication in low temperature. The major components were separated by RP-18 chromatography column using a 60-min water-acetonitrile-methanol-trifluoroacetic acid gradient (Li *et al.*, 2001).
2.3.8.2. Pharmacological Reviews

The protective effect of quercitrin on the response of osteoblastic MC3T3-E1 cells to oxidative stress was evaluated. The study suggested that quercitrin-induced protective effect against osteoblast dysfunction by oxidative stress is associated with increased activation of ERKs and p38 MAPK (Choi, 2010).

Two topoisomerase I inhibitory compounds from *Houttuynia cordata* were purified and identified as caffeic acid and quercitrin. Caffeic acid and quercitrin inhibited the activity of topoisomerase I with IC (50) values of about 0.15 and 0.05 mM, respectively. A concentration of 45 µM quercitrin caused 50% growth inhibition in human leukaemia U937 cells, but not on those of normal fibroblast NIH3T3 cells (Jang *et al.*, 2011).

The effects of quercitrin on tumor promotion in mouse JB6 cells, a validated model for screening cancer chemopreventive agents and elucidating the molecular mechanisms was evaluated. The results provide the first evidence that quercitrin contributes to the inhibition of neoplastic transformation by blocking activation of the MAPK pathway and stimulation of cellular protection signaling (Ding *et al.*, 2010).

2.3.9. Sinigrin

Sinigrin is a glucosinolate that belongs to the family of glucosides found in some plants of the Brassicaceae family such as Brussels sprouts, broccoli, and the seeds of black mustard (*Brassica nigra*).
2.3.9.1. Analytical Reviews

The comparison of extraction procedures to identify the most efficacious method and whether each extraction can also be used for the quantification of total isothiocyanates has been studied by HPLC. Four extraction techniques were compared for the quantification of sinigrin from *Brassica juncea* seed; boiling water, boiling 50% (v/v) aqueous acetonitrile, boiling 100% methanol and 70% (v/v) aqueous methanol at 70°C. For the quantification of sinigrin, boiling 50% (v/v) acetonitrile was found to be the most efficacious extraction solvent of the four tested yielding 15% more sinigrin than the water extraction (Cools *et al.*, 2012).

The glucosinolate profile of *Raphanus sativus* L based dietary supplements has been investigated by HPLC-PDA, LC-ESI-MS/MS and LC-APCI-MS/MS systems. Optimization of the MS/MS parameters and LC conditions was performed using sinigrin reference standard and rapeseed certified reference material respectively. The intact glucosinolates identified were then desulfated and quantified on an HPLC-PDA system as desulfo-glucosinolates (Ediage *et al.*, 2010).

2.3.9.2. Pharmacological Reviews

The hypothesis of AITC-containing cruciferous vegetables inhibit bladder cancer development was investigated. Upon addition of water sinigrin was readily hydrolyzed by the accompanying endogenous myrosinase. This myrosinase was also required for full conversion of sinigrin to AITC in *vivo*, but the matrix of MSP-1 had no effect on AITC bioavailability. Comparison between hydrated MSP-1 and pure sinigrin with added myrosinase suggested that the anticancer effect of MSP-1 was derived principally, if not entirely, from the AITC generated from sinigrin. (Bhattacharya *et al.*, 2010)

2.3.10. 6-gingerol

6-gingerol is a major pungent ingredient of ginger, also has potent antioxidant activity. 6-gingerol is the most abundant constituent of fresh ginger but it decreases during postharvest storage and processing, especially thermal processing.

IUPAC name: 5-hydroxy-1-(42 hydroxy-32-methoxyphenyl)-3-decanone
2.3.10.1. Analytical Reviews

The determination of 6-, 8-, 10-gingerol, and 6-shogaol in dried ginger (Zingiber officinale) and in the dried aqueous extract of ginger is developed. The levels of 6-, 8-, 10-gingerol, and 6-shogaol in the raw herb were 9.3, 1.6, 2.3, and 2.3 mg/g, respectively. The levels of gingerols found in the dried aqueous extract were between 5 and 16 times lower than those in the raw herb, but the level of 6-shogaol was higher (Lee et al., 2007).

The contents of 6-Gingerol in Rhizoma Zingiberis Recens was determined by using HPLC. Retention time of 6-gingerol was near 19 min, showing a good recovery (98.2%) and linear correlation (r = 0.9999). The contents of 6-gingerol were 1.35-2.87 mg.g⁻¹, and the water contents were 70.4-85.5% mL/g in Rhizoma Zingiberis Recens (Wang et al., 2002).

HPLC-MS method was developed and validated for simultaneous quantification of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in rat plasma after oral administration of ginger oleoresin. Plasma samples extracted with a liquid-liquid extraction procedure were separated on an Agilent Zorbax StableBond-C₁₈ column. The limit of quantification was in a range of 3.57-10.4 ng/mL (Wang et al., 2009).

2.3.10.2. Pharmacological Reviews

The chemoprotective effect of 6-gingerol against PAT-induced genotoxicity in HepG2 cells was investigated. The results showed that 6-gingerol significantly reduced the DNA strand breaks and micronuclei formation caused by PAT. Moreover, 6-gingerol effectively suppressed PAT-induced intracellular ROS formation and 8-OHdG level (Yang et al., 2011).
The effects of 6-gingerol on mushroom tyrosinase, tyrosinase activity and melanin content were determined spectrophotometrically. The results revealed that 6-gingerol effectively suppresses murine tyrosinase activity and decreases the amount of melanin in a dose-dependent manner (Huang et al., 2011).

The in vitro activities of the compounds 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol were evaluated for scavenging of 1,1-diphenyl-2-picrylhydrazyl. In the antioxidant activity assay, 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol exhibited substantial scavenging activities with IC (50) values of 26.3, 19.47, 10.47 and 8.05 microM against DPPH radical (Dugasani et al., 2010).

2.3.11. Piperine

Piperine is an alkaloid found naturally in plants belonging to the Piperaceae family, such as Piper nigrum L. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants.

IUPAC name: 1-[(5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl] piperidine

![Fig. 12: Chemical structure of piperine](image)

2.3.11.1. Analytical Reviews

RP-HPLC method for the selective determination of piperine, in pepper or its oleoresins is described and evaluated. Results obtained for a range of samples at four different detection also analysed by the spectrophotometric method, which invariably yielded higher results because of the contribution from other pepper alkaloids to the UV absorption (Wood et al., 1988).

HPLC method is developed for the analysis of piperine in rat plasma. The calibration plot was linear over the range studied (2–2000 ng/mL) with correlation coefficient of 0.9984. Limit of detection and limit of quantitation were 1 ng/mL and 3 ng/mL, respectively. Good overall recovery (85.5±6%) was obtained with 4 ml ethyl acetate
and extraction time of 3 min. Intra- and inter-assay coefficient of variation was found to be less than 7.5% (Sunil et al., 2002).

UFLC–ESI–MS/MS method and validating it for the simultaneous determination of piperine and piperlonguminine in rat plasma using terfenadine as the internal standard has been developed. The method was successfully applied to pharmacokinetic studies of piperine and piperlonguminine in rats after oral administration of alkaloids from P. longum L (Junhui et al., 2011).

2.3.11.2. Pharmacological Reviews

The effect on lipid peroxidation was also examined and IC 50 values were calculated. Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the fenton reaction resulting in increased generation of hydroxyl radicals. The results proved that piperine possesses direct antioxidant activity against various free radicals (Mittal and Gupta, 2000).

The effect of piperine in obesity-induced dyslipidemia was studied. Supplementing piperine with HFD significantly reduced not only body weight, triglyceride, total cholesterol, LDL, VLDL, and fat mass, but also increased the HDL levels, with no change in food intake. The results suggest that piperine possesses potential fat reducing and lipid lowering effects, without any change in food appetite, at a small dose of 40 mg / kg (Shah et al., 2011).

The possible contribution of the serotonergic system in the antidepressant-like effect of piperine in mice was investigated. The results showed that piperine significantly reduced the immobility time in the forced swim test and tail suspension test in mice. Piperine treatment also significantly potentiated the number of head-twitches of mice induced by 5-HTP. The results suggested that the antidepressant-like effect of piperine is mediated via the serotonergic system by enhancing 5-HT content in mouse brain (Mao et al., 2011).

2.3.12. Piperlongumine

Piperlongumine, a pyridone alkaloid isolated from fruits of Piper longum, differs from the typical piperidine derived alkamides in that it has an α-carbonyl group and a 3,4-double bond in the piperidine ring.
IUPAC name: 1-[(2E)-3-(3, 4, 5-Trimethoxyphenyl) prop-2-enoyl]-5,6-dihydropyridin-2(1H)-one

![Chemical structure of piperlongumine](image)

**Fig. 13:** Chemical structure of piperlongumine

### 2.3.12.1. Analytical Reviews

UFLC–ESI–MS/MS method and validating it for the simultaneous determination of piperine and piperlonguminine in rat plasma using terfenadine as the internal standard has been developed. The method was successfully applied to pharmacokinetic studies of piperine and piperlonguminine in rats after oral administration of alkaloids from *P. longum* L (Junhui et al., 2011).

HPLC and LC-MS methods were developed and used to measure the following piperamides in 10 commercial peppercorns and in 10 ground, black, white, green, and red peppers: piperanine, piperdardine, piperine, piperlonguminine, and piperettine. Four well-separated stereoisomeric forms of piperettine with the same molecular weight were present in 19 peppers. The results demonstrated the utility of the described extraction and analytical methods used to determine the wide-ranging individual and total piperamide contents of widely consumed peppers (Friedman et al., 2008).

Extraction methodology and HPLC-MS analysis of *Piper* (Piperaceae) was developed. HPLC analysis using a binary gradient of acetonitrile and water separated the major amide peaks between 5 and 12 min. Atmospheric pressure chemical ionization APCI-MS improved the detection limit to 0.2 ng, 10-fold below the 2 ng limit of the HPLC-diode array detector (DAD) based on linear standard curves between 0.1 and 250 microg/mL (Scott et al., 2005).
2.3.12.2. Pharmacological Reviews

Piperlonguminine/dihydropiperlonguminine inhibition on the production of amyloidbeta in human neuroblastoma cells (SK-N-SH) has been investigated. Piperlonguminine/dihydropiperlonguminine components (1:0.8) were extracted from futokadsura stem, and then used to treat SK-N-SH cells at three different concentrations: 3.13 µg/mL, 6.25 µg/mL and 12.50 µg/mL. The study suggested that piperlonguminine/dihydropiperlonguminine components could significantly inhibit the level of APP, Abeta42 and Abeta40 peptide without affecting the activity of beta-secretase and gamma-secretase in SK-N-SH cells (Qi et al., 2009).

The in-vitro antiplatelet effect of pipilartine isolated from the roots of P. tuberculatum, on human platelet aggregation induced in platelet-rich plasma by the agonists collagen, adenosine 5'-diphosphate (ADP), arachidonic acid (AA) and thrombin has been evaluated. The mechanism underlying the pipilartine antiplatelet action could be related to the inhibition of cyclooxgenase activity and a decrease in thromboxane A formation, similar to that occurring with aspirin (Fontenele et al., 2009).

Piperlongumine activity of potential inhibitory effect on washed rabbit platelet aggregation induced by collagen, arachidonic acid (AA) and platelet activating factor without any inhibitory effect on that induced by thrombin has been investigated. Among those seven synthetic derivatives, 1-(3,5-dimethylpiperidin-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3') had the most inhibitory effect on platelet aggregation induced by collagen, AA and PAF (Park et al., 2008).

The antiatherogenic effect and possible mechanisms of piper longumine has been studied. The study concluded the activity of piperlonguminine to inhibit the atherogenesis formation and development, which might be due to regulating the lipid metabolism and enhancing the antioxidation (Ma et al., 2008).

2.3.13. Guggul sterones (E&Z)

Guggulsterone is a plant steroid found in the resin of the guggul plant, Commiphora mukul. Guggulsterone can exist as either of two stereoisomers, E-guggulsterone and Z-guggulsterone.
IUPAC name: (8R,9S,10R,13S,14S)-17-Ethylidene-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15-decahydrocyclopenta[a]phenanthrene-3,16-dione.

Fig. 14: Chemical structure of Guggul sterone E (A) & Z (B)

2.3.13.1. Analytical Reviews

Simultaneous separation of E- and Z-guggulsterone was accomplished by HPLC on a C$_{18}$ column using methanol, acetonitrile, buffer and tetrahydrofuran as a mobile phase. The compounds were monitored at 248 nm on a PDA. The recoveries of E- and Z-isomers from serum samples were always greater than 90%. The calibration graph was linear over the range of 25-2500 ng/mL for Z- and E-isomers (Verma et al., 1998).

HPLC method has been developed and validated for the fingerprinting and quantitative determination of E- and Z-guggulsterones. The method involves extraction of the guggul resin from either the raw exudate or compounded tablets with ethyl acetate, concentration of the combined extracts and chromatography. The method has a validated quantitation range of 15-85 µg/mL for E-guggulsterone and 25-130 µg/mL for Z-guggulsterone with a precision of +/-2% S.D. and a recovery of >99.5% (Mesrob et al., 1998).
HPTLC method of analysis of E and Z stereoisomers of guggulsterone both as a bulk drug and in formulations was developed and validated. It was found to give compact spots for E- and Z-guggulsterone ($R_f$ value of 0.38 +/- 0.02 and 0.46 +/- 0.02, respectively) following double development of chromatoplates with the same mobile phase. The limit of detection and quantitation were 12, 10 and 24, 20 ng/spot, respectively, for E- and Z-guggulsterone (Arawal et al., 2004).

2.3.13.2. Pharmacological Reviews

Guggul sterones sensitizes hepatocellular carcinoma cells (HCC) to apoptosis mediated by tumor necrosis factor-related apoptosis inducing ligand (TRAIL) has been investigated. Guggul sterones induced upregulation of the death receptor DR5 for TRAIL. The effects seemed to be associated with eIF2α and CHOP activation, which are related to the endoplasmic reticulum (ER) stress response and apoptosis (Moon et al., 2011).

The short-term safety and efficacy of 2 doses of a standardized guggul extract in healthy adults with hyperlipidemia eating a typical Western diet has been studied. There were no significant changes in levels of total cholesterol, HDL-C, triglycerides, or VLDL-C in response to treatment with guggulipid in the intention-to-treat analysis. Guggulipid also appeared to cause a dermatologic hypersensitivity reaction in some patients (Szapary et al., 2003).

The anti-inflammatory effect of guggulsterone on cultured human middle ear epithelial cells was evaluated. The results show that the guggulsterone has inhibitory effect on TNF-α expression and COX-2 production and it may be mediated through its inhibition of nuclear factor-kB activation. The findings provide an insight into the molecular mechanisms underlying the anti-inflammatory activities of guggulsterone in relationship to otitis media (Song et al., 2010).

2.3.14. Berberine

Berberine is an isoquinoline alkaloid, which has a wide range of pharmacological and biochemical effects. It has been reported in roots, rhizomes and stem barks of Hydrastis canadensis (Goldenseal), Coptis chinensis (Coptis or Goldenthread), Berberis aquifolium (Oregon grape), Berberis vulgaris (Barberry) and Berberis aristata (Turmeric tree).
2.3.14.1. Analytical Reviews

Validated HPLC method, with photodiode array detection, has been developed for the analysis of commercial Goldenseal products. Samples were treated by sonication with acidified methanol/water. The method was validated for LOD, LOQ, linearity, reproducibility and recovery with good results (Li and Fitzloff, 2002).

HPLC method for the determination of berberine in plasma, urine and bile samples is developed. Berberine was determined in all samples using an octyl reversed-phase column with a mobile phase of 60–63% acetonitrile in 0.1% phosphoric acid (pH 6.0) and with UV detection at 267 nm (Chi and Horng, 1995).

RP-HPLC method for determination of concentration of berberine in human serum by RP-18 column and mobile phase of Methanol containing 0.15% triethylamine. The content of berberine was determined by the external standard method with UV detector at 347 nm. The linear range was 0.2–2 mg/L ($r^2 = 0.9996$). The detectable limit was 2.55 ng. The average recovery was 90.73% and the coefficients of variation were below 8.0% (Zhang et al., 1997).

RP-HPLC method for fast and simultaneous determination of four index contents, which are Genpioside, Baicalin, Berberine Hydrochloride and Ammonium Glyeyrrhizinate, in Qingwei Huanglian tablets, has been established. Separation was achieved by gradient elution on a Hypersil C18 column with the mobile phases of acetonitrile -0.5% triethylamine water-solution (pH 3.0) at a flow rate of 1.0 mL/min, detection wavelength of 230 nm, and room temperature (Li et al., 2007).
2.3.14.2. Pharmacological Reviews

The effect of berberine investigated on the transcriptional activity and the protein expression of p53 in p53-positive (wild-type, MCF7 cells) and p53-negative (mutant, MDA-MB231 cells) human breast cancer cells. Results showed that the basal level of p53 mRNA and protein expression was increased by berberine treatment. Results also indicated berberine may be used as an effective ingredient for anticancer products (Kim et al., 2011).

Berberine, up-regulated the expression of two different sets of C/EBP inhibitors, CHOP and DEC2, while down-modulating C/EBPα, PPARγ and other adipogenic markers and effectors in differentiating 3T3-L1 preadipocytes and mature adipocytes has been studied. The results suggested that the berberine-induced up-regulation of CHOP and DEC2 was attributable to selective activation of an unfolded protein response and modified extracellular environment, respectively (Pham et al., 2011).

The cytotoxicity activity exhibited by berberine in HepG2 cells mainly through upregulation of reactive oxygen species (ROS) production but was ineffective in normal Chang liver cells has been investigated. The results suggest that berberine may be a potential alternative against invasive hepatoma cells through PI3K-AKT and ERK pathways-dependent downregulation of MMP-9 expression (Liu et al., 2011).

The effect of berberine on androgen receptor signaling in prostate cancer was studied. Berberine decreased the transcriptional activity of androgen receptor. Berberine did not affect androgen receptor mRNA expression, but induced androgen receptor protein degradation (Li et al., 2011).

2.3.15. Glabridin

Glabridin is a major polyphenolic flavonoid specific for Glycyrrhiza glabra L. Glabridin is the main compound in the hydrophobic fraction of licorice extract. It is known for its beneficial effects on the skin due to its anti-inflammatory and antioxidant properties.

IUPAC name: 4-[(3R)-8, 8-Dimethyl-3, 4-dihydro-2H-pyrano [6, 5-f] chromen-3-yl]benzene-1, 3-diol]
2.3.15.1. Analytical Reviews

A reverse phase HPLC method was developed for the quantitation of glabridin in *Glycyrrhiza glabra*. Glabridin is detected by UV absorption at 280 nm after separation by the chromatographic system. Good linearity was obtained in the working range of the concentration (0.01–0.1 mg mL\(^{-1}\)), with correlation coefficients 0.999. The method was validated under ICH guidelines (Shanker *et al*., 2007).

The extraction and separation conditions of glycyrrhizic acid and glabridin from licorice were investigated. By changing the different extraction solvents, procedures, times and temperature, the optimum extraction condition was established as ethanol/water (30:70, v/v) as an extraction solvent, and 60 min dipping time under 50°C (Tian *et al*., 2008).

HPLC method for determination of glabridin diacetate and dihexanoate prodrugs was developed, validated and applied to the enzymatic and chemical hydrolysis studies. The chromatographic separation was achieved on a reverse phase C\(_{18}\) (Thermo Hypersil-Keystone, 250 x 4.6 mm, 5 µm) column using the mixture of acetonitrile and water as mobile phase at 1 mL/min flow rate, detected at 280 nm (Jirawattanapong *et al*., 2009).

Solid-phase extraction and LC-MS/MS method was developed and validated for the determination of glabridin in human plasma. Glabridin was extracted from plasma by using solid-phase extraction a C\(_8\) cartridge and analyzed by LC-MS/MS using mefenamic acid as an internal standard. The analyte were separated by a C\(_{18}\) column on LC, and monitored with a fragment ion of m/z 201 formed from a molecular ion of m/z 323 for glabridin (Aoki *et al*., 2005).
2.3.15.2. Pharmacological Reviews

The antimigration, anti-invasive effect of glabridin in human non-small cell lung cancer A549 cells has been studied. Glabridin exhibited effective inhibition of cell metastasis by decreasing cancer cell migration and invasion of A549 cells. In addition, glabridin also decreased A549-mediated angiogenesis (Tsai et al., 2010).

The antimigration, antiinvasive effect of glabridin, in MDA-MB-231 human breast adenocarcinoma cells has been studied. Glabridin exhibited effective inhibition of cell metastasis by decreasing cancer cell migration and invasion of MDA-MB-231 cells. Investigation revealed that the inhibition of cancer angiogenesis by glabridin was also evident in a nude mice model (Hsu et al., 2011).

LDL oxidation effect of glabridin was studied. The determination of the extent of LDL oxidation was accomplished by measuring the formation of thiobarbituric acid reactive substances. The oxidative stress level was assessed using a FORM system/CR 3000 instrument. The study concluded the dietary consumption of glabridin can partially protect LDL from oxidation (Carmeli et al., 2008).

Glabridin and glabrene licochalcone A, licoricidin and licoisoflavone B has been studied for inhibitory activity against the growth of Helicobacter pylori in vitro. These compounds proved as chemopreventive agents for peptic ulcer or gastric cancer in H. pylori-infected individuals (Fukai et al., 2002).

2.3.16. Glycyrrhizic acid

Glycyrrhizic acid is the main sweet-tasting compound from liquorice root. It is 30–50 times as sweet as sucrose (table sugar). In chemical terms, Glycyrrhizic acid is a triterpenoid saponin glycoside of glycyrrhizic (or glycyrrhizinic) acid.

IUPAC name: (3β,18α)-30-hydroxy-11,30-dioxoolean-12-en-3-yl 2-O-β-D glucopyranuronosyl-β-D-glucopyranosiduronic acid.
2.3.16.1. Analytical Reviews

HPTLC method has been developed to control the quality of raw as well as finished glycyrrhiza using glycyrrhizin as the bioactive marker. The method was validated in terms of specificity, linearity, precision, LOD, and LOQ. Linearity range was found to be 0.96-4.80 µg/spot. The amount of glycyrrhizin found in the extract was 9.1% (Gantait et al., 2010).

HPTLC method is developed for the simultaneous determination of glycyrrhizinic acid tripotassium salt, 18 alpha- and 18 beta-glycyrrhetinic acid in rat plasma, after oral administration of 3K-G (30 mg/kg once a day for 30 days). Systolic pressure and the volume of urine excreted in 24 hours were recorded during this period to observe any drug-induced effects (Vampa et al., 1992).

Glycyrrhizic acid content in Fen Gancao and its rough bark was determined by HPLC. The result showed that at least three unknown ingredients were detected in Cortex glycyrrhizae which were not in Fen Gancao, and glycyrrhizic acid content in the Cortex Glycyrrhizae is higher than that in Fen Gancao. It suggests that Cortex Glycyrrhizae can be used as the material not only to extract glycyrrhizic acid but also for making additives (Rong et al., 2006).

A HPLC method was established for the quantification of glycyrrhizic acid in traditional Chinese herbal medicines. It was found that the average content of glycyrrhizic acid of the Hejian decoction was higher than that of the Fenjian decoction (Yang et al., 2007).
2.3.16.2. Pharmacological Reviews

Effect of glycyrrhizin on streptozotocin induced diabetic changes and associated oxidative stress, including haemoglobin-induced free iron-mediated oxidative reactions has been investigated. Free iron in haemoglobin, iron-mediated free radical reactions and carbonyl formation in haemoglobin were pronounced in diabetes, and were counteracted by glycyrrhizin. Effects of glycyrrhizin and glibenclamide treatments appeared comparable (Sen et al., 2011).

The effects of repeated glycyrrhizin ingestion on the oral pharmacokinetics of midazolam, a probe drug for CYP3A activity in humans investigated. The geometric coefficient of variation for the AUC (0-infinity) of midazolam in the placebo group was 196.4 ng x h/mL (30.3%) and after glycyrrhizin treatment, 151.3 ng x h/ml (34.7%). Administration of glycyrrhizin resulted in a modest induction of CYP3A that was clinically relevant according to the bioequivalence analysis (Tu et al., 2010).

The parenteral glycyrrhizin preparation was investigated on highly pathogenic influenza A H5N1 virus replication, H5N1-induced apoptosis, and H5N1-induced pro-inflammatory responses in lung epithelial (A549) cells for its effect. Therapeutic glycyrrhizin concentrations substantially inhibited H5N1-induced expression of the pro-inflammatory molecules CXCL10, interleukin 6, CCL2, and CCL5 but interfered with H5N1 replication and H5N1-induced apoptosis to a lesser extent (Michaelis et al., 2011).

2.3.17. Boswellic acids

Boswellic acids are a series of pentacyclic triterpene molecules that are produced by plants in the genus Boswellia. Like many other terpenes, boswellic acids appear in the resin of the plant that exudes them; it is estimated that they make up 30% of the resin of Boswellia serrata.
HPLC method for determining the contents of five boswellic acids in *Boswellia serrata* has been developed. The five ingredients were separated well. The content ranges of alpha-boswellic acid, beta-boswellic acid, 3-acetyl-beta-boswellic acid, 11-keto-beta-boswellic acid and 11-keto-beta-acetyl- boswellic acid were 8.68-16.1, 53.5-246.9, 38.4-192.9, 4.48-5.81, 32.7-44.2 mg/g, respectively (Wang et al., 2011).

HPLC-MS method has been developed for the quantification of all major boswellic acids in serum. Average steady concentrations (ng/mL) in the range of 6.4-247.5 for KBA, 0-15.5 for AKBA, 36.7-4830.1 for αBA, 87.0-11948.5 for βBA, 73.4-2985.8 for AαBA and 131.4-6131.3 for AβBA were determined in the verum group. The quantified steady state levels suggest βBA to be a possible candidate for the anti-inflammatory and anti-edemateous effects of BSE. In general, the serum level analysis underlines the promising clinical results of BSE on cerebral edema (Gerbeth et al., 2011).
A simple reverse-phase HPLC method is developed for the estimation of boswellic acids, the active constituents in *Boswellia serrata* oleo-gum resin. The mean recoveries are 98.24% to 104.17% and 94.12% to 105.92% for 11-KBA and A-11-KBA, respectively. The inter- and intra-day variation coefficients are less than 5% (Shah et al., 2008).

LC/MS method has been developed for the simultaneous determination of KBA and AKBA, the most potent boswellic acids, in plasma and brain. The method involves matrix-assisted liquid-liquid extraction on Extrelut NT followed by separation on reversed-phase high-performance liquid chromatography and tandem mass spectrometry detection using atmospheric pressure chemical ionization. The developed method represents an appropriate tool to further study the time-dependent distribution of KBA and AKBA in plasma and brain (Reising et al., 2005).

### 2.3.17.2. Pharmacological Reviews

The inhibition of 5-lipoxygenase by boswellic acid extract in an experimental model of pulmonary fibrosis using bleomycin studied. The levels of lipoxygenase enzyme activity were significantly increased. Boswellic acid treated rats had reduced number of macrophages, neutrophils in bronchoalveolar lavage and protein. Moreover, the hydroxyproline content was significantly lowered in boswellic acid treated rats (Ali et al., 2011).

The higher derivatives including butyryloxy (BKBA) and hexanoyloxy (HKBA) derivatives of KBA were synthesized and compared the anticancer potential of HKBA with PKBA, detailed in vitro pro-apoptotic and in vivo anticancer activity. Target based studies showed that HKBA inhibited the enzymatic activity of topoisomerasenes I and II at low doses than that of PKBA. *In vivo* studies also revealed a low dose inhibitory effect of HKBA on ascitic and solid murine tumor models (Chashoo et al., 2011).

The apoptotic effects of boswellic acid, affected by inhibition of PI3K/Akt pathway were examined. Colon cancer HT29 cells were treated with 3-acetyl-11-keto-beta boswellic acid in the absence and presence of LY294002 or Wortmannin, inhibitors of PI3K. Apoptosis was determined by flow cytometry and caspase assay. Preincubation of the cells with LY294002 or wortmannin significantly enhanced the AKBA-induced
apoptosis up to 20-fold. AKBA may activate the PI3K/Akt pathway and inhibition of the PI3K pathway significantly enhances AKBA-induced apoptosis (Liu et al., 2009).

2.3.18. Aloe emodin

Aloe emodin is an anthraquinone present in aloe latex, an exudate from the aloe plant.

IUPAC name; 1,8-Dihydroxy-3-(hydroxymethyl)-9,10-anthracenedione

![Chemical structure of Aloe emodin](Fig. 19: Chemical structure of Aloe emodin)

2.3.18.1. Analytical Reviews

A RP-HPLC method is described for the simultaneous determination of four anthraquinones: rhein, aloe-emodin, emodin, and chrysophanol in *Senna alata* leaves. The solvent for extraction of anthraquinones from *S. alata* leaves was examined in order to increase the anthraquinone content of the leaf extract (Panichayupakaranant et al., 2009).

A reverse phase UPLC method was developed for the rapid quantification of five anthraquinone derivatives (aloe-emodin, rhein, emodin, chrysophanol and physcion) in rhubarb. The proposed method was found to be reproducible and convenient for quantitative analysis of five anthraquinone derivatives in three species of rhubarb and related preparations (Wang et al., 2008).

LC-ESI/MS/MS method has been developed and validated for simultaneous determination of five active constituents (magnolol, honokiol, rhein, emodin and aloe-emodin) from Da-Cheng-Qi decoction in rat plasma. After the addition of glicludone as the internal standard, plasma samples were prepared by one-step protein precipitation using methanol and separated by HPLC (Xu et al., 2008).

LC/MS and LC with diode array detection in the UV range for the determination of low levels of the anthraquinones aloe-emodin and aloin-A (barbaloin) in aloe-based
products has been developed. The wavelengths used for quantification of aloin-A, aloe-емodin, and emodin (internal standard) by DAD were 357, 257, and 289 nm, respectively (Elsohly et al., 2007).

### 2.3.18.2. Pharmacological Reviews

The possible modulation of defined markers of monocytic differentiation of aloe emodin on human U937 cell line has been investigated. The study showed that aloe emodin treated U937 cells exhibit a noticeably rise in transglutaminase activity. This enhanced enzyme activity correlates with -induced growth arrest and differentiation to functionally mature monocytes. The results showed that aloe emodin can promote the macrophage differentiation of U937 cells (Tabolacci et al., 2011).

The antineoplastic properties of aloe emodin on highly metastatic B16-F10 melanoma murine cells were studied. The study demonstrated inhibitory effects of aloe emodin on melanoma cell proliferation and invasion power, accompanied by the stimulation of cell differentiation parameters (Tabolacci et al., 2010).

The efficacy of aloe emodin-8-O-glycoside on alleviating insulin resistance and augmenting glycogen synthesis in L6 myotubes and 3T3L1 adipocytes was investigated. The study shows that aloe emodin-8-O-glycoside enhances glucose transport by modulating the proximal and distal markers involved in glucose uptake and its transformation into glycogen (Anand et al., 2010).