various diseases[10]. It is surprising to note that a large number of herbal medicines in this papyrus are still important in modern medicine. Opium, myrrh, frankincense, fennel, cassia, senna, thyme, aloe, linseed, and castor oil are few examples that were used then in much the same manner as they are used today[9].

Plant medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulation. The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in herbals. In more recent history, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century. Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, and quinine, in addition to morphine, of which some are still in use[11].

1.2. The move to synthetic drugs in 19th century

By the year 1900, the flourishing of synthetic chemistry was responsible for the shift in the use of pure chemicals replacing gradually most of the crude drugs. However, plant drugs continued to provide every day new entities to combat disease. The introduction of synthetic drugs by the start of 19th century and specifically during and after World War I was paralleled by a decline in the number of phytopharmaceuticals. This was evidenced by the reduction in the number of monographs on plant drugs in different compendia with the exception of Chinese pharmacopoeia[7].

In case of synthetic chemical drugs their manufacture, sale, and supply as medicines come under the strict control of different Medicines Acts. The latter, are implemented to ensure the quality parameters of dosage forms namely safety, efficacy, and maintenance of stability during shelf-life of product. The botanical products used in medicine on the other hand, often fail to fulfil such parameters for a number of reasons. One of the major problems confronting quality control testing of botanical products is their complexity with regard to the number of components and that the actual active constituents may not be necessarily the components specified in the analysis. Failure to prove safety is another pitfall of using botanical products[12].

1.3. "The Back to Nature" move for medication with botanical products

In the past few decades synthetic drugs have been condemned for many adverse drug reactions and for inducing certain diseases. Accordingly, in the past 50 years and more specifically after the thalidomide tragedy[13], there has been a strong call for back to nature for medication and revival of botanical medicines. The claims have been for better safety and efficacy. One of the major reasons cited for the current revival of natural medicines is the belief that "naturalness" is a guarantee of harmlessness and that herbal remedies have been in use for centuries and have the reputation of being safe and efficacious. However, critical examination of the literature on herbal preparations clearly indicates that claims of safety and efficacy are not always justified[7].

EGb 761 extract has been standardized to contain 6% total terpene trilactones (3.1% ginkgolides and 2.9% bilobalide) and 24% flavonoids. This is found to be the optimum for their health benefits[20]. The flavonoids are almost exclusively flavonol- O-glycosides, a combination of the phenolic aglycones quercetin, kaempferol, or isorhamnetin, with glucose or rhamnose or both at different positions of the flavonol moiety. EGb 761 contains several other components, including proanthocyanidins and organic acids. Moreover, the content of ginkgolic acids in EGb 761 is limited to 5 ppm owing to the allergenic properties of these compounds[14]. Table 1 shows different classes of compounds present in EGb 761.

Table 1. Different classes of compounds present in EGb 761[22].

Compound class	%	Compound class	%
Flavonol glycosides	24.0	High molecular mass compounds	4.0
Terpene trilactones	6.0	Inorganic constituents	5.0
Proanthocyanidins	7.0	Water, solvent	3.0
Carboxylic acids	13.0	Various	3.0
Catechins	2.0	Unknown	13.0
Non-flavonol glycosides	20.0	Alkylphenols (Ginkgolic acids)	≤5 ppm

Very little is known about which components of EGb 761 are efficacious, and thus the molecular basis for the action of *Ginkgo biloba* L. constituents on the Central Nervous System (CNS) is poorly understood. The major components of the extract are flavonoids and terpene trilactones. The flavonoids are assumed not to penetrate the Blood Brain Barrier (BBB), whereas nothing is known about whether terpene trilactones penetrate the BBB. However, it is generally assumed that the lipophilic character of terpene trilactones renders these compounds permeable to the BBB. Therefore, it appears that terpene trilactones are partially responsible for the effects of *Ginkgo biloba* L. extracts on the CNS[14].

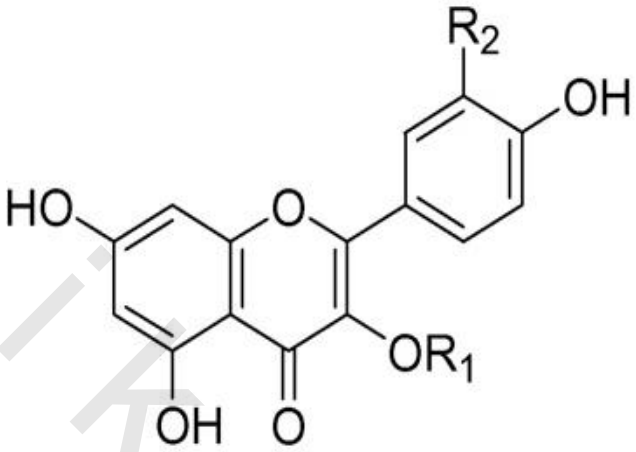
2.4. Constituents responsible for *Ginkgo biloba* L. activity

Analysis of the active ingredients of *Ginkgo biloba* L. is a challenging task, because of the diversity of the composition, seasonal variation of the flavonoid content, and the effect of place of growth. Pharmacological studies reveal that *Ginkgo biloba* L. mainly contains two groups of active ingredients: flavonoids and terpenoids.

2.4.1. *Ginkgo* flavonoids

Flavonoids are polyphenolic compounds probably found in all green plants[23-25]. Flavonoids are derived from the shikimate and acetate-malonate pathways. Approximately

35 flavonoids have been isolated from *Ginkgo biloba* L.[26]. The flavonoids in Ginkgo include flavones, flavonol glycosides, acylated flavonol glycosides, biflavonoids, flavan-3-ols and proanthocyanidins. Flavonol glycosides, present in *Ginkgo biloba* L. leaves, are more abundant than the other flavonoids and most of them being derivatives of quercetin, kaempferol, and isorhamnetin aglycones. The aglycones themselves occur only in relatively low concentration. Figure 4 shows the structure.



Ginkgo Flavonoids	R1	R2
Rutin	Rutinose	OH
Quercetrin	Rhamnose	OH
Quercetin	H	OH
Kaempferol	H	H
Isorhamnetin	H	OCH3

Figure 4. Stuctures of selected flavonols and flavonol glycosides in *Ginkgo biloba* L..

The great number of different flavonoids is not a result of the variability of the 2-phenylchromone framework but of the different glycosides found in *Ginkgo*. Nevertheless, only glucose and rhamnose can be found as sugar molecules and the variety of mono-, di-, and triglycosides in different binding patterns[27]. Recently, the flavonol glycosides have gained renewed interest and it has been suggested that gastrointestinal tract absorption of flavonoids is not limited to the aglycone form[28]. Medicinal extracts made from dried *Ginkgo* leaves are usually standardised to contain 24% flavonol glycosides and these compounds are considered to be of importance for their beneficial effects[20]. Flavonoid concentration in *Ginkgo biloba* L. leaves is influenced by ecological factors primarily during the young stage of *Ginkgo* development. Among all ecological factors, light and temperature are the most important[27]. Five coumaroyl esters of flavonoids have been isolated and identified[29]. These compounds are unstable because of the presence of two centres of instability as shown in figure 5. Accordingly stability indicating method of assay for flavonol glycosides is essential.

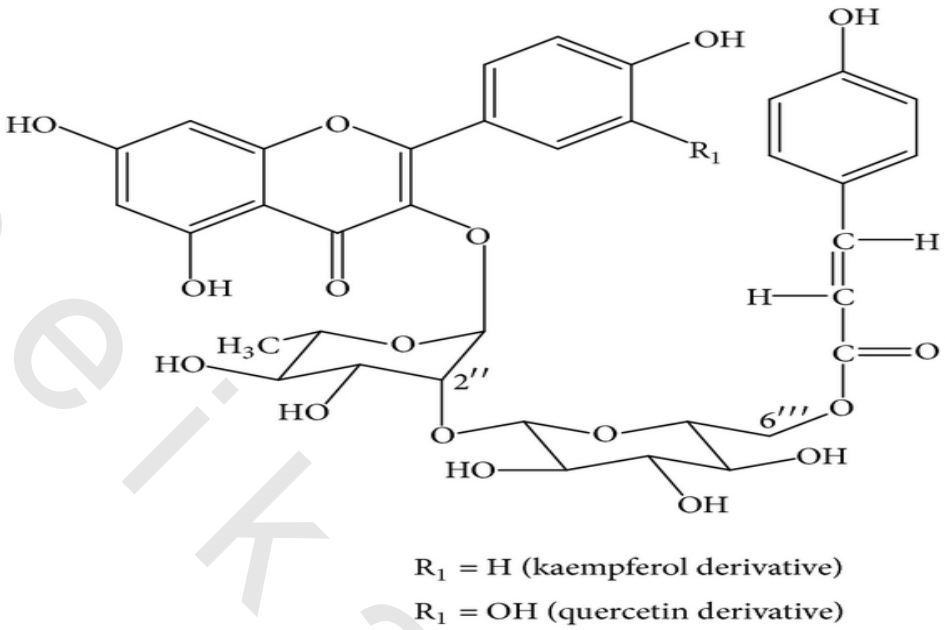
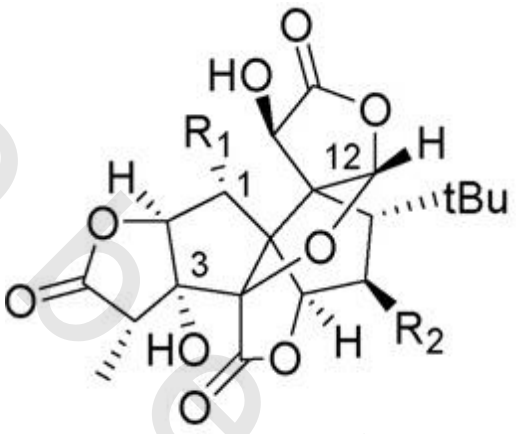


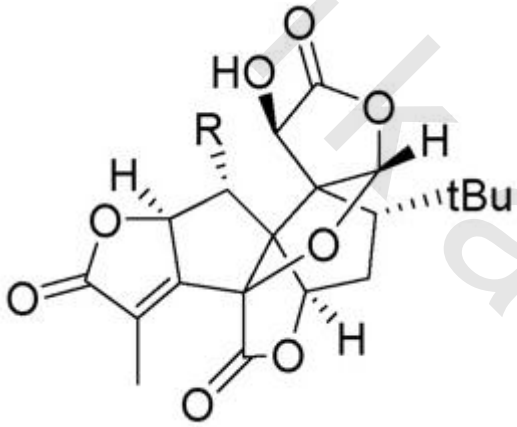
Figure 5. Two major flavonol-acyl-glycosides of *Ginkgo biloba* L.[30].

2.4.2. *Ginkgo* terpenoids

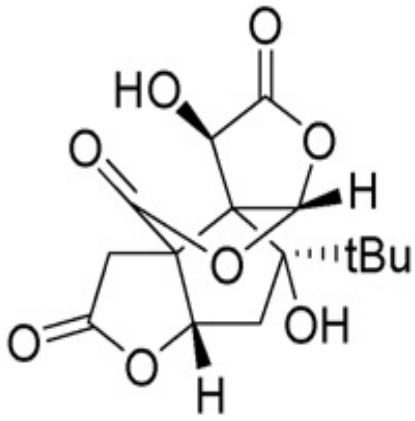
Terpene trilactones are a family of compounds with unique chemical structures, first recognised in an extract of *Ginkgo biloba* L.[19]. They are compounds with cage like structure. The ginkgolide A, B, C, J, M, K, and L are reported from the leaves of *Ginkgo biloba* L.. In addition to the ginkgolides (diterpenes), a bilobalide (sesquiterpene) is also found in *Ginkgo biloba* L.. Bilobalide is closely related to the ginkgolides. Two new ginkgolides P and Q were isolated from the leaves of *Ginkgo biloba* L. recently[31]. Ginkgolides and bilobalide are collectively known as terpene trilactones. Terpene trilactones are reported to be present only in *Ginkgo biloba* L.. Terpene trilactones are the only natural products possessing t-butyl group in their structure until now. Therefore, terpene trilactones has received more attention among other constituents of *Ginkgo biloba* L.. The large interest in *Ginkgo* terpene trilactones is due to their chemical uniqueness and their importance for quality control. Ginkgolides are potent and selective platelet-activating-factor antagonists but all attempts to turn pure ginkgolide B, the most active one, into a drug have failed so far. For the overall beneficial activity of *Ginkgo* extracts multiple compound classes and synergism are responsible[22].



	R1	R2
Ginkgolide A	H	H
Ginkgolide B	OH	H
Ginkgolide C	OH	OH
Ginkgolide J	H	OH



Ginkgolide-K: R=OH
Ginkgolide-L: R=H



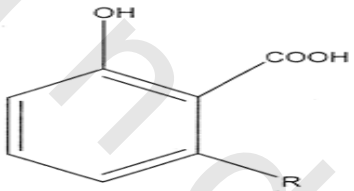
Bilobalide

Figure 6. Structures of ginkgolides and bilobalide in *Ginkgo biloba* L.[22].

2.5. Negative markers of *Ginkgo biloba* L.

2.5.1. Ginkgolic acids

The determination of ginkgolic acids in *Ginkgo* products is an important quality control parameter[32]. Ginkgolic acids (6-alkyl salicylic acids substituted with different long alkyl side chain) were proved existed in *Ginkgo biloba* L. leaves and EGb 761 (Figure 7)[33]. Ginkgolic acid C 15:1 and ginkgolic acid C 17:1 are the major ginkgolic acids in *Ginkgo* leaves[34]. Ginkgolic acids are claimed to be allergenic and should be absent from phytopreparations. Due to the different manufacturing processes, ginkgolic acids occur in different concentrations and proportions in commercial extracts. Nevertheless several producers limit their presence to a certain maximum in commercial extracts[35]. Thus, for drug safety, a maximal concentration (< or = 5 ppm) of ginkgolic acids is requested by different monographs in different compendia[36]. Recently studies have shown that ginkgolic acid concentrations vary from 0.5 to 4.8% for *Ginkgo biloba* L. leaves, and from 3.5 to 12.8% for *Ginkgo biloba* L. sarcotesta[37]. On the other hand, molluscicidal, insecticidal, and larvicidal activities against mosquito have also been reported for these compounds[37]. Recently Four new ginkgolic acids were isolated from the leaves of *Ginkgo biloba* L.[38].



Ginkgolic acids	Number of carbon atoms	Number of double bonds	R
Ginkgolic acid C 13:0	13	0	$C_{13}H_{27}$
Ginkgolic acid C 15:0	15	0	C_{15}:H_{31}
Ginkgolic acid C 15:1	15	1	$C_{15}H_{29}$
Ginkgolic acid C 17:1	17	1	$C_{17}H_{33}$
Ginkgolic acid C 17:2	17	2	$C_{17}H_{31}$
Ginkgolic acid C 17:3	17	3	$C_{17}H_{29}$

Figure 7. Ginkgolic acids stuctures[39].

2.5.2. Ginkgotoxin

Ginkgotoxin is a neurotoxic compound occurring in the seeds and in a less amount in the leaves of *Ginkgo biloba* L.. It is an antivitamin structurally related to vitamin B6 that causes severe neuronal disorders in mammals after ingestion. Symptoms of this poisoning called 'gin-nan sitotoxism' are mainly epileptic convulsions, paralysis of the legs, and loss of consciousness. There are even reports of death due to overconsumption of *Ginkgo* seeds, which are the main source of ginkgotoxin[40,41].

2.6. Medicinal importance of *Ginkgo biloba* L.

Studies confirmed that *Ginkgo biloba* L. leaf extract has been very useful in the treatment of Alzheimer's disease[42-44], neurodegenerative disease[45], cerebral insufficiency[46], eye ailments[47], hypertension[48], age-related memory deficit and oxidative stress[49,50]. In addition, it has been demonstrated to be effective in preventing apoptosis[51,52] and free radicals[53,54]. It is very useful antioxidant[55] and can be used for enhancing sexual pleasure in individuals suffering from sexual dysfunction[56,57]. *Ginkgo biloba* L. extracts were also tested in cancer research, but very little is known so far [23,24,58]. The whole extract provides maximum benefits for the previous reported indications as compared with individual compounds isolated from the plants[31].

2.7. *Ginkgo biloba* L. drug interaction, side effects, and dosage

People mistakenly think that all herbs are safe, because of the fact that they are natural, and the use of herbal medication is growing. Aspects of the efficacy, safety, and quality of herbal products are the subjects of on-going debates. Concurrent administration of herbs may interfere with the effects of drugs[59].

The marketing and consumer use of herbs and dietary supplements has risen dramatically over the past two decades. It is estimated that more than 50% of patients with chronic disease or cancers even use herbs and dietary supplements. Nearly one fifth of patients take herbs and dietary supplements products concomitantly with prescription medications users[60].

The most important potential clinical problem with *Ginkgo* is caused by its inhibition of the platelet-activating factor; this makes the use of *Ginkgo* in conjunction with warfarin (Coumadin), aspirin, or other antiplatelet agents a matter of clinical judgment[61]. *Ginkgo* should be discontinued between 36 hours and 14 days before surgery. Herbal medications that may increase the risk of bleeding if used concurrently with *Ginkgo* include the following: feverfew, garlic, ginseng, dong quai, red clover, and other natural coumarins. The unprocessed *Ginkgo* leaf contains ginkgolic acids that are toxic. Hypersensitivity to *Ginkgo* preparations is a contraindication to use. *Ginkgo* is generally well tolerated, with side effects being rare, usually mild, and including nausea, vomiting, diarrhea, headaches, dizziness, palpitations, restlessness, weakness, or skin rashes. Although no studies have been performed to support any restrictions on the use of *Ginkgo* during pregnancy or lactation, it seems prudent not to administer *Ginkgo* in the absence of any data.

Table 2. *Ginkgo biloba* L. documented drug interactions.

Drug	Results of interaction	Comments
Aspirin[62,63]	Spontaneous hyphema	Ginkgolides are potent inhibitors of Platelet activating factor.
Theophylline[62,64]	Therapeutic failure	This is due to the increase in the metabolic activity and the clearance of theophylline by *Ginkgo*.
Warfarin[62,63,65]	Intracerebral haemorrhage	It is of a major safety concern, as Warfarin is a narrow therapeutic index drug.
Thiazide diuretic[66]	Hypertension	This effect may be due to the metabolic inhibition of thiazide by *Ginkgo*.
Nifedipine[62]	Sever headache	*Ginkgo* affects its pharmacokinetics and pharmacodynamics properties.

The recommended dosage of an oral standardized dry extract of *Ginkgo* (24% *Ginkgo* flavonol glycosides, 6% terpene lactones) is 120–240 mg daily[67]. The dosage for patients who have tinnitus and peripheral vascular disease is no more than 160 mg per day, taken in two or three doses. An initial period of six to 12 weeks is recommended to assess the effectiveness of *Ginkgo*, although results have been seen as early as four weeks[68].

2.8. Content variation of *Ginkgo biloba* L. constituents in relation to age, gender, and collection time of the leaves

The content of terpene trilactones and flavonol glycosides in the leaves of young trees are higher than those in old trees, and they are higher in male trees than in female trees[69].For terpene trilactones the concentration was lowest in spring and then gradually increases until a maximum in late summer or early autumn is reached[70]. Flavonoid content raises progressively from May to October but ginkolide content reach peak value on September and came down slightly on October[71]. The suitable seasons for the harvest of *Ginkgo biloba* L. leaves are from September to October (Figure 6 and 7).

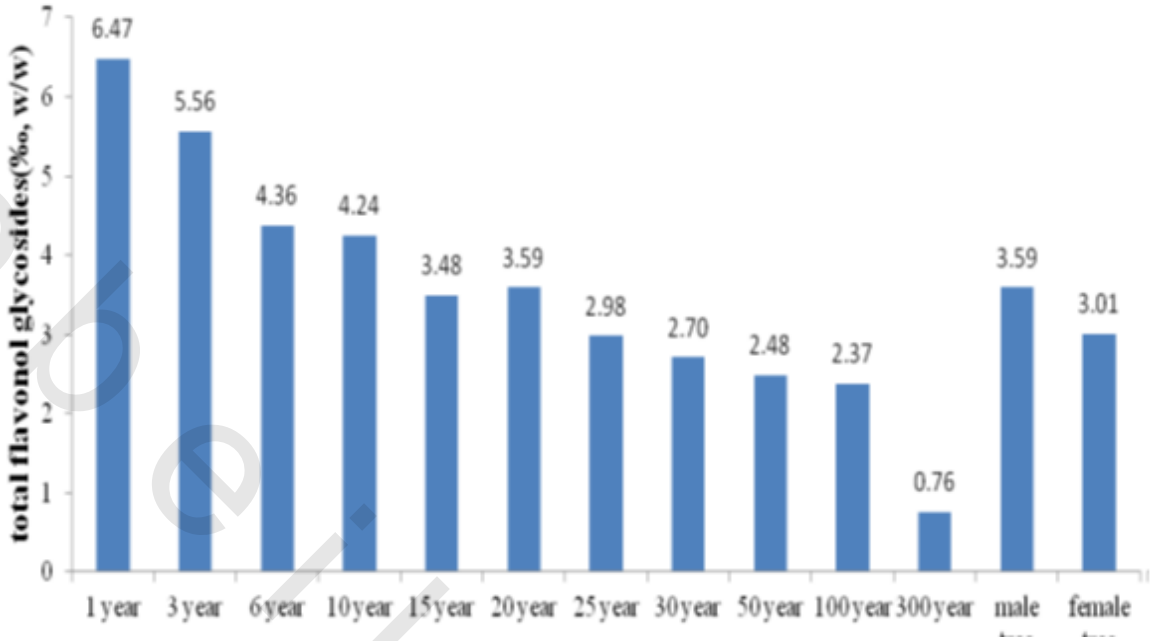


Figure 8. Comparison of the total flavonol glycosides in the *Ginkgo biloba* L. leaves collected from different ages and genders[(69)].

Figure 9. Comparison of the total terpene trilactones in the *Ginkgo biloba* L. leaves collected from different ages and genders[(69)].

2.9. *Ginkgo biloba* L. nowadays

Nowadays *Ginkgo* is one of the most intensely studied medicinal plants, with more than 3000 scientific papers published on the topic between 2001 and 2009 alone[(21)]. *Ginkgo biloba* L. is also one of the top 10 selling herbs in health food stores in the United States. In Germany and several other European countries, *Ginkgo biloba* L. is registered as a drug and is among the top five most commonly prescribed medications. In Germany, *Ginkgo biloba* L. extracts must meet the requirements of the German Commission E monograph

which specifies what the extract must contain[72]. Extracts sold in other countries, however, are not classed as drugs and so are not required to conform to the standards of those that have been shown to be effective. Thus, there is a large variety of extracts available[73].

3. Regulation of dietary supplements internationally

In 1994 the United States Congress enacted the Dietary Supplement Health and Education Act (DSHEA). The law created a new regulatory framework for the safety and labelling of dietary supplements and separated them from drugs and conventional foods.

According to the DSHEA definition, a dietary supplement is ‘‘a product (other than tobacco) that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, a herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing total daily intake, or a concentrate, metabolite, constituent, extract or combination of these ingredients.’’[74]

Manufacturers of dietary supplements, unlike manufacturers of pharmaceuticals, are not required to provide evidence of safety and efficacy based on rigorous pre-market clinical testing. Nor are they required to register or obtain Food and Drug Administration (FDA) approval before their products reach consumers. DSHEA places the burden on the FDA to prove that a ‘‘dietary supplement presents a significant or unreasonable risk or illness or injury’’ prior to any marketplace removal. This innocent until proven guilty approach has consequences in and of itself. Under this reactive regulatory scheme, harmful dietary supplements might not be removed from market unless a significant number of consumers are harmed. The only exception to this standard is that manufacturers of ‘‘new dietary ingredients’’ (those not marketed in Unites States before October 15, 1994) must notify the FDA at least ‘‘75 days before being introduced or delivered for introduction into interstate commerce. This process is a notification provision only and it does not require, as with drugs, pre-market approval from FDA[75]. This regulatory structure has led to problems with the consistency and safety of herbal products[76].

Manufacturers may make three types of claims for dietary supplements: health claims, structure-function claims and nutrient-content claims. Health claims are FDA approved claims to be used under specific conditions, whereas, structure-function claims are not approved by the FDA, and this must be stated in a ‘disclaimer’ (‘the FDA has not evaluated the claim’). Manufacturers of dietary supplements that make structure-function claims must submit a notification to the FDA no later than 30 days after marketing the dietary supplement. In 1997, a study on the regulation of label claims and statements on dietary supplements was conducted by the Commission on the Dietary Supplements Labels[77].

The quality of consumer information about the product is as important as the finished herbal product. Warnings on the packet or label will help to reduce the risk of inappropriate uses and adverse reactions. The primary source of information on herbal products is the product label. Currently, there is no organization or government body that certifies a herb or a supplement as being labelled correctly. It has been found that herbal remedy labels often cannot be trusted to reveal what is in the container. The word

"standardized" on a product label is no guarantee of higher product quality, since there is no legal definition of the word "standardized." Consumers are often left on their own to decide what is safe and effective for them and the lack of consistent labelling on herbal products can be a source of consumer frustration. Certain information such as "the product has been manufactured according to Pharmacopoeial standards," listing of active ingredients and amounts, directions such as serving quantity (dosage) and frequency of intake of the drug, must be included on the labels of all herbal products and packages. The label should also indicate the method of extraction and possible side effects. It should indicate that the product's content has been standardized to contain a particular amount of a specified biochemical constituent. In addition to the above information, the label should include the name and origin of the product, its intended use, net quantity of contents, other ingredients such as herbs and amino acids, and additives, for which no daily values have been established, storage conditions, shelf life or expiry date, warnings, disclaimer, and name and address of manufacturer, packer, or distributor[78].

The DSHEA of 1994 gave the FDA authority to establish current good manufacturing practices (cGMP) for dietary supplements. However, it was not until June 22, 2007, 13 years later, that the FDA announced a final rule establishing regulations to require cGMP for dietary supplements, the Dietary Supplements Final Rule. Prior to that date, although it was obligatory for manufacturers to notify the FDA that they planned introducing a dietary supplement into the marketplace and to provide information about the safety of the product, except for new dietary ingredients, the manufacturer did not have to prove that the dietary supplement was either safe or efficacious. In addition, adverse event reports did not have to be reported[79].

In Egypt, the Egyptian Drug Authority (EDA) is an organization within the Ministry of Health that is responsible for safeguarding people health by regulating safety and quality of medicines (human and veterinary), biological, medical devices, cosmetics, dietary supplements, and pesticides. The EDA has three sub-organizations that work cooperatively and synergistically to assure the achievement of the EDA mission. The Central Administration of Pharmaceutical Affairs (CAPA), National Organization for Drug Control & Research (NODCAR), and National Organization For research & Control of Biologicals (NORCB).

Regulatory requirements for the quality of herbal products vary depending on the country and the regulatory category. The same herbal product can be marketed as a drug in Europe and as a dietary supplement in the United States [77,80,81].

4. Methods used for the assessment of botanicals

The assessment of botanical dietary supplements is very complicated. Unlike conventional drugs, herbal products provide a complex mixture of biologically active entities, with possible therapeutic benefits, and often a complete description of all individual constituents is not known. Additionally, herbs are irregular because their chemical profile may vary depending on multiple factors, for example, origin, the part of the plant, vegetative phase, growing, harvesting, processing, and storage conditions[75].

4.1. Compendial methods

During the past decade, several compendial monographs on the quality control of bulk herbal materials were published. With the existence of monographs, identification and quality control of the herbal material can be performed based on macroscopic and microscopic techniques and on the use of marker compounds. The latter are classified into seven categories, namely active principles, active markers, group markers, chemical fingerprints, analytical markers, 'phantom' markers, and negative markers[82]. However, the use of markers might not always be suitable due to the lack of unique chemical compounds and the synergic effects between the constituents[83].

4.1.1. USP monographs

The USP was first published in 1820. In 1942 USP developed over 600 botanical monographs. Gradual omission of botanical monographs was adapted by USP committees (1942-1995). The feasibility of establishing standard monographs and information for botanical in response to DSHEA was possible starting from 1995. In 2003 United States Pharmacopoeia 27- National Formulary 22 (USP 27-NF 22) included a dietary supplements section separate from drug standards, in addition to 200 monographs for botanicals, non-botanicals, and vitamin-mineral combination products covering almost 900 dietary supplements. In 2009 USP 32 was published with more than 450 standard monographs and general chapters and USP introduced the USP dietary supplement compendium. In 2012 USP Dietary Supplements Compendium (DSC) has been significantly updated and expanded into a two-volume set. It contains 50 new dietary supplement monographs, 560 monographs which are all redesigned, 160 excipients used in dietary supplement, and 26 safety reviews. USP offers also independent verification services for dietary supplement finished products, dietary ingredients, pharmaceutical ingredients, and excipients. Products and ingredients that meet all USP verification are awarded use of the distinctive USP Verified Marks. Participation is voluntary and available to manufacturers worldwide[84].

4.1.2. WHO monographs

The World Health Organization (WHO) published 28 monographs in the first volume, which provide valuable information. The monographs are based on a systematic review of scientific literature from 1975_1995. Review articles, bibliographies in review articles, pharmacopoeias and other reference books were used. Some 100 experts in more than 40 countries commented on the draft monographs. In 1996, a WHO Consultation on Selected Medicinal Plants was held and 16 experts and medicine regulatory authorities from the WHO member states participated. Following discussion, 28 of 31 draft monographs were approved. Each monograph contains two parts: pharmacopoeial summaries for quality assurance, including botanical features, distribution, identity tests, purity requirements, chemical assays, and active or major chemical constituents, the second part summarizes clinical applications, pharmacology, contraindications, warnings, precautions, potential adverse reactions, and posology. Volume 2 includes 30 monographs and volume 3 in this series was published in 2007 and includes 31 monographs. Volume 4 was published in 2009 and includes 28 monographs[81].

4.1.3. Commission E monographs

In Germany, the Commission E was established by the German government in 1978, which is an expert committee on herbal remedies, including physicians, pharmacists, pharmacologists, toxicologists, representatives of the pharmaceutical industry, and lay persons. Commission E evaluated more than 300 botanicals and botanical combinations for their safety and efficacy. After the evaluations, draft monographs were prepared and following a suitable period for comments and their considerations these were published as monographs and are used as the basis for regulatory decisions. Three types of monographs were published. Positive (approved) monographs permitting the use of the botanical as a non-prescription medicine, negative (unapproved) monographs which do not permit the sale of the botanical because the health risks were too great, and neutral monographs permitting the sale of the botanical because there are no safety concerns, but the documentation is too limited to prove benefit. These monographs were based on actively collected bibliographic data, which were evaluated by the members of Commission E to determine the safety and efficacy. The bibliographic data were presented by manufacturers of botanical products, completed by data obtained from literature search. The information that was reviewed can include for example traditional use, chemical data, experimental, pharmacological, and toxicological studies, clinical studies, field and epidemiological studies, patient case records, and additional studies (for example performed by the manufacturers)[81].

4.1.4. ESCOP monographs

ESCOP was founded in 1989 as the European umbrella organization of national associations for phytotherapy and is a non-governmental organization. The aims of ESCOP are to advance the scientific status of phytomedicines and to assist with harmonization of their regulatory status at the European level. ESCOP prepares monographs which consist of the following elements: definition, constituents, clinical particulars (including therapeutic indication, posology and method of administration, contra-indications, special warnings and special precautions for use, interactions with other medicaments and other forms of interaction, pregnancy and lactation, effects on ability to drive and use machines, undesirable effects, and overdose[81]. By the end of the 1990s ESCOP had published 60 monographs on the medicinal uses of plant drugs. The second edition of ESCOP monographs, published in 2003, achieved wide recognition for its authoritative information on the therapeutic uses of herbal medicines and summaries of pharmacological, clinical and toxicological data. As part of ESCOP's program to expand and update the range of monographs the supplement 2009 adds twenty-seven new ones to the eighty published in 2003, as well as eight monographs revised to include recent data from herbal research.

4.1.5. Pharmacopoeia of the People's Republic of China Monographs

The most comprehensive document available on herbal medicines is the 2010 edition of the ‘Pharmacopoeia of the People's Republic of China’ containing monographs on 2136 species. To meet the increasing quality standards, many of the monographs includes different chromatographic teqniques such as Thin Layer Chromatography (TLC), High Pressure Liquid Chromatography (HPLC), and Gas Chromatography (GC)[81]. A total

of 282 chemical makers are listed in the Chinese Pharmacopoeia (2005 edition) for the quality control of Chinese herbal medicines[82].

4.2. Pitfall of compendial monographs for quality assessment of *Ginkgo biloba* L. extract

Pharmacopoeial monographs play an important role in the quality assurance of botanicals and herbal medicinal products. However, discrepancies may exist between the different pharmacopoeias[81].

Monographs for *Ginkgo* raw materials (leaf and extract) can be found in various pharmacopoeias, including the USP-NF, Ph. Eur., and the BP. The USP-NF mono-graph for powdered *Ginkgo* extract specifies a flavonoid content of 22–27%, calculated as flavonol glycosides and a maximum content of 5 ppm for ginkgolic acids. In addition, specifications are provided for the terpene trilactones, bilobalide, and ginkgolide A, B, and C, but the ranges for these differ between the USP-NF and the BP/ Ph. Eur. The USP-NF also provides monographs for *Ginkgo* tablets and *Ginkgo* capsules. Although the USP-NF, BP, and Ph. Eur. stipulate a required range for flavonol glycosides in *Ginkgo* extract, the prescribed assays quantify flavonol aglycones. This means that these pharmacopoeial methods are not capable of detecting adulteration of *Ginkgo* extract with free flavonol aglycones[21].

The current approach for standardization of flavonols in *Ginkgo* extracts is by calculation of the total flavonol glycoside content from the aglycone concentration in extracts after acid hydrolysis [22] (Figure 9). The hydrolysis step is necessary as the original layout of flavonol glycosides in *Ginkgo* in form of glycosides is very complex and challenging from separation and analysis perspective. However, this entails a degree of non-specificity in the flavonol glycosides testing procedure and makes this group of phytochemical class the target for adulteration. Typical adulteration are done to enhance the total flavonol glycosides by artificially spiking the botanical extracts that can release *Ginkgo* flavonol aglycones quercetin, kaempferol, and isorhamnetin after hydrolysis[20]. Although this procedure is relatively simple and widely accepted, the aglycones already present in extracts and calculations based on the average glycoside mass result in exaggerated reported flavonol glycoside content. The presence of glycosides, which may hydrolyse during extraction and/or incorrect storage, is therefore useful quality control indicator. An increase in the ratio of aglycones to glycosides in extracts signifies degradation[28,85].

As a parameter for quality/authenticity the USP method also mandates to monitor a quercetin/kaemferol/isorhamnetin ratio (Q/K/I) of the hydrolysed extract based on their respective peak areas. The kaempferol peak must be 0.8-1.2 times the size of the quercetin peak, and the peak for isorhamnetin must be not less than 0.1 times the size of quercetin peak. The quercetin/kaempferol/isorhamnetin ratio could vary on the growing location of *Ginkgo*, harvest season, and processing conditions. It is possible to manipulate the ratio by adulteration with non- *Ginkgo* extracts that can produce *Ginkgo* flavonol aglycones. However, it is more challenging to match naturally occurring *Ginkgo* unhydrolyzed flavonoid fingerprints through adulteration with non-*Ginkgo* extracts[20].

Quantitative analysis of flavonol glycosides which are relatively hydrophilic, have been largely overlooked since such compounds are usually poorly absorbed from gastrointestinal tract following administration and thus not considered to contribute to clinical activity. However, other studies suggest that the non-flavonoid components in *Ginkgo biloba* L. extracts could increase the absorption and improve the bioavailability of flavonol glycosides but decreases the absorption and reduce the bioavailability of flavonol aglycones[86] Also studies that are more recent have indicated that flavonol glycosides are indeed more readily absorbed than their aglycone counterparts are and thus their presence/ absence requires monitoring and assessment. Although reference standards for all flavonol glycosides are not available, relevant flavonol glycoside markers can be chosen for analytical techniques to ensure comprehensive standardization[28,85].

To produce a (flavonol glycosides/terpene trilactones) 24/6 grade *Ginkgo* extract, the quality of leaves is critical. When *Ginkgo* leaves are harvested at unfavourable times, procurement of more *Ginkgo* leaves to produce optimized extracts that are standardized to 24% flavone glycosides levels is required. This results in driving the cost of the end product relatively high and thus inviting one more reason for adulteration. These facts make it very important to pay extra attention to monitor the quality of the purchased extracts[20].

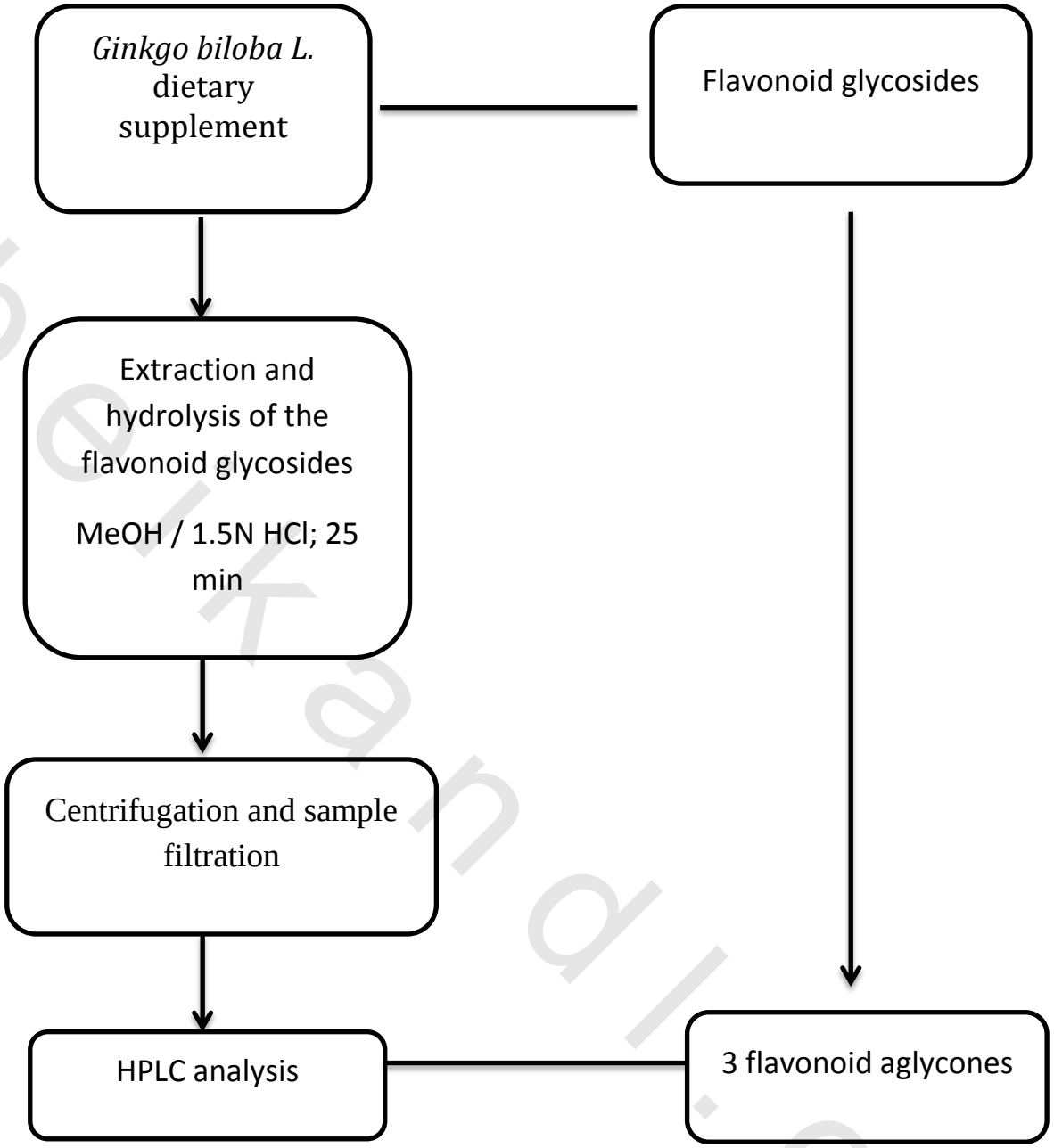


Figure 10. Work-up procedure for HPLC determination of flavonoid aglycones.

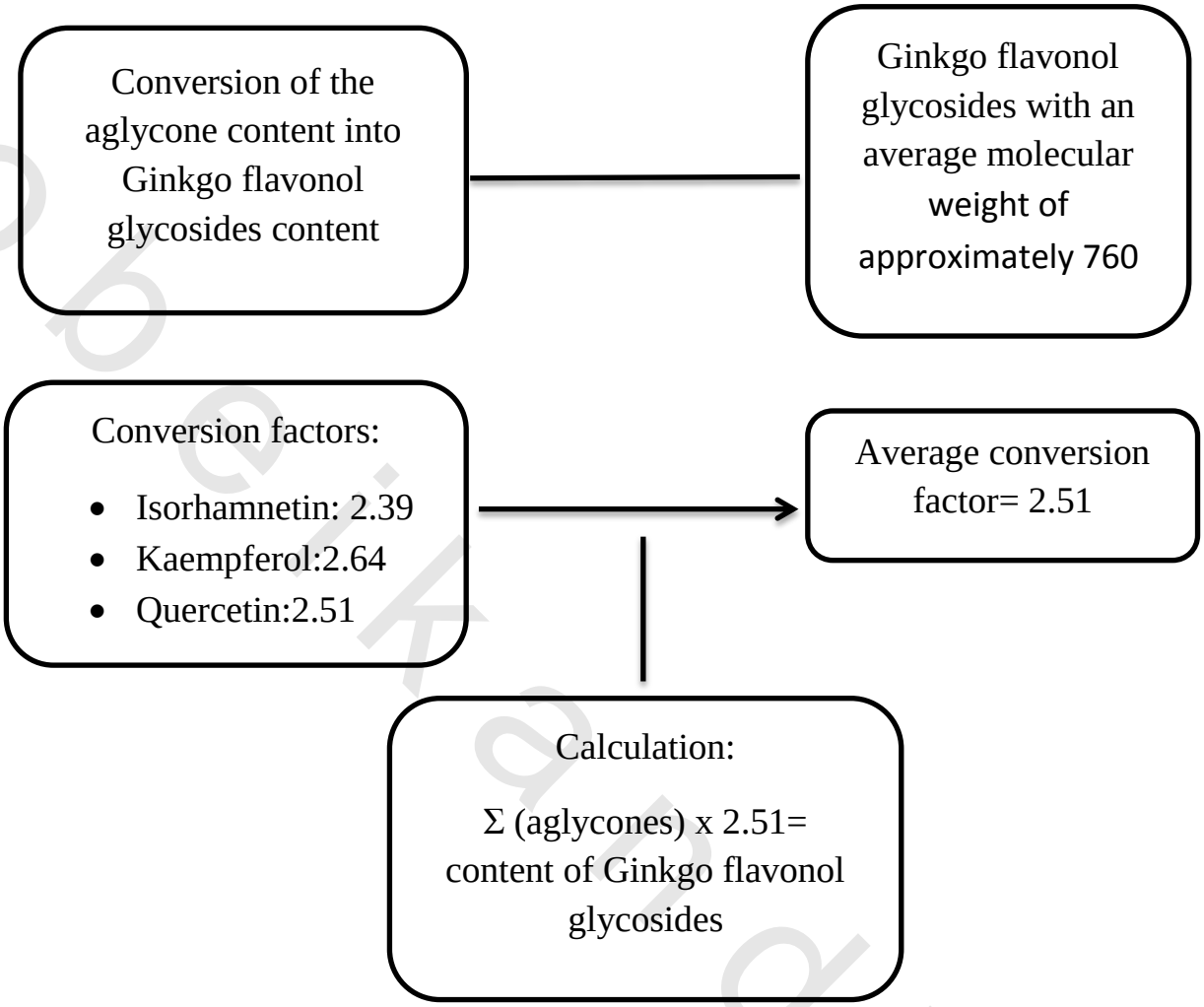


Figure 11. Calculation of *Ginkgo* flavonol glycosides content.

4.3. Chromatographic and tandem methods

Usually, herbs contain hundreds of chemical constituents but only few components are bioactive. Consequently, it is essential to identify and measure all of the bioactive constituents of medicinal plants to ensure the reliability and repeatability of clinical research and enhance quality control from the pharmacologically beneficial and/or hazardous perspectives. However, the inherent complexity and variability of botanical extracts has presented significant challenges for separation and detection methods enabling rapid analysis of the chemical composition of medicinal plants[87]. In order to prove constant composition of herbal preparations, adequate analytical methods have to be applied such as photometric analysis, TLC, HPLC, GC, and Fingerprinting[4]. Quantitative and qualitative methods have been devised for the quality control of herbal materials in recent years. Both methods have advantages and disadvantages.

Quantitative analysis aims to separate and identify the marker compounds from herbs or herbal preparations and then use them as indicators or standards to assess quality.

Determining the effective or principal chemical constituents and the toxic compounds is crucial to the quality control of herbs and herbal preparations. But for quality control of complex systems, the determination of only a few compounds cannot, realistically speaking, give a comprehensive and accurate assessment of all active constituents in herbal medicinal products; such an approach is inherently inadequate for quality control and stability evaluation of herbal medicinal products[88].

Qualitative analysis is typically used to demonstrate the general characteristics of herbal materials or their herbal preparations with regard to quality, consistency, and stability. [88] Qualitative chromatographic fingerprinting has been used over the last decade for the authentication and quality control of herbs. Chromatographic fingerprinting techniques can be used to characterize both the marker compounds and the unknown components in a complex system, a strategy recommended to assess the quality and consistency of botanical products by FDA, the European Medicines Evaluation Agency (EMEA), and State Food and Drug Administration of China (SFDA). This strategy, however, is not sufficient to control the overall quality of botanical products because it does not quantitate active components. Recently, some combinative methods using HPLC fingerprinting and quantitative determination have been developed and validated for quality control of herbal preparations[89].

Based on the chemical and physical properties of specific compounds, a number of different detection techniques including ultraviolet detection (UV) or photodiode array detection (PDA), fluorescence detection, refractive index, evaporative light scattering detection and mass spectrometry (MS) have been used for detecting the components in mixtures. Compared to other detection methods, MS can not only allow determination of chemical structure of natural compounds with known and unknown structures, but also offers excellent sensitivity. In combination with PDA, liquid chromatography mass spectroscopy can provide on-line structural information for each individual peak in a chromatogram at low amount of samples within relatively short analysis time. In addition, liquid chromatography mass spectroscopy can also interface with nuclear magnetic resonance and thus offers advantages for the more accurate and confirmative structure analysis of the unknown compounds in complex mixture[87].

4.4. Available analytical methods used for the quality assessment of *Ginkgo biloba* L. dietary supplements

Many studies have been conducted on assessment of dietary supplements containing *Ginkgo biloba* L. extracts. Some concentrated on the determination of terpene trilactones[90]. while others concentrated on the determination of flavonol glycosides concentration either by the approximate compendial method which involves hydrolysis of flavonoids to aglycones [20,85,91,92] or by determination of the concentration of the intact flavonol glycosides but, it does not serve a real purpose[20,85,91,93]. No quantitative procedure for all major flavonol glycosides has yet been published because they are not commercially available. A profile of the genuine flavonol glycosides can detect poor storage or adulteration. Some studies used HPLC either qualitatively or quantitatively [28,94,95] and others used HPLC mass spectrometry techniques[85,93,96-98]. Also some studies have been conducted to determine the quality of the botanical dietary supplements by the

estimation of the ginkgolic acids concentration in the extract[99,100]. Simultaneous determination of flavonol glycosides and terpene trilactones has been done [91,93,96,97,101-103]. There are new approaches to determine the quality of botanical dietary supplements by using fingerprint profile [92,98,104] and chemometric methods[105].