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Stability of Active Ingredients of Traditional Chinese Medicine (TCM)

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Studies on stability of active ingredients are fundamental and critical for the rational development of Traditional Chinese Medicine (TCM) in view of its modernization and worldwide use. The stability of both active and marker constituents of plants used in TCM is reviewed for the first time. More than 100 papers, mostly written in Chinese, have been reviewed. Studies concerning plant constituents were analyzed according to their chemical classification of active ingredients. In addition, several crude drugs of animal origin are also reported. Stability of active ingredients is summarized during extraction and/or storage of the herbal drug preparations, and under stress conditions (pH, temperature, solvents, light, and humidity) and in the presence of preservatives, antioxidants, and metals.

Keywords: Traditional Chinese Medicine (TCM), active ingredients, stability, alkaloids, phenols, terpenoids.

Traditional Chinese Medicine (TCM), as one of the most representative traditional medicine systems, has contributed significantly to the health of Chinese and East Asian people for over two thousand years [1-7]. Now it still plays an indispensable role in local health care system [8-15]. In recent years, the advantages of TCM for certain diseases have been recognized and studied all around the world [16-19].

In the last two decades active principles and/or marker constituents from plant and animal TCMs have been investigated using an interdisciplinary approach, which includes phytochemistry, analytical chemistry, pharmacology, and molecular biology. In some selected cases, isolated constituents, such as polyphenols, alkaloids, terpenoids, polysaccharides, amino acids, polypeptides and enzymes, have also been developed and marketed as either new drugs or perspective pre-drugs [20-24].

Quality, and consequently safety and efficacy profiles, of Herbal Drugs (HDs) and Herbal Drug Preparations (HDPs) of TCM is strictly related to the presence of the active ingredients in defined amounts. However the integrity of these structures can be susceptible to stress conditions during collection, storage and extraction leading to irreversible chemical modifications, which can cause a dramatic decrease of the activity of the HDs or HDPs or even create toxic constituents. For these reasons, the stability of these active ingredients is considered as the basis of safety and efficacy, as is also stated by the Chinese Pharmacopeia.

In China the current methods to evaluate stability of the constituents of TCM are similar to those reported for synthetic drugs through the guidelines of SFDA (State Food and Drug Administration). These are also similar to those issued by the FDA and ICH and include stress tests, accelerating tests, and long-term stability tests [25-27]. Factors tests (stress tests) observe the changes using various kinetic models under certain stress conditions, such as acidic or alkaline pHs, oxidation, light, and humidity [28].

On the basis of accelerated and long-term stability tests, the expiration of drug substance in the plant material (Herbal Drug, HD) and in the preparations (HDPs) will be set. Furthermore, some characteristic properties such as color, flavor and odor, can also be evaluated, even if these are not reported in the present review.

A variety of well known analytical methods, such as TLC, HPLC-DAD, HPLC-MS, NMR, IR, NIR, and UV-Vis, are employed in the evaluation of the stability of the characteristic ingredients of TCM and track the possible degradation products, to deduce degradation pathways [29-32].

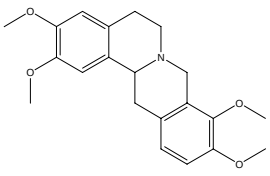
In this paper, for the first time, to the best of our knowledge, the studies concerning the stability of active ingredients represented by the characteristic constituents of herbal drugs and their preparations from TCM are reported and reviewed according to their origin (plant or animal drugs) and chemical classification.

1. Alkaloids

Alkaloids are naturally occurring constituents containing nitrogen [33,34], produced by a large variety of organisms, including bacteria, fungi, plants, and animals and many of them represent the characteristic constituents of TCM possessing diverse physiological activities.

The degradation of alkaloids is mainly caused by oxidation of nitrogen atoms. These reactions may be triggered by environmental conditions, such as light, temperature and moisture.

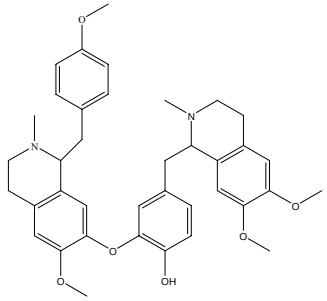
Isoquinoline alkaloids: Tetrahydropalmatine (**1**) is the characteristic constituent of *Rhizoma corydalis* (Yanhusuo), which is the dried tuber of *Corydalis yanhusuo* W. T. Wang. The drug is reported to alleviate pain and as having hypnotizing effects. Xu and coworkers found that tetrahydropalmatine in the dry extract of *Rhizoma corydalis* (Yanhusuo) can degrade at high temperature in the presence of air [35,36].



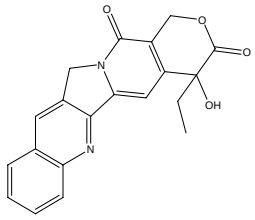
Tetrahydropalmatine (**1**)

The degradation mechanism was oxidative. The reaction rate was extremely slow below 30°C, but increased rapidly above 60°C. The long-term stability tests evidenced that tetrahydropalmatine was stable in the range 10-30°C for 12 months. *Plumula Nelumbinis* (Lianzixin) is a traditional Chinese medicine, which is the dried young cotyledons and ripe seed of *Nelumbo nucifera* Gaertn. This HD has good therapeutic effects on heat-shock, dysentery, diarrhea, and against vomiting. Liensinine, isoliensinine and neferine (**2**) are the main alkaloids from the extract of *Plumula Nelumbinis* (neferine>40%). Neferine is a bisbenzylisoquinoline alkaloid with a phenolic hydroxyl group which is susceptible to oxidation. As the most abundant compound, its stability in liquid extracts was studied by Lu and coworkers [37]. Neferine is unstable at acid and alkaline pHs, and to irradiation, moisture, andFe^{3+}, but is not affected by temperature. Oxidant and acidic media, Fe^{3+} and irradiation could catalyze conspicuously the decomposition reaction. High humidity causes moisture absorption by the dry powder of neferine, which leads to degradation, as occurs in the liquid state. As a consequence, the product should be stored only in cool and dry conditions.

Camptothecin (CPT, **3**) is a well-known anti-cancer constituent isolated from *Fructus Camptothecae Acuminatae* (Xishuguo), which is the fruit of *Camptotheca acuminata* Decne. Thermal and light stability of CPT in phosphate buffers was studied using HPLC and first order derivative spectrophotometry. CPT degradation followed first order kinetics and was catalyzed by alkali (calculated energy was 87.6l KJ/mol). Decomposition by irradiation followed zero order kinetics [38].

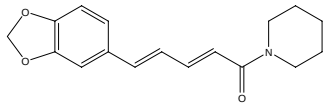


Neferine (**2**)



Camptothecin (**3**)

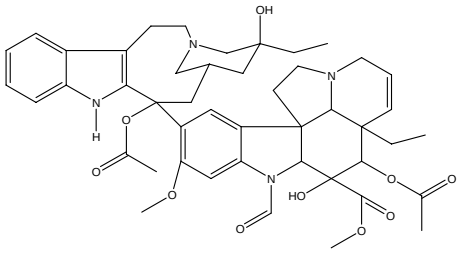
Piperidine alkaloids: Piperine (**4**), a pyrrolidine alkaloid, is the major active ingredient of *Fructus Piperis* (Hujiao), which is the dried fruit of *Piper nigrum* L. It possesses several activities, such as anti-inflammatory, anti-convulsivant, and anti-cancer [39]. High-speed counter current chromatography and atmospheric-vacuum distillation have been applied to extract and purify piperine. The stability studies revealed that piperine was stable in an acidic medium and for a short period of heating (60ºC applied for 2 h) [40]. Packaging was essential



Piperine (**4**)

to preserve stability, due to the significant decrease of piperine content by ambient irradiation [40].

Indole alkaloids: Vincristine (**5**) is very efficacious on various types of acute leukemia and malignant lymphomas, and represents the active constituent of *Herba Catharanthi Rosei* (Changchunhua), which is the whole herb of *Catharanthus roseus* (L.) Don [41]. Zuo and coworkers studied the stability of vincristine sulfate in 0.9% NaCl solution used for injections [42].



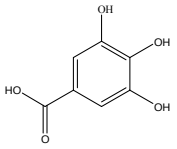
Vincristine (**5**)

The studies indicated that the degradation of vincristine sulfate was a first order reaction ($T_{0.9}$ at 25ºC and 32ºC was 12.51 h and 8.82 h, respectively) concluding that the injectable preparation of vincristine sulfate in 0.9% NaCl solution is stable for 8 hours [42].

2. Polyphenols

Polyphenols are a wide group of secondary constituents, characterized by the presence of one or more phenol units. Polyphenols, mostly derived from the shikimate pathway, are generally classified according to their basic skeletons [43]. It is well known that polyphenols have antioxidant characteristics and potential health benefits, such as anti-cancer, antibacterial, anti-inflammatory, and cholesterol-lowering [44-47].

C6-C1 derivatives: Gallic acid (**6**) is one of the most simple polyphenols, having anti-hepatitis B virus activity. The compound is widespread in food and HDs, such as the Chinese medicine *Radix et Rhizoma Rhei* (Dahuang), which is the dried rhizome and root of *Rheum palmatum* L., *R. tanguticum* Maxim, ex Balf., and *R. officinale* Baill., and from the Chinese medicine *Fructus Corni* (Shanzhuyu), which is the dried flesh of *Cornus officinalis* Sieb. et Zucc. Studies on the stability of gallic acid in aqueous solutions have been carried out by the classical isothermal accelerated test using RP-HPLC [48]. It was found that gallic acid is unstable in basic and neutral aqueous media and under strong oxidizing conditions, but stable in acidic aqueous media, after strong irradiation and high temperature. Protection by antioxidants could strongly affect the stability of gallic acid.

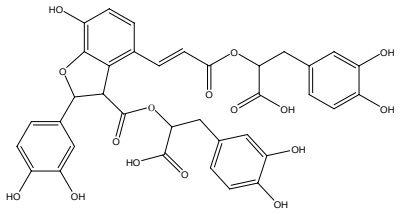


Gallic acid (**6**)

C6-C3 derivatives and their glucosides: C6-C3 derivatives are widely distributed in medicinal plants of TCM, such as *Flos Lonicerae Japonicae* (Jinyinhua) from the family Caprifoliaceae, *Herba Erigerontis* (Dengzhanxixin) and *Herba Taraxaci* (Pugongying) from the family Asteraceae, *Radix et Rhizoma Salviae Miltiorrhizae* (Danshen) from the family Lamiaceae, *Fructus Canarli* (Qingguo) from the family Burseraceae, *Radix Angelicae Sinensis* (Danggui) and *Rhizoma Chuanxiong* (Chuanxiong) from the family Apiaceae [1-5]. In addition, many foods like wine, coffee and tea represent other important sources of these constituents. A variety of biological activities are reported for these constituents such as radical scavenging, anti-inflammatory, antiviral, immunomodulatory, anti-clotting and anti-tumor.

Most of the compounds contain a carboxyl ester bond, which can undergo hydrolysis in alkaline or strong acidic conditions, while they are stable in weak acidic media. Some of them are sensitive to light and heat, probably due to the oxidation of the phenolic hydroxyl groups.

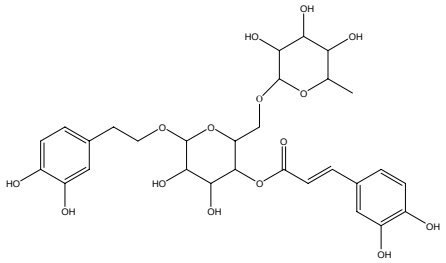
Salvianolic acid B (lithospermic acid B, **7**) is the most representative constituent of this class of constituents, having a good clinical effect on heart, brain, liver and kidney [49].



Salvianolic acid B (lithospermic acid B, **7**)

Chromatography on macroporous resin and semi-preparative HPLC were successfully adopted to isolate salvianolic acid B from *Rhizoma Salviae Miltiorrhizae* (Danshen), which is the dried rhizome and root of *Salvia miltiorrhiza* Bge. The compound, the structure of which consists of four units of caffeic

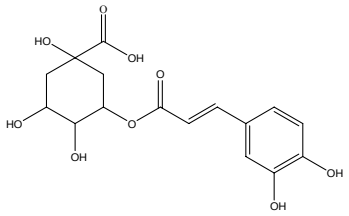
acid, is one of the most important active ingredients of the drug, and many researchers have investigated its stability using various methods [50-56]. Using 1mg/mL aqueous solutions at 20ºC, it has been found that salvianolic acid B is stable under acidic conditions (pH=2), but degraded significantly when the pH value rises to pH> 6. The degradation of salvianolic acid B was related to the pH and temperature of the solutions and followed first order kinetics. Hydrolysis of the ester moiety and ring cleavage of the benzofuranyl unit represented the two main pathways, as found by the degradation products indentified by HPLC-MS and NMR. After hydrolysis, salvianolic acid B can be converted to danshensu and alkannic acid, while protocatechualdehyde represents the product obtained by oxidative degradation. Salvianolic acid E was obtained by opening the ring of benzofurane. Salvianolic acid B had a faster degradation rate in alkaline intestinal juice rather than in simulated acidic gastric juice [50-56].



Forsythoside (**8**)

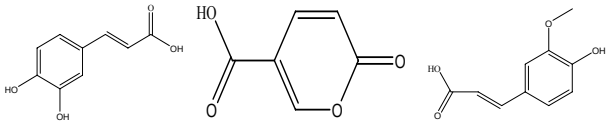
Forsythoside (**8**) is the bioactive constituent of *Fructus Forsythiae* (Lianqiao) from *Forsythia suspensa* (Thunb.) Vahl. It has strong inhibitory effect against various pathogenic bacteria, fungi and viruses [57]. Being a derivative of caffeic acid, forsythoside can be successfully separated with macroporous resins. Both the ester and glucosidic bonds are susceptible to strong acidic and alkaline media, and to high temperature (>80ºC) [58]. If the pH is above 9.40, ester hydrolysis can occur and the degradation products are caffeic acid, D-glucose, and L-isodulcitol. The stability of forsythoside was good at pH=4.03 and lower pHs.

Chlorogenic acid (**9**) is a widespread caffeoylquinic acid ester, well known for many activities, including anti-viral and antihypertensive properties [59]. The stability of chlorogenic acid in the extract of *Flos Lonicerae Japonicae* (Jinyinhua), the dried flower and bud of *Lonicera japonica* Thunb., was investigated using the classical accelerated test. The hydrolysis of chlorogenic acid can be catalyzed by both acidic and basic media [60]. The most stable aqueous solution was found at pH=3.0. After comparison of different organic solvents, ethanol was elected as the best for chlorogenic acid, having a good profile of stability [59].



Chlorogenic acid (**9**)

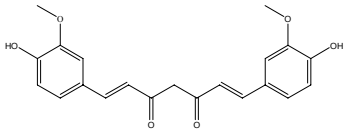
Caffeic acid (**10**) and *p*-coumaric acid (**11**) are two further common active ingredients in a variety of medicinal plants and animals used in TCM. Lu and coworkers studied their stability in a 70% v/v methanol extract of *Spora Lygodii* (Haijinsha), the mature sporophytes of *Lygodium japonicum* (Thunb.) Sw. [61]. Decomposition and structural transformations of these constituents occurred under irradiation, so light was considered a determinant factor concerning their stability. The content of caffeic acid decreased rapidly with increasing temperatures, while minor changes were found for *p*-coumaric acid. Furthermore, in acidic conditions, other phenolic derivatives present in the extract of *Spora Lygodii* could hydrolyze to caffeic acid, which resulted in significant increases in the content of caffeic acid, while the content of *p*-coumaric acid remained unchanged.



Caffeic acid (**10**) *p*-Coumaric acid (**11**) Ferulic acid (**12**)

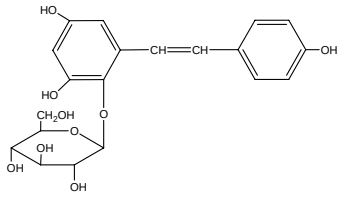
Ferulic acid (**12**) is the active ingredient of a variety of commonly used TCMs, such as *Radix Angelica Sinensis* (Danggui), which is the dried root of *Angelica sinensis* (Oliv.) Diels, and *Rhizoma Chuanxiong* (Chuanxiong), which is the dried rhizome of *Ligusticum chuanxiong* Hort. The compound has several therapeutic effects, including the inhibition of the release of 5-HT from platelets [62]. Ferulic acid is very sensitive to light and decomposes rapidly in 80% v/v ethanol solution after 2 hours decoction. The crude drug of *Rhizoma Chuanxiong* is stable in dry conditions [63-64]. 70% v/v methanol is the best solvent medium for ferulic acid if compared with other organic solvents. In addition, small amounts of glacial acetic acid can significantly stabilize ferulic acid in solution [63-64].

Curcuminoids represent a group of rare dione pigments with a C6-C3 backbone structure, present in *Rhizoma Curcumae Longae* (Jianghuang), the dried rhizome of *Curcuma longa* L. They have anti-inflammatory, antioxidant, anti-lipidemic and anti-cancer activities, widely used in medicine and health food products [65]. Different methodologies have been applied for the isolation of such molecules, including activated charcoal and acid-base extraction. Different studies on the stability of curcumin (**13**) and related molecules, such as demethoxycurcumin and bis-demethoxycurcumin, have been reported under different stress conditions (temperature, pH, metal ions, with different surfactants, oxidizing and reducing agents) [66-68]. Curcumin is photosensitive, and is unstable at high temperature, and in strong acidic or alkaline media. Sucrose, maltose, Zn^{2+}, Fe^{2+} and Fe^{3+} have an hyperchromic effect on its UV-vis absorptions, while discoloration is caused by sodium benzoate, citric acid, tartaric acid and Cu^{2+}. Vitamin C, Na^{+}, K^{+}, and Mg^{2+} have slight effects on curcumin. PEG400 and DMSO could degrade it, and furthermore sodium bisulfite, sodium sulphite, and sodium thiosulfate can significantly accelerate its degradation rate. Glutathione and SDS can stabilise curcumin. Under the same conditions, the stability of curcuminoid derivatives follow the order: bis-demethoxycurcumin>demethoxycurcumin>curcumin. However, bis-demethoxycurcumin and demethoxycurcumin are less active, probably due to their weaker electrophilic reactivity when compared with curcumin, and demethoxycurcumin was found as the natural stabilizer for curcumin [66-68].



Curcumin (**13**)

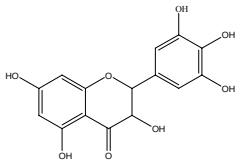
C6-C2-C6 derivatives: Stilbenes are simple phenolic derivatives present in diverse mosses and plants. 2,3,5,4'-Tetrahydroxystilbene-2-*O*-β-D-glucoside (THSG) (**14**) is the active principle of *Radix Polygoni Multiflori* (Heshouwu), which is the dried root of *Polygonum multiflorum* Thunb. [69]. THSG is well known for its anti-aging and hypolipidemic activities and can be isolated pure (99.9%) by liquid-liquid extraction, silica gel column chromatography and repeated crystallizations. THSG in solution (0.1 mg/mL) undergoes rapid degradation in either acidic or strong alkaline conditions, after irradiation and in methanol solutions [70]. Strong acidic conditions result in the hydrolysis of THSG to form glucose and the aglycone, which can further degrade into small molecules. In the presence of strong alkaline media, THSG can be easily oxidized into quinones, whereas in neutral or weak basic conditions it is quite stable.



2,3,5,4'-Tetrahydroxystilbene-2-*O*-β-D-glucoside (THSG) (**14**)

C6-C3-C6 derivatives: Flavonoids have a characteristic C6-C3-C6 backbone structure, and represent an important group of active ingredients of TCM. Flavonoids are commonly known for their antioxidant and radical scavenger activities. However, they are known for many other activities, such as anti-cancer and heart disease [71-72]. The general structure of flavonoids is represented by a 2-phenyl-chromone constituted of three rings (A-C rings) [73].

Dihydromyricetin (**15**) is the characteristic constituent of *Ampelopsis Grossedentata* (Tengcha), the leaf and stem of *Ampelopsis grossedentata* (Hand-Mazz) W.T.Wang, a widely used TCM, traditionally employed to cure dysentery and diarrhea [74].

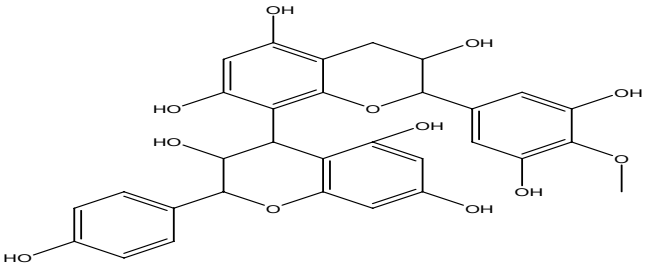


Dihydromyricetin (**15**)

Dihydromyricetin possesses several common activities typical of flavonoids, for example, free radical scavenging, antioxidant, anti-thrombosis, anti-tumor, and anti-inflammatory, and in addition, has excellent liver protecting effects. It can be isolated (99% pure) using high-speed counter current chromatography. HPLC analysis in different aqueous media determined the maximum stability of the compound at pH=4, while oxidative degradation increased with increasing pH values and oxidation was immediate from pH 9 upward.. In addition, strong irradiation and high temperature accelerated the oxidation of dihydromyricetin [75].

Oligomeric proanthocyanidins (**16**) are widely recognized as powerful natural antioxidants, and they are widely spread in various TCMs, for example,

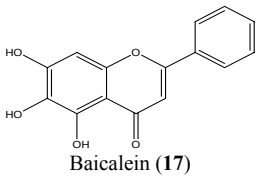
Fructus Crataegi (Shanzha), the dried fruit of *Crataegus pinnatifida* Bge. and *C. pinnatifida* Bge. var. *major* N. E. Br.; *Semen Ginkgo* (Baiguo), the dried seed of *Ginkgo biloba* L.; *Radix Puerariae Lobatae* (Gegen), the dried root of *Pueraria lobata* (Willd.) Ohwi; *Rhizoma Drynariae* (Gusuibu), the dried rhizome of *Drynaria fortune* (Kunze) J. Sm.; *Radix et Rhizoma Glycyrrhizae* (Gancao), the dried root or rhizome of *Glycyrrhiza uralensis* Fisch., *G. inflata* Bat., and *G. glabra* L.; *Herba Epimedii* (Yinyanghuo), the dried aerial parts of *Epimedium brevicornum* Maxim., *E. sagiuarum* (Sieb. et Zucc.) Maxim., *E. pubescens* Maxim., *E. wushanense* T. S.Ying, and *E. koreanum* Nakai [76]. Studies carried out on fractions of oligomeric proanthocyanidins (purity 95.0%) extracted from *Semen Vitis Viniferae* (Putaozi), the seed of *Vitis vinifera* evidenced a stability in acidic conditions, but degradation occurred at increasing pH values up to alkaline conditions [77].



A typical oligomeric proanthocyanidin (**16**) of *Semen Vitis Viniferae*

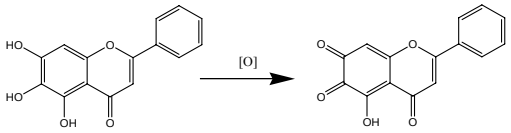
Temperatures above 60°C can also affect the stability of oligomeric proanthocyanidins, while their stability can be increased in the presence of some antioxidants, such as ascorbic acid and Na_2SO_3 [77].

Baicalein (**17**) is another very common flavonoid in TCMs and represents the anti-inflammatory bioactive ingredient of *Radix Scutellariae* (Huangqin), the dried root of *Scutellaria baicalensis* Georgi [78]. Studies with isolated baicalein (purity 97.2%) in different pH media evidenced its stability in acidic conditions, the optimum being pH 4.28 [79-81].



Baicalein (**17**)

In addition, baicalein and flavonoids containing *o*-dihydroxy, *o*-trihydroxy, and a 3,4'-dihydroxy moiety are easily oxidized to colored quinones in alkaline media (Figure 2) [82-83]. Other studies showed that the decomposition of baicalein and its 7-glucuronide



(Yellow) (Green)

Figure 2: Structural changes of baicalein in alkaline media (Ph = 10)

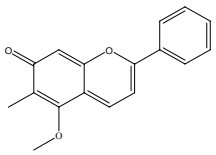
can be accelerated by increasing temperature, especially near to their melting points [84]. Baicalin also has poor stability in the presence of light. The complex between baicalin and zinc showed similar results to those reported for the stress conditions [85].

Similar results have been found by Jing and co-workers for the stability of the total flavonoid fraction of *Fructus Hippophae* (Shaji), the dried fruit of *Hippophae rhamnoides* L. [83]. Different results were found according to reaction time. When the extract was treated with an alkaline solution for less than five hours and then neutralized, the stability did not change significantly when compared with the TFH solution at pH=7, probably due to a reversible reaction of the phenolic moieties to quinone structures. However, an irreversible reaction was observed by UV spectrophotometry when the time of the alkaline treatment was more than 5 hours.

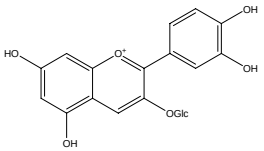
Other studies of the flavonoid fraction of *Cacumen Platycladi* (Cebaiye), the leaf of *Platycladus orientalis* (L.) Franco confirmed the above results. Good stability was found in weakly acidic conditions (pH 5-6) [86-87]. Similar results were also found for flavonoids isolated from *Flos Paulowniae* (Maopaohua) [88-89], whilst the flavonoids of *Herba Tagetis Patulae* (Kongquecao), the whole herb of *Tagetes patula,* were unstable after either irradiation or increased temperature (>60 °C) [90].

Sanguis Draconis (Xuejie), the resin of *Daemonorops draco* Bl., is a valuable Chinese medicine for surgery [91]. Its major bioactive ingredient, dracorhodin (**18**), is structurally related to flavonoids and is unstable if submitted to dry heat [92]. The results of classic thermal accelerated tests indicated that the kinetic constants increased conspicuously with increasing temperatures. A first order kinetic relationship was found by the Arrhenius equation; expiration was set at 2.23 years at 25ºC.

The extract of *Herba Emiliae* (Yidianhong), the stem and leaf of *Emilia sonchifolia* DC., is largely employed for its many pharmacological effects, such as its anti-inflammatory, analgesic and immunological properties [93]. Anthocyanin **19** represents the characteristic constituent of this TCM



Dracorhodin (**18**)



Anthocyanin (**19**)

and can be isolated using microwave extraction. The compound is stable in 80% ethanol solution below 80°C, but very sensitive to acids under boiling water bath conditions, which caused hydrolysis to the aglycone [94]. In addition, the aqueous solution of the anthocyanin is not stable in the presence of sucrose, citric acid, sodium benzoate and potassium sorbate, because of degradation of the chromophore moiety.

Another TCM containing anthocyanins is *Caulis Parthenocissi Tricuspidatae* (Pashanhu), which is the dried stem and root of *Parthenocissus tricuspidata* (S. et Z.) Planch., used for its effects in arthromyodynia and chronic bronchitis [95]. The constituents are very stable in the presence of sodium sulphite, citric acid, and glucose, but unstable in the presence of sodium benzoate and sodium chloride [95].

The anthocyanins from the fresh crude drug *Radix Rehmanniae* (Dihuang), the fresh root of *Rehmannia glutinosa* Libosch., were evaluated for their stability at different pH values and temperatures, under ultraviolet irradiation, and in the presence of metal ions, oxidizing and reducing agents [96]. The complex was stable in mild alkaline conditions, but degraded slightly in the present of ultraviolet light and reducing agents. Heat, oxidants, and several ions, Sn^{3+}, Fe^{2+}, Si^{2+}, Pb^{2+}, Cu^{2+}, also had a great impact on stability, and generated colored sediments [96].

Several studies were carried out to evaluate the stability of anthocyanins of *Gladiolus Gandavensis* (Tangchangpu), the corm of *Gladiotus huhridus* Bort, to sunlight, heat, ultraviolet light (365 nm), acidic and basic solutions, metal ions, reducing agents, oxidizing agents, sugars and preservatives [97]. It was found that these anthocyanins are stable in strong acidic conditions (pH=1) and to heat, while reducing agents, oxidizing compounds, sucrose, and sodium benzoate led to their degradation. In addition, anthocyanins and many other flavonoids can easily form colored complexes with Pb^{2+}, Al^{3+}, Fe^{3+}, Mg^{2+}, and other cations, that oxidized the compounds to quinones, changing the colour of the aqueous

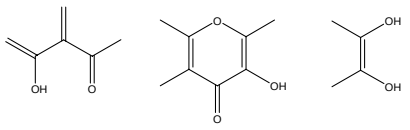
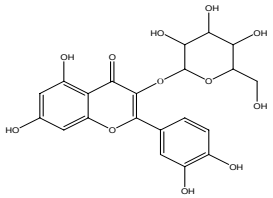


Figure 3: Structural moieties of flavonoids capable of interacting with cations

solutions [97]. The structural units can interact with such cations to give complexes with the OH in the 5 position of ring A, the carbonyl at C-4 of ring C, by the 3-OH and the carbonyl of ring and the catecol moiety of ring B, as shown in Figure 3.

Hyperoside (**20**), the 3-*O*-galactoside of quercetin, represents the main and characteristic constituent of a series of widely used TCMs, including *Herba Hyperici Perforate* (Guanyelianqiao), the whole herb of *Hypericum perforatum* L., *Herba Polygoni Avicularis* (Bianxu), the dried aerial parts of *Polygonum aviculare* L., *Fructus Evodiae* (Wuzhuyu), the dried fruit of *Evodia rutaecarpa* (Juss.) Benth., *Semen Cuscutae* (Tusizi), the dried seed of *Cuscuta chinensis* Lam. *Folium Crataegi* (shanzhaye), the dried leaf of *Crataegus pinnatifida* Bge. var. *major* N. E. Br. and *C. pinnatifida* Bge. These TCMs have numerous therapeutic effects, including decreasing blood lipids, cardiovascular and cerebrovascular protection and immunological properties [98]. Wang and coworkers found that strong acidic conditions (1 M HCl) cause the degradation of hyperoside in the extract of *Herba Hyperici Perforate* [99]. A certain level of degradation of this compound was also detected in the presence of strong oxidants, high temperature and moisture.



Hyperoside (**20**)

Formononetin-7-*O*-β-D-glucoside-6"-*O*-malonate is one of the major flavonoids of *Radix Hedysari* (Hongqi), the dry roots of *Hedysarum polybotrys* Hand.-Mazz., a health food, but also a very famous TCM. The most suitable conditions to optimize the stability of the compound were represented by an acidic aqueous methanol solution at pH=3 [100]. In conclusion, the studies on flavonoids suggested that the optimum conditions for maintaining their stability are represented by low temperature, weak acidity, and protection from light and oxidants.

3. Terpenoids

Terpenoids are widespread constituents in nature, including higher plants, fungi, micro-organisms, insects and marine organisms. Terpenoids represent very important bioactive ingredients of TCM and play an indispensable role in the cosmetic and food industries, due to the aromatic properties of those having smaller molecular weight.

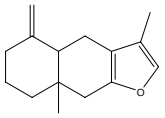
Volatile terpenoids: Volatile terpenoids (mainly mono- and sesquiterpenes) represent the constituents of essential oils, which are generally extracted by steam distillation. Essential oils are very effective TCM ingredients, such as *Herba Menthae* (Bohe), *Herba Ocimi Basilici* (Luole), *Herba Pogostemonis* (Guanghuoxiang) from Lamiaceae; *Fructus Foenicull* (Xiaohuixiang), *Radix Angelicae Sinensis* (Danggui), *Radix Angelicae Dahuricae* (Baizhi), *Rhizoma* Chuanxiong (Chuanxiong) from Apiaceae; *Folium Artemisiae Argyi* (Aiye), *Herba Artemisiae Scopariae* (Yinchen), *Rhizoma Atractylodis* (Cangzhu), *Rhizoma Atractylodis Macrocephalae* (Baizhu) from Asteraceae; *Pericarpium Citri Reticulatae* (Chenpi), *Exocarpium Citri Rubrum* (Juhong), *Semen Citri Reticulatae* (Juhe), *Fructus Aurantii* (Zhiqia), *Fructus Aurantii Immaturus* (Zhishi) from Rutaceae; *Borneolum* (Bingpian), *Cortex Cinnamoni* (Rougui) from Lauraceae; and *Rhizoma Zingiberis Recens* (Shengjiang), *Rhizoma Curcumae Longae* (Jianghuang), and *Rhizoma Curcumae* (Ezhu) from Zingiberaceae.

Various medical applications have been ascribed to the essential oils, such as accelerating blood and lymph circulation, and antihypetensive, antibacterial, antifungal, anti-inflammatory, insecticide, and anti-viral properties.

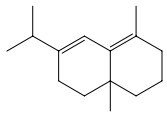
In general, essential oils are unstable towards air and light, and they can resinify, changing in color and viscosity.

The essential oil of *Fructus Evodia* (Wuzhuyu), the dried fruit of *Evodia rutaecarpa* (Juss.) Benth., has good anti-bacterial and analgesic effects [101]. Zhen and coworkers established the relationship between refractive index and degree of oxidation of this essential oil [102]. The degradation follows first order kinetics, as observed by the classic isothermal test. At 293K, the experimental values $t_{0.9}$, $t_{0.7}$, and $t_{0.8}$ were approximately 5 months, 10 months, and 16 months, respectively.

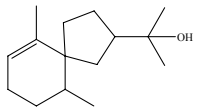
Atractylone (**21**) is the bioactive constituent of *Rhizoma Atractylodis Macrocephalae* (Baizhu), the dried rhizome of *Atractylodes macrocephala* Koidz. and *Rhizoma Atractylodis* (Cangzhu), the dried rhizome of *A. lancea* (Thunb.) DC. and *A. chinensis* (DC.) Koidz. Atractylone represents the marker of several Chinese patented medicines. Pure atractylone (99%) is unstable at room temperature and can be self-oxidized rapidly to butenolide I and butenolide III. Its residual content in the essential oil stored at room temperature is 31% and 15% of the initial value after 0.5 h and 2 h, respectively [103].



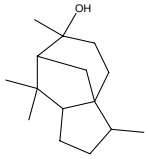
Atractylone (**21**)



β-Selinene (**22**)

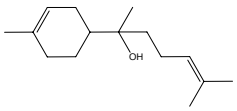


Hinesol (**23**)



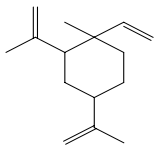
β-Eudesmol (**24**)

Deng and coworkers investigated the instability of the constituents of the essential oil obtained from *Rhizome Atractylodis Macrocephalae*. In the study 17 main constituents were identified by GC/MS. After a long-term storage period (about 6 months), the content of the four main components (total content more than 4%), β-selinene (**22**), hinesol (**23**), β-eudesmol (**24**), α-bisabolol (**25**), and one of the unknown compounds decreased dramatically. Isomerization of some compounds was also observed [104].

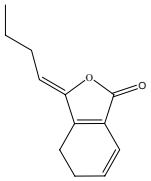


α-Bisabolol (**25**)

β-Elemene (**26**) is a characteristic sesquiterpene of the essential oil extracted from *Rhizoma Curcumae* (Ezhu), the dried rhizome of *Curcuma phaeocaulis* Val., *C. kwangsiensis* S. G. Lee et C. F. Liang and *C. wenyujing* Y. H. Chen et C. Ling [105]. Supercritical carbon dioxide extraction was carried out to obtain elemene mixture, which consists of three isomers, β-, γ- and δ- elemene having similar physico-chemical properties. Sun and coworkers reported that β-elemene is more stable than γ-elemene, which can be converted to β-elemene and other compounds. The stability of each isomer was also related to the different percentages of the three isomers [106].



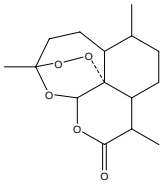
β-Elemene (**26**)



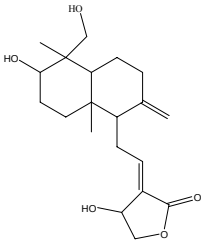
Ligustilide (**27**)

Ligustilide (**27**) is the active ingredient, commonly extracted by steam distillation of many TCMs, including *Rhizoma Chuanxiong* (Chuanxiong), which is the dried rhizome of *Ligusticum chuanxiong* Hort., *Radix Angelicae Sinensis* (Danggui), which is the dried root of *Angelica sinensis* (Oliv.) Diels, and *Rhizoma et Radix Ligustici Sinensis* (Gaoben), the dried rhizome or root of *Ligusticum sinense* Oliv.,and *L. jeholense* Nakai et Kitag,. Ligustilide has spasmolytic and vasodilator activities [107]. In the essential oil of *Rhizoma Chuanxiong*, more than 45 components have been identified by GC-MS analysis, which represent 93.2% of the total constituents [108]. The stability of ligustilide in the volatile oil of *Rhizoma Chuanxiong*, as well as during the extraction process, has been extensively studied. The major constituents, ligustilide (27.4%) and 2-propylene-1-hexanoic acid-3-ene (41.8%), are both unstable during storage (3 months at 37ºC). This was reflected by a decrease of ligustilide and an increase in 2-propylene-1-hexanoic acid-3-ene, this probably being the degradation product of ligustilide. Several isomerization products were also observed [109]. Chloroform and cyclohexane can stabilise ligustilide. Ligustilide (purity>98%) was stable in chloroform at -20°C, if protected from light. Strong acidic/basic solutions can irreversibly modify the constituents during extraction and the best conditions were low-temperatures, dark conditions, and a pH=5 [110].

Iridoids, non volatile sesquiterpenoids and diterpenoids: Iridoids contain a hemi-acetal moiety, which can easily be hydrolyzed and/or oxidized [111]. Iridoids are major constituents of *Herba Hedyotis Diffusae* (Baihuasheshecao), the whole herb of *Hedyotis diffusa* Willd. and *Oldenlandia diffusa* (Willd.) Roxb. Aqueous solutions of iridoids can turn blue in plastic containers and studies have suggested that to preserve the structure of iridoids they need to be stored at temperatures lower than 60°C for not more than 6 hours. Iridoids with carboxyl groups at C-4 were found to be more stable than those having carboxymethyl groups, which can be easily hydrolyzed in acidic media [111].



Artemisinin (**28**)



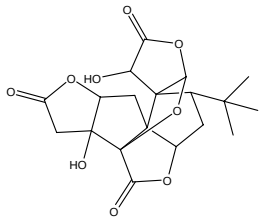
Andrographolide (**29**)

Artemisinin (**28**) is a unique sesquiterpene endoperoxide lactone. It is considered to be the best and most well-known clinically effective drug from a Chinese medicine, *Herba Artemisiae Annuae* (Qinghao), obtained from the dried aerial parts of *Artemisia annua* L. Artemisinin has been successfully used all around the world to cure malaria since the middle of the last century [112]. The highly unusual endoperoxide moiety present in artemisinin, which is essential for the activity, has been focused as the possible degradation target, in addition to the lactone moiety, which is not stable in acidic media. Artemisinin is surprisingly stable in neutral solvents heated to 150°C. Dihydroartemisinin represents the natural metabolite of artemisinin and it is chemically more vulnerable than artemisinin. Artemether, arteether, artesunate and artelinic acid, which are acetal-type prodrugs, are susceptable to moisture and acidic conditions [113].

Several researchers have studied the stability of andrographolide (**29**) and its succinate salt (DAS), respectively [114-116]. Andrographolide is the characteristic constituent of *Herba Andrographis* (Chuanxinlian), the dried aerial parts of *Andrographis paniculata* (Burm.f.) Nees., which can be obtained by supercritical fluid extraction. Both andrographolide and DAS are not stable to either heat or to acidic and alkaline conditions, but are not sensitive to light and oxygen, as reported by HPLC-MS analyses, which led to the characterization of two products of degradation. DAS, in aqueous methanol solution, degraded by 1% at 25°C in one month. The $t_{0.9}$ of andrographolide at 25°C was about 4 months at pH=3, which was found as the most stable condition. Andrographolide was degraded rapidly in protic solvents, but was stable in organic solvents, such as chloroform. In biological media, it was found that the compound was more stable in bovine serum than in mice homogenate [114-116].

Ginkolides are the characteristic and unique trilactone diterpene constituents of *Folium Ginkgo* (Yinxingye), obtained from the leaves of *Ginkgo*

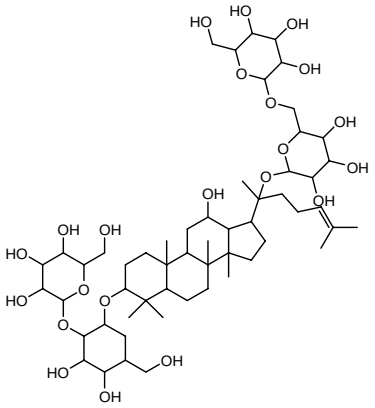
biloba L. Liquid-liquid extraction and supercritical fluid extraction can be adopted successfully to extract these constituents. This herbal drug represent one of the most known and used TCMs, mentioned in the book of Liu Wen-Tai in 1505 AD and has gained wide interest for its biological activities, especially for the treatment of memory related impairments [117].



Ginkgolide B (**30**)

In particular, ginkgolide B (**30**) is the most effective natural inducer of platelet aggregation [117]. Evaluation of stability performed on pure ginkgolide B in an injection solution evidenced that the molecule is tolerant to acidic media, oxidation and light exposure, but can be degraded in alkaline conditions and high temperature [118]. Two degradation products were found in the samples submitted to a long-term stability test (two years) at room temperature; one was recognised as ginkgolide A, and the other an unknown compound with a molecular ion [M-H-H_2O] at m/z 405 in its MS [118].

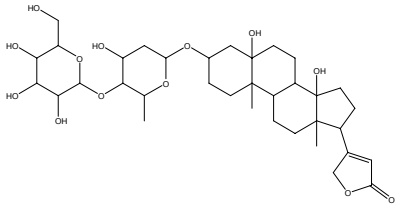
Triterpene and steroidal saponins: Saponins are a large class of secondary constituents found in both vegetable and animal sources. Specifically, they are amphiphilic molecules having a polar part, represented by sugars (one or more moieties), combined with a lipophilic triterpene or steroidal part [119-121]. Steroidal saponins are widely distributed in plants of the Liliaceae and animal sources, while saponins having a triterpenoid skeleton are commonly extracted from plants, for example, species of Araliaceae, Apiaceae, and Fabaceae. Saponins have been shown to have antibacterial, antipyretic, sedative, anti-cancer, cardioactive and corticosteroid effects. Most saponins from TCMs can be separated and isolated with macroporous resins, silica gel and C18 preparative columns. Saponins are stable in mild conditions (pH near neutral and/or temperatures below 50°C), but can be hydrolyzed in strong acidic and alkaline media. Under mild conditions, the stability is quite good and most saponins are stable, such as those of *Herba Gynostemmae Pentaphylli* (Jiaogulan), the whole herb of *Gynostemma pentaphyllum* (Thunb.) Mak.,



Ginsenoside Rb1(**31**)

and momordicosides in *Fructus Momordicae Charantiae* (Kugua), obtained from the fruit of *Momordica charantia* L., and *Radix Panacis Quinquefolii* (Xiyangshen), from the dried root of *Panax quinquefolium* L. [122-126].

In particular, ginsenoside Rb1(**31**), the characteristic and active constituent of *Radix et Rhizoma Ginseng* (Renshen), the dried root or rhizome of *Panax ginseng* C. A. Mry., *Radix Panacis Quinquefolii* (Xiyangshen) and *Radix et Rhizoma Notoginseng* (Sanqi), the dried root or rhizome of *Panax notoginseng* (Burk.) F. H. Chen, isolated by macroporous resins and silica gel chromatography has been extensively studied. Song and coworkers investigated the stability of ginsenoside Rb1 in different pH media, including gastric and intestinal juices [127]. The results showed that ginsenoside Rb1 was relatively stable at intestinal pH, but degraded rapidly at the pH of the stomach.



Periplocin (**32**)

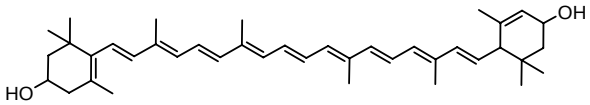
Periplocin (**32**) is a cardiac glycosides form *Cortex Periplocae* (Xiangjiapi), the dried root bark of *Periploca sepium* Bge., a traditional Chinese herb medicine used to treat chronic congestive heart failure [128]. Periplocin (99.5%) was isolated using macroporous resin and silica gel and its stability in mimetic gastrointestinal fluids was investigated by TLC and HPLC-MS analyses [129]. The degradation was pH-dependent, but was not influenced by the presence of pepsin and trypsin. Periplocin hydrolyzed

rapidly to its aglycone, periplogenin, in mimetic gastric fluid in the fasting state, but no decomposition in mimetic gastric fluid, small intestinal fluid and large intestinal fluid in the feeding state was found [129].

Tetraterpenoids (Carotenoids): Carotenoids are characteristic polyisoprenes having conjugated double bonds, well known for their antioxidant proprieties and as pigments. Due to their peculiar structure, carotenoids are very unstable in the presence of light, oxidants or very polar solutions, mainly attributed to photocatalytic oxidation of the chromophore moiety of these molecules [130].

Lutein (**33**) and lutein esters are widely used for their anti-oxidant properties, but also for their therapeutic action on cataract and age-related macular degenerations [131]. Lutein esters can be isolated by supercritical CO_2 fluid extraction from *Flos Calendulae* (Jinzhanju), the dried flower of *Calendula officinalis* L.

Li and coworkers studied the stability of lutein and lutein esters after irradiation, heat treatment, in acidic and alkaline media, and in the presence of metal ions, oxidizing and reducing agents [132]. Generally lutein was less stable than its ester under the same thermal and photostability conditions. The studies showed that irradiation had a great influence on the stability of these molecules and only residual (0.35%) luteolin was present after exposing to sunlight for 0.5 h. Lutein and its esters were stable to heat, even if temperature could accelerate the degradation reaction in the presence of light. It was found that strong acidic media and certain metal ions, like Fe^{3+}, Fe^{2+} and Cu^{2+} could modify these structures. Degradation can also occur, even if slightly, in the presence of H_2O_2; reducing agents (such as Na_2SO_3) have a minor impact [132].



Lutein (**33**)

4. Other constituents of animal origin

Polysaccharides: *Stichopus Japonicus Selenka* (Cishen) is a marine animal medicine used in TCM since the Ming Dynasty for its tonic and replenishing effects. The bioactive ingredient is an acidic mucopolysaccharide (SJAMP) having good anti-tumor, anti-coagulation and immunological enhancing properties [133,134]. The stability of SJAMP at high temperature, humidity and after irradiation was investigated and humidity was ascribed as the main cause of its instability [135].

Polypeptides: Hirudin (Shuizhi) is an antithromboticpeptide composed of 64-66 amino acid residues derived originally from the salivary gland of the medicinal leech, *Hirudo medicinalis* [136]. These acidic, low molecular weight peptides were adopted in Chinese medicine about one thousand years ago to relieve congestion. It was one of the most active specific thrombin inhibitors, with strong anticoagulant, antithrombotic and anti-inflammatory activities. Today, as recombinant hirudin (rHV3), it is used as an inhibitor to prevent phlebothrombosis, and is available on the USA and German markets. Stability of hirudin, based on accelerating and long-term tests was evaluated by its bioactivity. Major determinative factors in its stability were humidity and temperature, while light had a minor effect [136]. Samples under accelerated and long-term experiments maintained their bioactivity.

Ahalysantinfarctasum is a polypetptide consisting of several arginines, characteristic of *Agkistrodon Halys* (Fushe), a TCM widely used for rheumatism. Its chemical stability was assessed by enzyme activity under different temperatures and at different pH values. The inactivation of ahalysantinfarctasum was caused by an acid-base catalytic reaction under first order kinetics. The maximum value of activation energy was found at 55°C and 60°C at pH 6 and 7.6, respectively [137].

5. Conclusions

TCMs have more than 5000 years of history and during these millennia have played and still play an important and indispensable role in treating diseases in the health care for Chinese and East Asian people. This increasing recognition, especially in Europe, is shown by the acceptance of TCM by the European Pharmacopeia and the decision by this institution to organise an ad hoc working group on TCM to develop monographs (more than 70 are expected to be published in the next years). This increasing interest is also reflected by the increasing modernization of TCM and, in particular, the increasing development of stability studies of active ingredients in order to have a rational development of TCMs in view of its worldwide acceptance and use. Stability of active or marker constituents of TCMs from animal or plant sources is reviewed for the first time.. More than 100 papers, mostly of them written

in Chinese, have been reviewed and commented upon. Studies concerning plant constituents were analyzed according to their phytochemical classification of active ingredients. In addition several studies of constituents from animal origin, such as polysaccharides and polypeptides are also reported. Stability of active ingredients as pure drug substances or in the herbal drug or in extracts is summarized during extraction and/or storage of the herbal drug preparations. In addition, the performance of these constituents under stress conditions, including different pHs, storage temperatures, solvents, light and humidity, is reported and, in some cases, the kinetics of degradation are also described. For some active principles used for oral administration, their stability in the presence of simulated gastric or intestinal fluids are reported, while for injectable preparations, the effects of serum proteins, physiological solutions and glucose are described. Finally, the presence of some preservatives, antioxidants, antimicrobials and metals is reviewed, in order to evidence the effects on the drug substances.

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