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**DKTK**   
DRUŠTVO KEMIČARA I  
TEHNOLOGA KOPRIVNICA

# Biljke i biljni pripravci za zdravlje i ljepotu: Znanstveni pogled



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# Biljke kroz život za zdravlje i ljepotu



Kamilica ili  
komorač  
protiv  
grčeva

Čaj od  
šipka za  
umirenje  
želuca

Kozmetički  
proizvodi s  
biljnim  
ekstraktima

Alkoholni  
napici s  
biljem- za  
sve

Soja za  
pomoć kod  
regulacije  
hormona

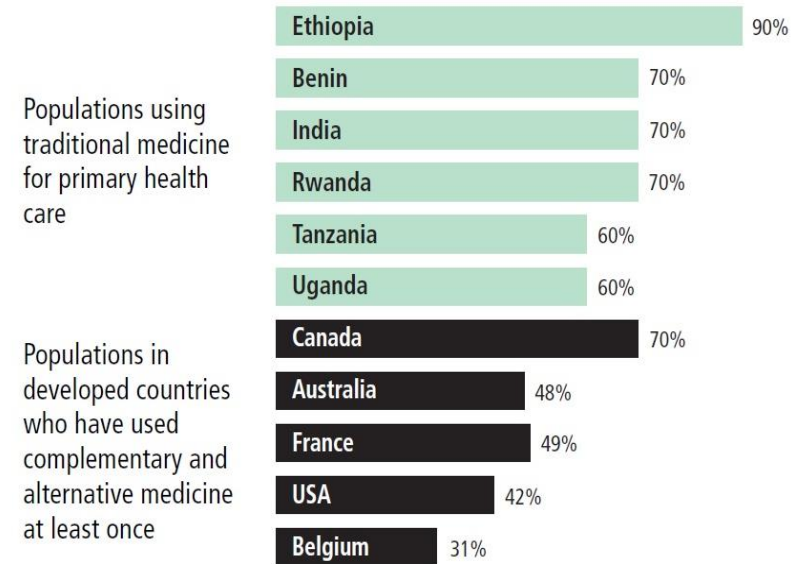
Ginko za  
kognitivne  
funkcije

# Upotreba biljaka u liječenju poznata od davnina

- Najstariji pisani tragovi o upotrebi biljaka u liječenju stari oko 5000 godina
- Upotreba zabilježena u zapisima gotovo svih poznatih kultura širom svijeta



**Figure 1** Many developing country populations use TM to help meet health care needs, while many populations in developed countries have used CAM at least once



Sources: Eisenberg DM et al, 1998; Fisher P & Ward A, 1994; Health Canada, 2001; World Health Organization, 1998; and government reports submitted to WHO.

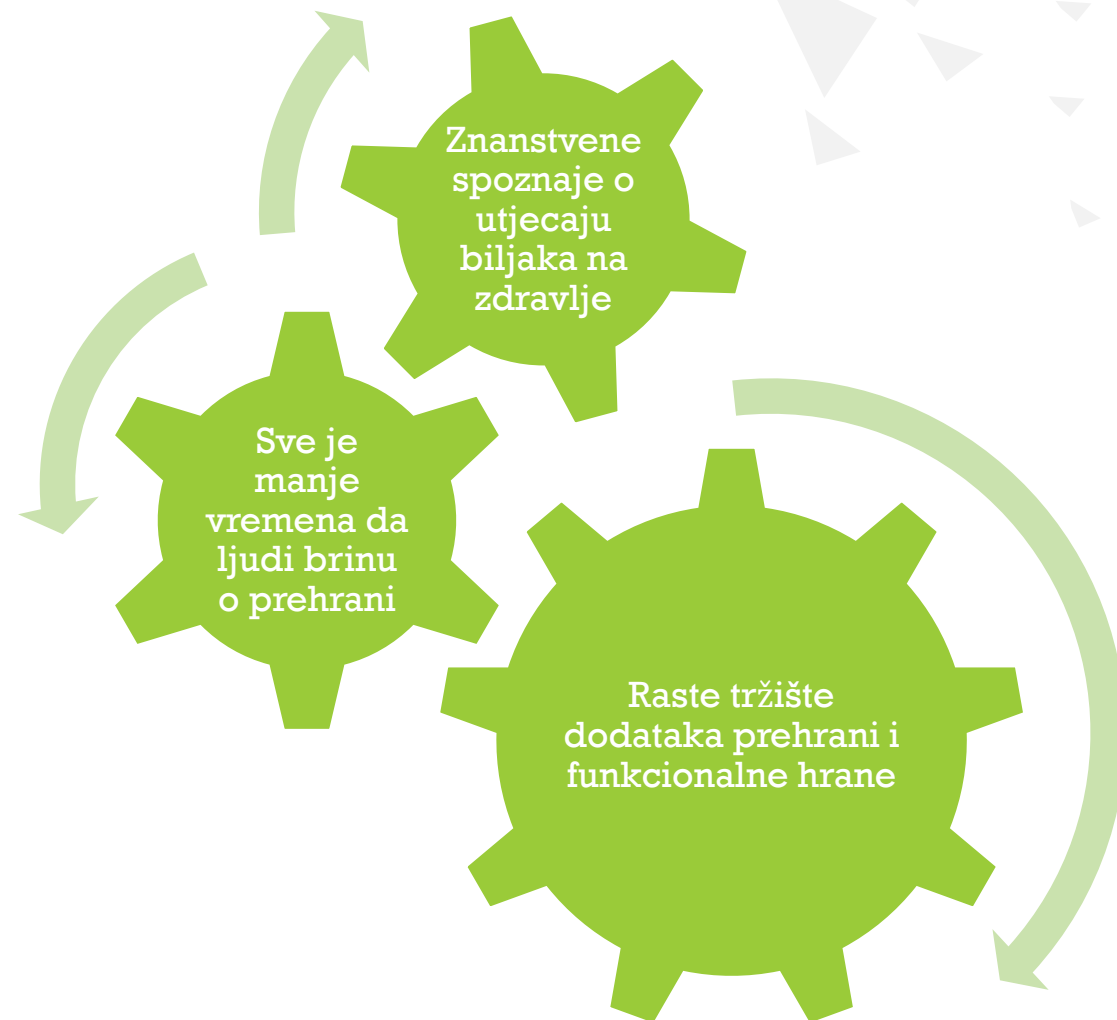


# Suvremena upotreba biljaka

Krajem 19. st. i početkom 20. st. s razvojem organske kemije i sintetičkih lijekova biljke padaju u drugi plan

Krajem 20. st. i početkom novog milenija → trend 'zdravog' načina života → ponovno okretanje prirodi

- Raste tržište dodataka prehrani i kozmetičkih proizvoda baziranih na biljkama
- Raste tržište funkcionalne hrane
- 'Superhrana'
- 'Super biljke'

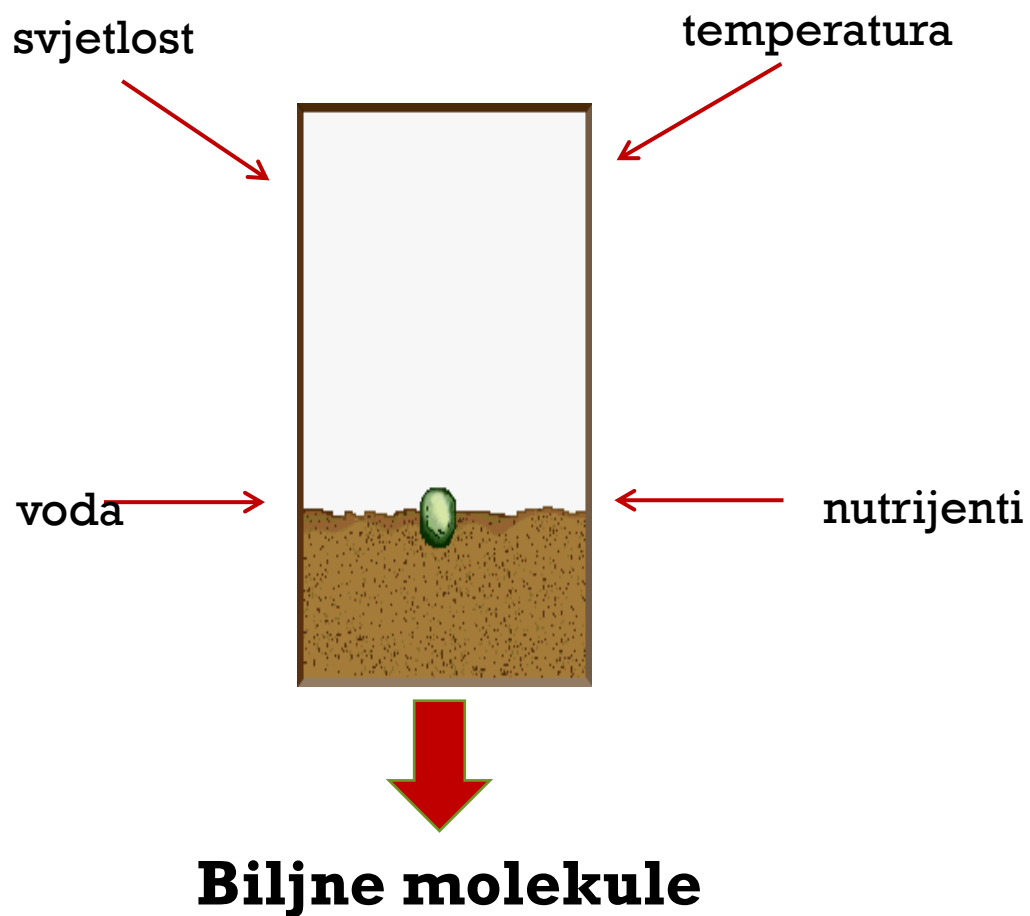


# Gdje je tu znanost?



- Od ukupnog broja biljaka (300 000) većina znanstvenika se bavi modelnim biljkama (manje od 100)
- Fitokemikalije se određene u oko 15-20% biljaka
- Biosintetski putovi i biološka uloga fitokemikalija najčešće nepoznata

# Fitokemikalije (biljne molekule)



Bioaktivne molekule- pokazuju biološku aktivnost- štite biljku, prenose signale, privlače životinje za oprašivanje i sl.

Zbog svoje specifične kemijske strukture imaju:

Antioksidativni učinak-> štite od slobodnih radikala

Antibakterijski učinak -> uništavaju bakterije

Antiviralni učinak -> uništavaju viruse

Odgovorni za upotrebu biljaka, od pradavnih vremena, u liječenju, ublažavanju bolesti, u kozmetici, održavanje higijene i sl.



#### Karotenoidi

- U crvenom, žutom, narandastu voću, povrću, cvijeću...
- Antioksidativna aktivnost, preveniraju nastanak karcinoma, oboljenja krvožilnog sustava te pomažu imunosustavu



#### Fitosteroli

- U sjemenkama suncokreta, sezamu, koštunjičavom voću, soji...
- Zaštita od karcinoma debelog crijeva, smanjuju kolesterol → slične kolesterolu te sprečavaju apsorpciju kolesterola



#### Saponini

- U leguminozama, špinatu
- Pomažu imunosustavu, smanjuju razinu kolesterola te štite od nastanka krvožilnih oboljenja



#### Glukozinolati

- U biljkama iz porodice krstašica- zelje, cvijetaća, brokula...
- Pomažu kod upalnih oboljenja te smanjuju rizik od nastanka nekih vrsta hormonalno uvjetovanih karcinoma



#### Polifenoli

- U različitom voću i povrću -- > posebno obojenom
- Antioksidativna, antibakterijska, antiviralna svojstva, štite od nastanka karcinoma, krvožilnih oboljenja i sl.



### Alkaloidi

Nađeni u velikom broju biljaka, oko 10-25% biljaka ih sadrži  
Mnogi lijekovi su alkaloidi ali i većina droga- morfin, THC...



### Terpeni

- U raznovrsnom bilju, ulaze u sastav eteričnih ulja
- Neki imaju antitumorska svojstva
- Upotreba u aromaterapiji



### Fitoestrogeni

- Žitu, leguminozama te proizvodima od žita
- Slični spolnim hormonima te imaju ulogu u zaštiti od tumora uzrokovanih hormonima- karcinom dojke, maternice, prostate ali također pozitivno utječu na kardiovaskularne bolesti, bolesti mozga, osteoporozi...



### Sulfidi

Komponente koje sadrže sumpor, prisutne u biljkama iz porodici ljiljana- luk, poriluk, šparoge, češnjak...

Antibakterijska, antioksidativna svojstva, smanjuju kolesterol, štite od nekih vrsta karcinoma...



### Kumarini

- Nađeni u različitim biljkama
- Neki mogu imati ulogu u zaštiti od virusa HIV-a, antitumorska svojstva, antiseptička, za tretman astme...



REVIEW ARTICLE

## Rutin as Neuroprotective Agent: From Bench to Bedside

Barbara Budzynska<sup>1</sup>, Caterina Faggio<sup>2</sup>, Marta Kruk-Slomka<sup>1</sup>, Dunja Šamec<sup>3</sup>, Seyed Fazel Nabavi<sup>4</sup>, Antoni Sureda<sup>5,6</sup>, Kasi Pandima Devi<sup>7</sup> and Seyed Mohammad Nabavi<sup>4,\*</sup>

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**Abstract:** Flavonoids are major dietary constituents of plant-based food found ubiquitously in plant kingdom where they are usually present in substantial amounts. Rutin is a flavonol-type polyphenol which consists of the flavonol quercetin and the disaccharide rutinoside. Rutin has been reported to exert diverse biological effects such as antitumor and antimicrobial mainly associated to its antioxidant and anti-inflammatory activities. Mental, neurological, and behavioural disorders are an important and growing cause of morbidity. Most of these disorders combine a high prevalence, early onset, progressive clinical course, and impairment of critical brain functions making them a major contributor to the global disease burden. In the present work, the biological *in vitro* and *in vivo* effects and the potential therapeutic applications of rutin in neurodegenerative processes are reviewed, as well as their bioavailability and pharmacokinetics, which are essential for a better understanding of its biological effectiveness. Moreover, the present review also provides an overview of the molecular mechanisms through which rutin proposed to exert its neuroprotective effects.

**Keywords:** Alzheimer's disease, Antioxidant, Flavonoid, Neurodegenerative diseases, Polyphenols, Rutin.



Review

## Neuroprotective Potential of Biflavone Ginkgetin: A Review

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**Abstract:** Neurological disorders are becoming more common, and there is an intense search for molecules that can help treat them. Several natural components, especially those from the flavonoid group, have shown promising results. Ginkgetin is the first known biflavonoid, a flavonoid dimer isolated from ginkgo (*Ginkgo biloba* L.). Later, its occurrence was discovered in more than 20 different plant species, most of which are known for their use in traditional medicine. Herein we have summarized the data on the neuroprotective potential of ginkgetin. There is evidence of protection against neuronal damage caused by ischemic strokes, neurotumors, Alzheimer's disease (AD), and Parkinson's disease (PD). Beneficial effects in ischemic strokes have been demonstrated in animal studies in which injection of ginkgetin before or after onset of the stroke showed protection from neuronal damage. AD protection has been the most studied to date. Possible mechanisms include inhibition of reactive oxygen species, inhibition of  $\beta$ -secretase, inhibition of A $\beta$  fibril formation, amelioration of inflammation, and antimicrobial activity. Ginkgetin has also shown positive effects on the relief of PD symptoms in animal studies. Most of the available data are from *in vitro* or *in vivo* animal studies, where ginkgetin showed promising results, and further clinical studies should be conducted.

**Keywords:** Alzheimer's disease; biflavonoids; ginkgetin; neuroprotection; ginkgo



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Invited review

## Apigenin as neuroprotective agent: Of mice and men

Seyed Fazel Nabavi<sup>a</sup>, Haroon Khan<sup>b,\*</sup>, Grazia D'onofrio<sup>c</sup>, Dunja Šamec<sup>d</sup>, Samira Shiroomi<sup>e</sup>, Ahmad Reza Dehpour<sup>e,f</sup>, Sandro Argüelles<sup>g</sup>, Solomon Habtemariam<sup>h</sup>, Eduardo Sobarzo-Sanchez<sup>i,j</sup>

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### ABSTRACT

Neurodegenerative disorders (NDDs) such as Alzheimer's and Parkinson's diseases are the most common age-related pathologies that affect millions of people all over the world. To date, effective therapy for NDDs is not available and current approaches to disease management include neuroprotection strategy with a hope of maintaining and enhancing the function of surviving neurons. Of course, such an approach by its own will not offer a cure but is likely to delay the disease progression by ameliorating the increase of neurotoxic agents such reactive oxygen species (ROS) as well as the associated inflammatory cascades. In this regard, natural products including flavonoids that offer neuroprotection through multiple mechanisms have gained a lot of interest in recent years. In this communication, evidences from the various experimental models and clinical trials on the therapeutic potential of one promising flavonoid, apigenin, is presented. Its chemistry, mechanism of action and potential benefits in the various examples of NDDs are discussed in the light of drug discovery aspects.

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Review

## Flavonoids Target Human Herpesviruses That Infect the Nervous System: Mechanisms of Action and Therapeutic Insights

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# Ginko (*Ginkgo biloba* L.)

- U tradicionalnoj kineskoj medicini lišće i plodovi koriste se već nekoliko stoljeća s dokumentiranom upotrebom koja datira od 1280. do 1368. godine.
- Više od 500 godina ajemenci se pripisuju različita ljekovita svojstva za tretiranje astme, kašalja i infekcije mjehura, dok se lišće uglavnom koristi za liječenje kožnih infekcija i plućne disfunkcije.
- Najpoznatija svojstva ginkga su njegova blagotvorna djelovanja na **kognitivne tegobe** → Standardizirani ekstrakt formula- EGb761 koristi se diljem svijeta (24% flavonoida, 6% terpenskih trilaktona (TTL) manje od 5 ppm ginkgolične kiseline).
- No zna li se točno koja je aktivna komponenta?



# Ginko (*Ginkgo biloba* L.)

Ekstrakt koji se ože koristiti za pripravu dodataka prehrani po standardu sadrži : **24% flavonoida**, **6% terpenskih trilaktona (TTL)** manje od 5 ppm ginkgolične kiseline

**FLAVONOIDI**- velika skupina biljnih metabolita- uključuje oko 8000 različitih spojeva s različitim kemijskim strukturama- **različito biološko djelovanje**

U ginku prema literaturi je prisutno **110 različitih flavonoida** čiji sadržaj ovisi o dijelu biljke, starosti lišća, starosti samog stabla i sl.

Jedna od skupina flavonoida u ginku – **biflavonoidi**- dimerne strukture flavonoida

## ISTRAŽIVANJA NA SVEUČILIŠTU SJEVER:


Projekt financiran sredstvima Hrvatske zaklade za znanost: „Uloga biflavonoida u biljkama: *Ginkgo biloba* L. kao modelnu sustav” HRZZ-UIP-2019-04-1018 (voditelj D. Šamec)





# Biflavonoidi kao ključni faktori u zdravstvenim benefitima ginka?



 Highly Cited Paper



Review

## Biflavonoids: Important Contributions to the Health Benefits of Ginkgo (*Ginkgo biloba* L.)

Dunja Šamec <sup>1,\*</sup>, Erna Karalija <sup>2</sup>, Sabina Dahija <sup>2</sup> and Sherif T. S. Hassan <sup>3</sup>

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**Abstract:** Ginkgo (*Ginkgo biloba* L.) is one of the most distinctive plants, characterized by excellent resistance to various environmental conditions. It is used as an ornamental plant and is recognized as a medicinal plant in both traditional and Western medicine. Its bioactive potential is associated with the presence of flavonoids and terpene trilactones, but many other compounds may also have synergistic effects. Flavonoid dimers—biflavonoids—are important constituents of ginkgophytochemicals. Currently, the presence of 13 biflavonoids has been reported in ginkgo, of which amentoflavone, bilobetin, sciadopitysin, ginkgetin and isoginkgetin are the most common. Their role in plants remains unknown, but their bioactivity and potential role in the management of human health are better investigated. In this review, we have provided an overview of the chemistry, diversity and biological factors that influence the presence of biflavonoids in ginkgo, as well as their bioactive and health-related properties. We have focused on their antioxidant, anticancer, antiviral, antibacterial, antifungal and anti-inflammatory activities as well as their potential role in the treatment of cardiovascular, metabolic and neurodegenerative diseases. We also highlighted their potential toxicity and pointed out further research directions.

**Keywords:** *Ginkgo biloba* L.; biflavonoids; ginkgetin; isoginkgetin; bilobetin; sciadopitysin; amentoflavone; bioactive compounds



Citation: Šamec, D.; Karalija, E.; Dahija, S.; Hassan, S.T.S. Biflavonoids: Important Contributions to the Health Benefits



Review

## Neuroprotective Potential of Biflavone Ginkgetin: A Review

İ. İrem Tatlı Çankaya <sup>1</sup>, Hari Prasad Devkota <sup>2</sup>, Gokhan Zengin <sup>3</sup> and Dunja Šamec <sup>4,\*</sup>

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**Abstract:** Neurological disorders are becoming more common, and there is an intense search for molecules that can help treat them. Several natural components, especially those from the flavonoid group, have shown promising results. Ginkgetin is the first known biflavonoid, a flavonoid dimer isolated from ginkgo (*Ginkgo biloba* L.). Later, its occurrence was discovered in more than 20 different plant species, most of which are known for their use in traditional medicine. Herein we have summarized the data on the neuroprotective potential of ginkgetin. There is evidence of protection against neuronal damage caused by ischemic strokes, neurotumors, Alzheimer's disease (AD), and Parkinson's disease (PD). Beneficial effects in ischemic strokes have been demonstrated in animal studies in which injection of ginkgetin before or after onset of the stroke showed protection from neuronal damage. AD protection has been the most studied to date. Possible mechanisms include inhibition of reactive oxygen species, inhibition of  $\beta$ -secretase, inhibition of A $\beta$  fibril formation, amelioration of inflammation, and antimicrobial activity. Ginkgetin has also shown positive effects on the relief of PD symptoms in animal studies. Most of the available data are from in vitro or in vivo animal studies, where ginkgetin showed promising results, and further clinical studies should be conducted.

**Keywords:** Alzheimer's disease; biflavonoids; ginkgetin; neuroprotection; ginkgo





# Biološka aktivnost biflavonoida



antioxidants



## Article

# A Comparative Analysis of Radical Scavenging, Antifungal and Enzyme Inhibition Activity of 3'-8''-Biflavones and Their Monomeric Subunits

Iva Jurčević Šangut <sup>1</sup>, Bojan Šarkanj <sup>1</sup>, Erna Karalija <sup>2</sup> and Dunja Šamec <sup>1,\*</sup>

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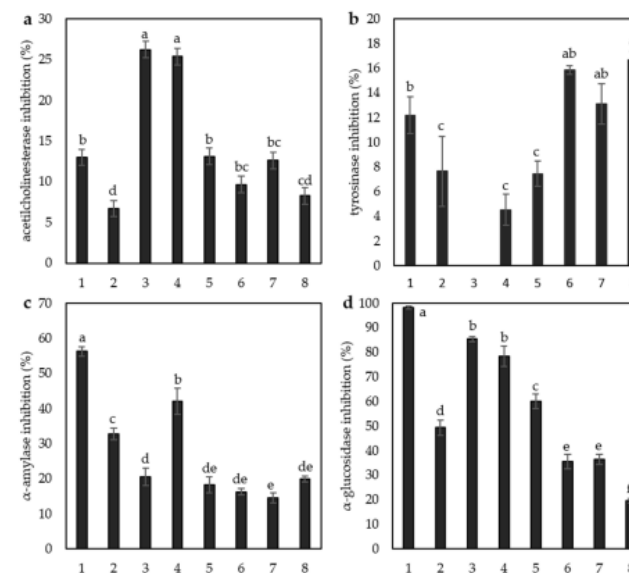
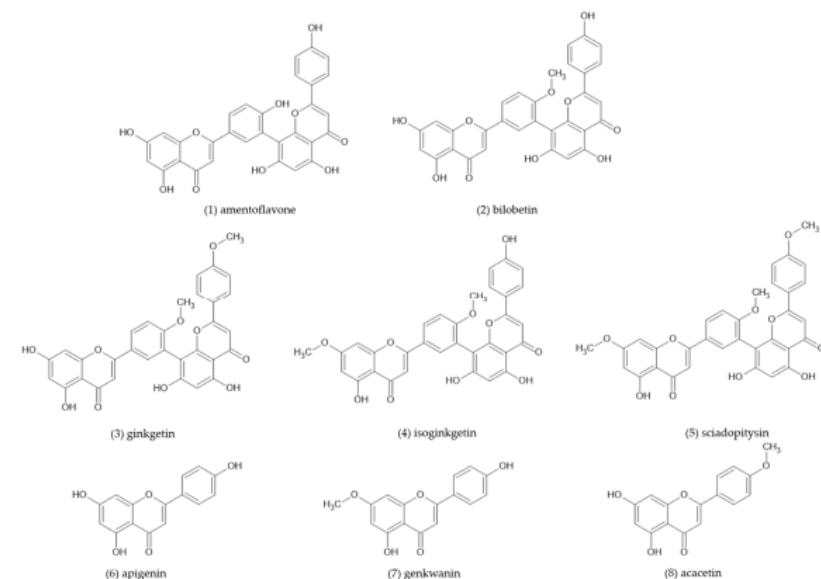
**Abstract:** Biflavonoids are dimeric forms of flavonoids that have recently gained importance as an effective new scaffold for drug discovery. In particular, 3'-8''-biflavones exhibit antiviral and antimicrobial activity and are promising molecules for the treatment of neurodegenerative and metabolic diseases as well as cancer therapies. In the present study, we directly compared 3'-8''-biflavones (amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin) and their monomeric subunits (apigenin, genkwanin, and acacetin) and evaluated their radical scavenging activity (with DPPH), antifungal activity against mycotoxigenic fungi (*Alternaria alternata*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Fusarium graminearum*, and *Fusarium verticillioides*), and inhibitory activity on enzymes (acetylcholinesterase, tyrosinase,  $\alpha$ -amylase, and  $\alpha$ -glucosidase). All the tested compounds showed weak radical scavenging activity, while antifungal activity strongly depended on the tested concentration and fungal species. Biflavonoids, especially ginkgetin and isoginkgetin, proved to be potent acetylcholinesterase inhibitors, whereas monomeric flavonoids showed higher tyrosinase inhibitory activity than the tested 3'-8''-biflavones. Amentoflavone proved to be a potent  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitor, and in general, 3'-8''-biflavones showed a stronger inhibitory potential on these enzymes than their monomeric subunits. Thus, we can conclude that 3'-8''-dimerization enhanced acetylcholinesterase,  $\alpha$ -amylase, and  $\alpha$ -glucosidase activities, but the activity also depends on the number of hydroxyl and methoxy groups in the structure of the compound.

**Keywords:** 3'-8''-biflavones; amentoflavone; bilobetin; ginkgetin; isoginkgetin; sciadopitysin; apigenin; genkwanin; acacetin



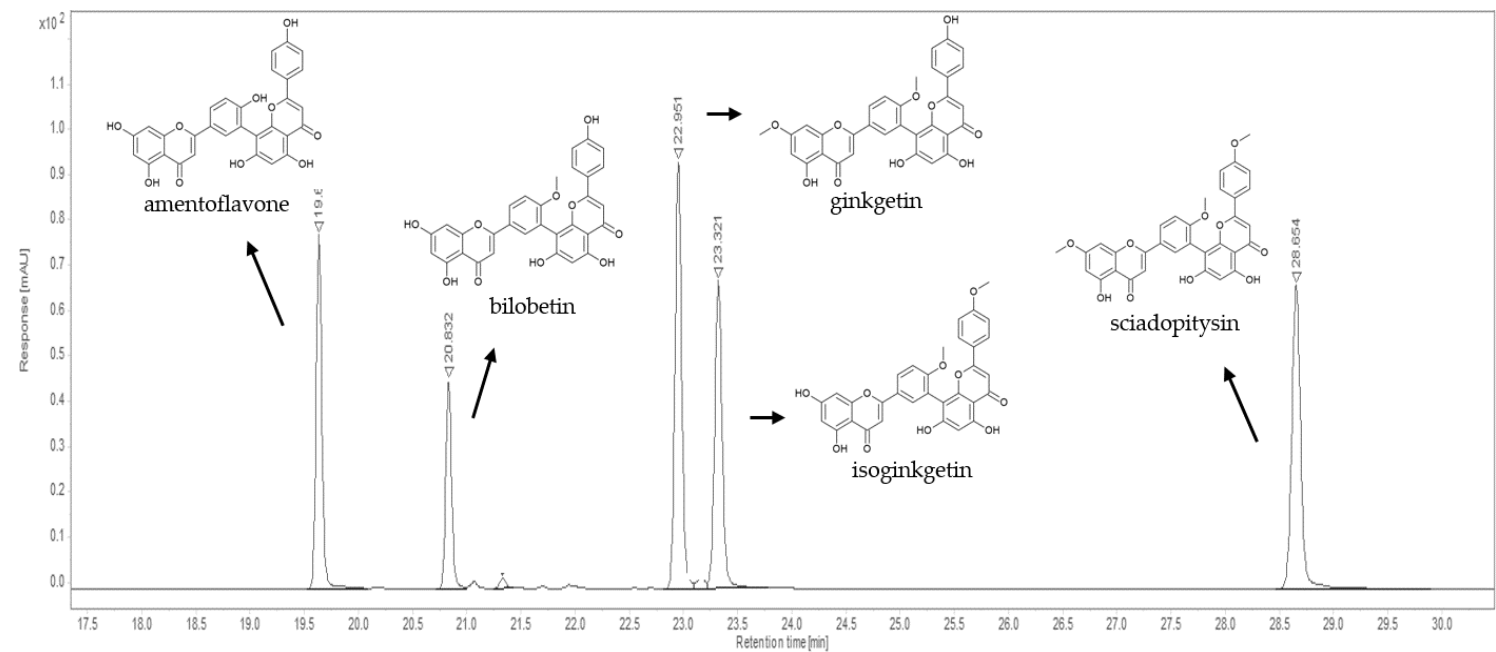
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Citation: Jurčević Šangut, I.; Šarkanj, B.; Karalija, E.; Šamec, D. A Comparative Analysis of Radical Scavenging, Antifungal and Enzyme Inhibition Activity of 3'-8''-Biflavones and Their Monomeric Subunits.

Antioxidants 2023, 12, 1854. https://



**Figure 4.** Enzyme inhibition activity of (1) amentoflavone, (2) bilobetin, (3) ginkgetin, (4) isoginkgetin, (5) sciadopitysin, (6) apigenin, (7) genkwanin, and (8) acacetin against (a) acetylcholinesterase, (b) tyrosinase, (c)  $\alpha$ -amylase, and (d)  $\alpha$ -glucosidase at a concentration of 100  $\mu$ M. Values with different letters differ significantly at  $p < 0.05$ .

# Razvijena HPLC-DAD metoda za profiliranje biflavonoids



## Communication Tissue-Specific Profiling of Biflavonoids in Ginkgo (*Ginkgo biloba* L.)

Marija Kovač Tomas , Iva Jurčević and Dunja Šamec

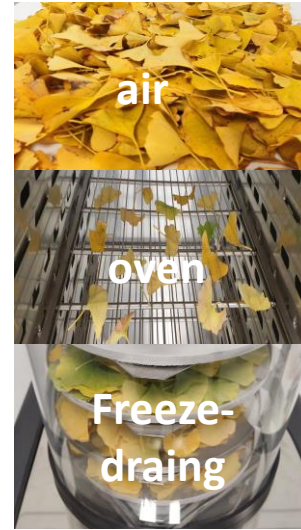
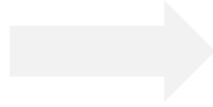
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**Abstract:** Biflavonoids are flavonoid dimers that are much less studied than monomeric flavonoids. Their precise distribution among plants and their role in plants is still unknown. Here, we have developed a HPLC-DAD method that allows us to separate and simultaneously determine the five major biflavonoids (amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin) in ginkgo (*Ginkgo biloba* L.). We performed tissue-specific profiling of biflavonoids in ten different plant parts: tree bark, twigs bark, twigs without bark, buds, leaf petioles, leaf blades, seed stalks, sarcotesta, nutshells, and kernels. We did not detect biflavonoids in plant parts not in direct contact with the environment (twigs without bark, nutshells, and kernels). We found the highest total biflavonoids content in leaves, where sciadopitysin was predominant. In contrast, in the bark, amentoflavone was the predominant biflavonoid, suggesting that more methylated biflavonoids accumulate in leaves and seeds. This is probably related to their biological function, which remains to be determined.

**Keywords:** amentoflavone; bilobetin; ginkgetin; isoginkgetin; sciadopitysin; ginkgo; tissue-specific profiling; HPLC-DAD

	amentoflavone	bilobetin	ginkgetin	isoginkgetin	sciadopitysin
retention time (min)	19.634	20.832	22.951	23.321	28.654
characteristic UV spectrum					
maximum UV apsorption (nm)	196, 226, 268, 336	196, 230, 270, 334	196, 228, 270, 332	196, 228, 270, 332	194, 232, 270, 330

# Optimizacija ekstrakcije – utjecaj načina sušenja



Article

## Influence of Air Drying, Freeze Drying and Oven Drying on the Biflavone Content in Yellow Ginkgo (*Ginkgo biloba* L.) Leaves

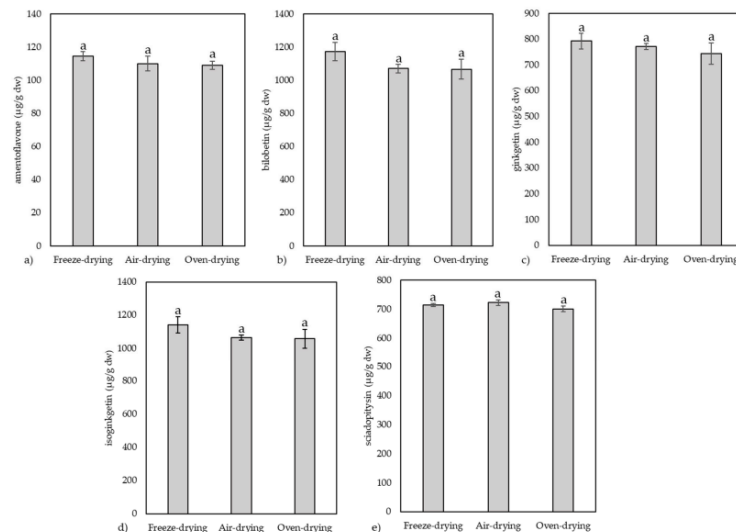
Iva Jurčević Šangut , Lana Pavličević and Dunja Šamec \*

Department of Food Technology, University North, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia; ijurcevic@unin.hr (I.J.Š.)

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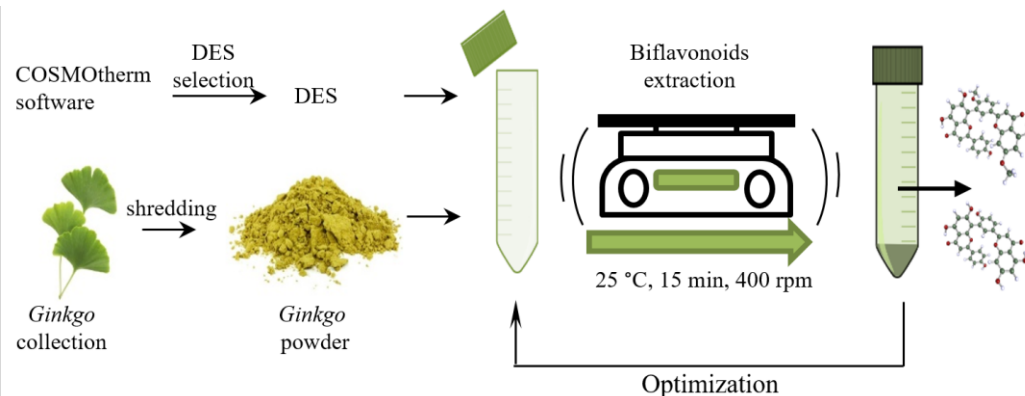
**Abstract:** Drying herbs is a crucial method for stabilizing and preserving their essential properties and bioactive compounds. Although freeze drying is the preferred method for most herbs, it is expensive due to high energy consumption and operating costs. Biflavonoids are dimeric flavonoids that have recently been recognized as potential molecules possessing biological activities, such as antiviral and antimicrobial activity, and as effective molecules for the treatment of neurodegenerative and metabolic diseases and for cancer therapies. In this study, we performed a comparative analysis of freeze drying, air drying and oven drying to evaluate their effects on biflavonoid content in yellow ginkgo leaves (*Ginkgo biloba* L.). After drying, we performed spectrophotometric analysis to determine the browning index, pigments, phenolic compounds and antioxidant activity, while HPLC-DAD was used for the identification and quantification of individual biflavones (amentoflavone, bilobetin, ginkgetin, isoginkgetin and sciadopitysin). The most abundant biflavonoids were isoginkgetin and bilobetin, the amounts of which exceeded 1000 µg/g dw in all leaf samples. They were followed by ginkgetin and sciadopitysin, the amounts of which were about 30% lower. The drying method did not influence biflavone content or the total carotenoids, total polyphenols and total flavonoids. Consequently, our study suggests that all three methods may be used for the preparation of yellow ginkgo leaves as a source of biflavones and other bioactive compounds.

**Keywords:** air drying; biflavonoids; freeze drying; ginkgo; oven drying

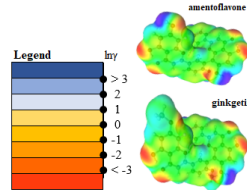




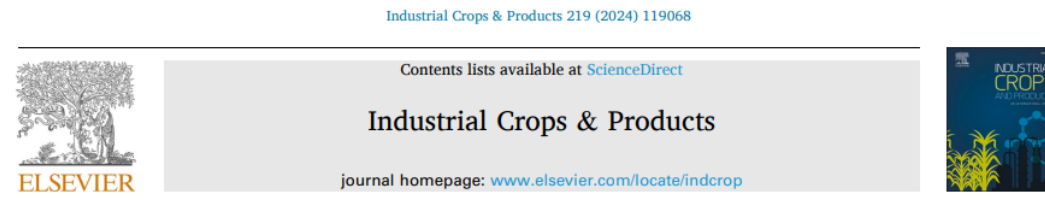
# Optimizacija ekstrakcije- upotreba zelenih otapala za ekstrakciju



	water fraction % (w/w)						water fraction % (w/w)						water fraction % (w/w)				
	10	20	30	50	10		10	20	30	50	10		10	20	30	50	10
<b>Hydrophilic DES</b>																	
1 B:CA						31	ChClU:Gly					61	Me:Cam				
2 B:Glc						32	ChCl:Xyl					62	Me:Cam				
3 B:Gly						33	ChCl:Xyl					63	Me:EU				
4 B:Ox:A:Gly						34	CA:Fru					64	Me:SA				
5 B:MA						35	CA:Fru:Gly					65	Me:C8				
6 B:MA:Glc						36	CA:Glc					66	Me:C10				
7 B:MA:Pro						37	CA:Glc:Gly					67	Me:C18.2				
8 B:EG						38	CA:Suc					68	Me:Ty				
9 B:Arg						39	CA:Suc					69	Ty:C8				
10 B:His						40	Fru:EG					70	Ty:C10				
11 B:Lys						41	Glc:EG					71	Ty:CoU				
12 B:Xyl						42	Glc:Fru										
13 B:Suc						43	Glc:Fru:EG										
14 ChCl:CA						44	Glc:Fru:U										
15 ChCl:CA						45	Glc:Gly										
16 ChCl:EG						46	Gly:Sol										
17 ChCl:Fru						47	MA:Fru										
18 ChCl:Glc						48	MA:Fru:Gly										
19 ChCl:Glc						49	MA:Glc										
20 ChCl:Gly						50	MA:Glc:Gly										
21 ChCl:MA						51	MA:Suc										
22 ChCl:Mal						52	MA:Suc										
23 ChCl:Ox:A						53	Pro:Glc:Gly										
24 ChCl:Pro:MA						54	Pro:MA										
25 ChCl:Sol						55	Suc:EG										
26 ChCl:Sol						56	Suc:Glc:Fru										
27 ChCl:Suc						57	Suc:Glc:U										
28 ChCl:Suc						58	Sor:EG										
29 ChCl:U						59	So:EG										
30 ChCl:U:EG						60	Xyl:EG										



Using COSMOtherm software, a total of 250 DESs were tested and 15 tested and selected



## Extraction of polyphenolic compounds from ginkgo leaves using deep eutectic solvents: A potential solution for the sustainable and environmentally friendly isolation of biflavonoids

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### ARTICLE INFO

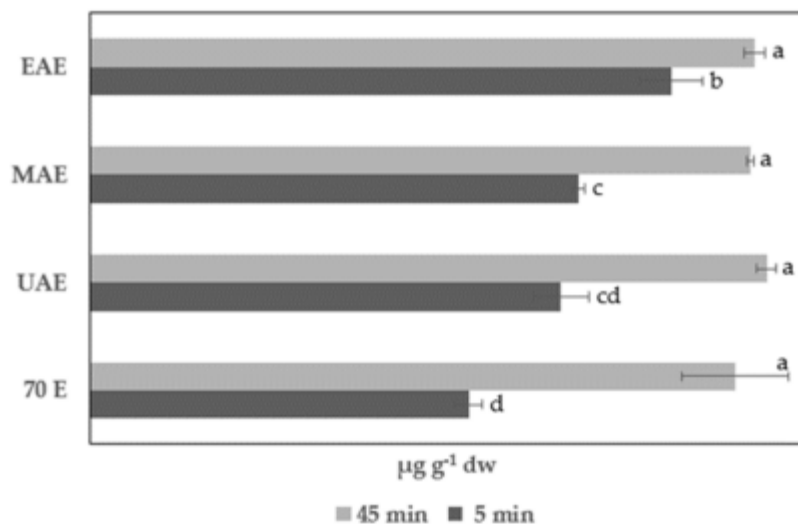
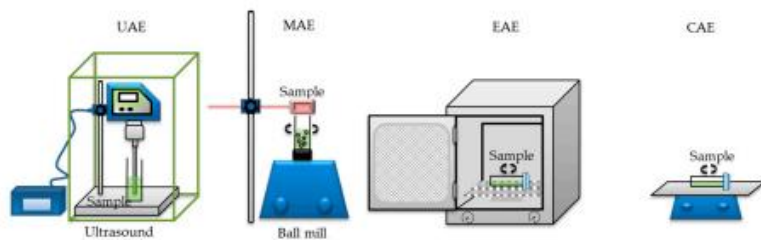
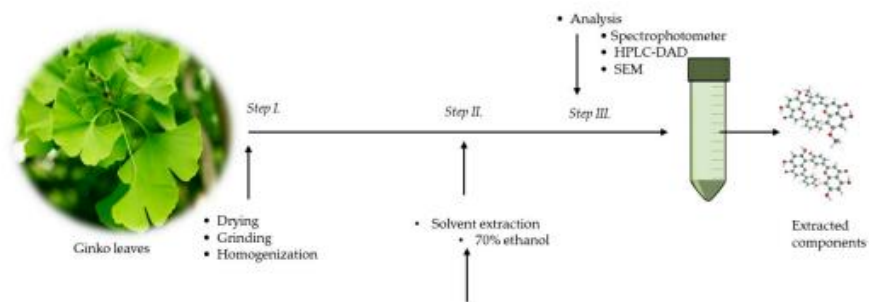
**Keywords:**  
Biflavonoids  
Ginkgo  
DES  
COSMOtherm  
Extraction

### ABSTRACT

Ginkgo (*Ginkgo biloba* L.) is one of the oldest trees on earth and its leaves are traditionally used as herbal medicine. The beneficial effects of ginkgo leaves are attributed to the presence of flavonoids and terpenic trilactones. Monomeric flavonoids are frequently studied, but recently much attention has been paid to biflavonoids, dimeric flavonoids, due to their biological activity and potential pharmaceutical use. The development of a suitable and effective extraction protocol focusing on biflavonoids is increasingly in demand. Therefore, in this study, we developed an optimized extraction protocol using deep eutectic solvents (DESs) to extract flavonoids from ginkgo leaves, with special attention to biflavonoids. Initially, 250 DESs were screened using COSMOtherm software to identify those that could strongly solubilize biflavonoids and flavonoids, and the results obtained indicated 15 DESs. Among them, betaine: ethylene glycol (B:EG) 1:2 with 30 % H<sub>2</sub>O (w/w) showed higher extraction efficiency than the extraction performed with 80 % methanol. In a next step, the extraction with the selected DES was further optimized using a Box-Behnken experimental design. The results obtained showed that the optimum extraction conditions using the selected DES were as follows: Extraction time 45 minutes, the ratio of the initial mass of the shredded leaves to the volume of the DES 20:1 and the temperature 20 °C. Finally, the concentrations of the 5 most abundant biflavonoids in ginkgo leaves (amentoflavone, ginkgetin, isoginkgetin, bilobetin and sciadopitysin) in the extracts were determined using HPLC-DAD. The results showed that B:EG 1:2 with 20 % H<sub>2</sub>O (w/w) and 30 % H<sub>2</sub>O (w/w) were suitable for the extraction of all five biflavonoids.



# Optimizacija ekstrakcije – moderne metode ekstrakcije



Article

## Comparative Analysis of Enzyme-, Ultrasound-, Mechanical-, and Chemical-Assisted Extraction of Biflavonoids from Ginkgo Leaves

Anita Šalić<sup>1</sup>, Lina Šepić<sup>1</sup>, Iva Turkalj<sup>1</sup>, Bruno Zelić<sup>1,2</sup> and Dunja Šamec<sup>2,\*</sup>

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**Abstract:** The biflavonoid extraction from ginkgo (*Ginkgo biloba* L.) leaves using solvent-based extraction with 70% ethanol, alone and in combination with enzyme-assisted, ultrasound-assisted, mechanical-assisted, and chemically assisted methods was investigated and the influence of extraction duration was explored. The total content of polyphenols, flavonoids, and phenolic acids in the extracts was determined spectrophotometrically, while individual biflavonoids were identified and quantified using HPLC-DAD. Amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin were identified in all our extracts. Among these, sciadopitysin emerged as the most prevalent biflavonoid with an amount above 1 mg g<sup>-1</sup> dw, followed by isoginkgetin. Comparative analysis of the extraction methods revealed that, except for chemically assisted extraction, similar levels of compounds were obtained after 45 min of extraction. However, enzymatic (EAE) and mechanical-assisted extraction (MAE) exhibited significantly higher individual (EAE: 19–41% higher; MAE: 22–67% higher) and total biflavonoid (EAE: 29% higher; MAE 50% higher) levels after just 5 min, suggesting their potential to abbreviate extraction duration and facilitate the efficient retrieval of target compounds. However, as extraction efficiency varies between individual biflavonoids, our findings also underscore the importance of considering specific compounds and extraction kinetics in the optimization of ginkgo leaf extraction processes.

**Keywords:** amentoflavone; assisted extraction; biflavonoids; bilobetin; ginkgo; ginkgetin; isoginkgetin; sciadopitysin

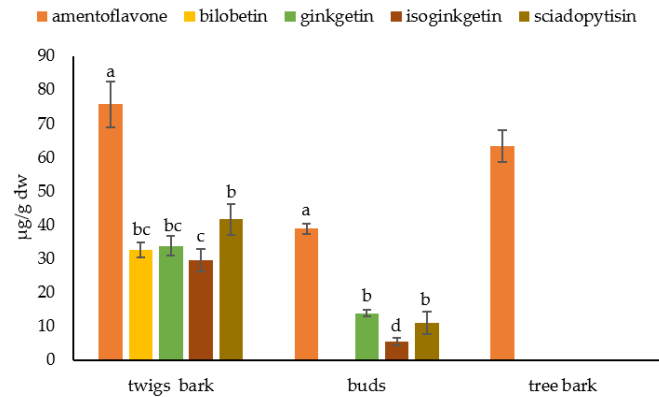


**Citation:** Šalić, A.; Šepić, L.; Turkalj, I.; Zelić, B.; Šamec, D. Comparative Analysis of Enzyme-, Ultrasound-, Mechanical-, and Chemical-Assisted Extraction of Biflavonoids from Ginkgo Leaves. *Processes* **2024**, *12*, 982.

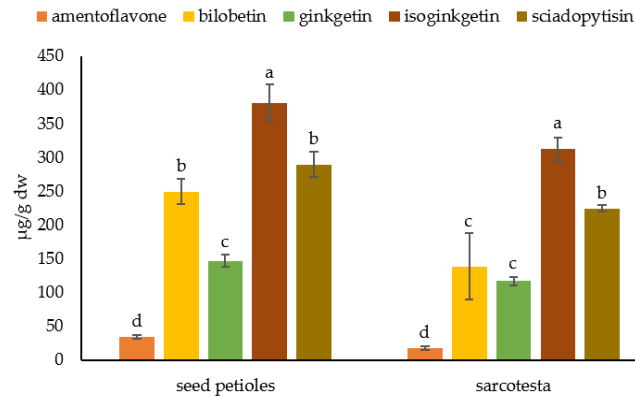
# Sadržaj biflavonoida u različitim tkivima ginka



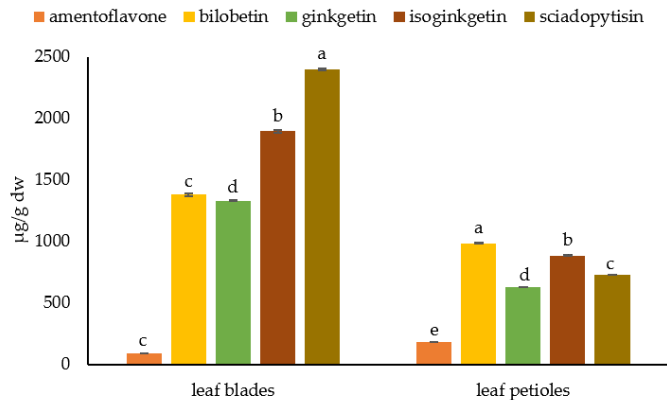
## Twigs, buds



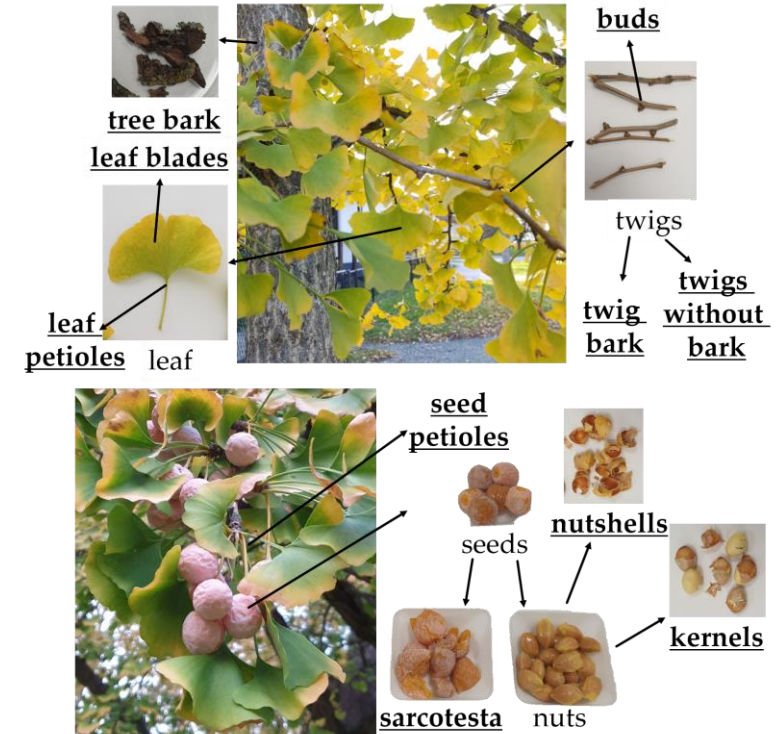
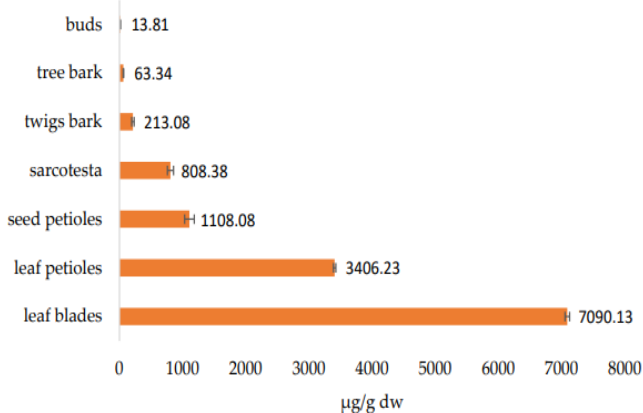
## Seed petioles and sarcotesta



## Leaves blades and petioles

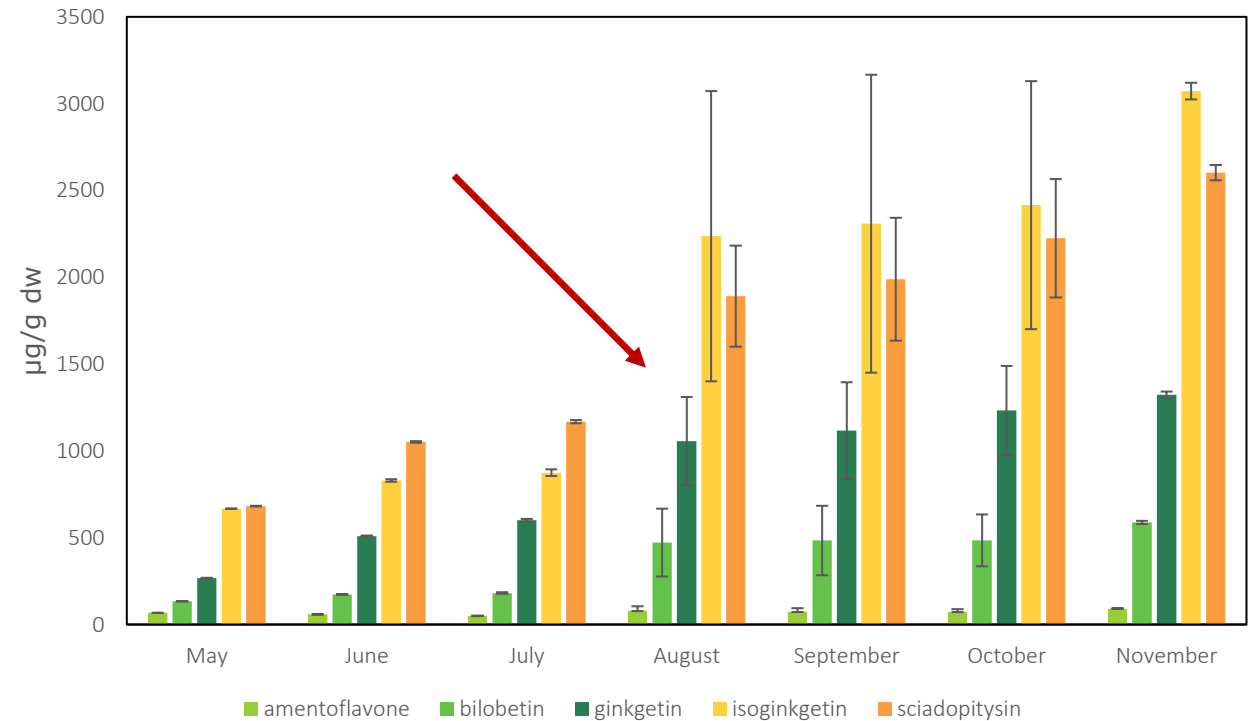
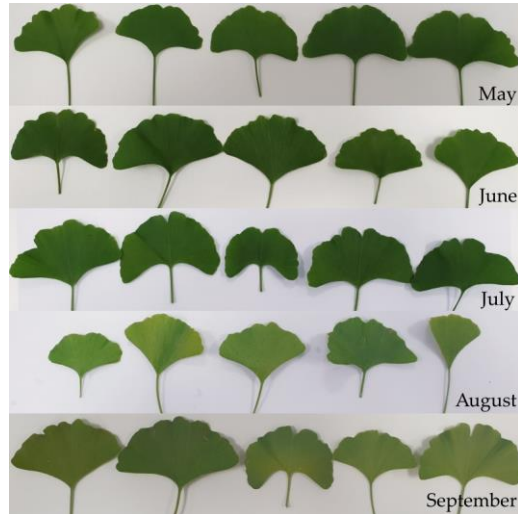


## Total biflavonoids



→ Biflavonoidi nisu identificirani u djelovima biljke koji nisu u direktnom kontaktu s okolinom

# Sezonska varijacija u sadržaju biflavonoida



→ Više biflavonoida u žutom lišću  
intenzivna akumulacija tijekom srpnja/kolovoza

# Ključne poruke

- Biljke se upotrebljavaju za liječenje, održavanje higijene i ljepotu koliko i postoji čovječanstvo
- Većina biljaka nije znanstveno istražena te nema znanstvenih dokaza o (ne)djelovanju – pitanje jel zbilja „prirodno“, „biljno“ opravdava višu cijenu?
- Valja biti oprezan kod upotrebe biljnih proizvoda te se informirati o podrijetlu, sastavu, načinu ekstrakcije, načinu upotrebe i sl..



Most men, like plants, possess hidden qualities  
which chance discovers.

*(François de La Rochefoucauld)*



# Hvala na pažnji!

Istraživanja na Sveučilištu Sjever financirana su iz sredstava Hrvatske zaklade za znanost za projekt „Uloga biflavonoida u biljkama: *Ginkgo biloba* L. kao modelnu sustav” HRZZ-UIP-2019-04-1018 (voditelj doc. dr. sc. Dunja Šamec)”



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Koprivnica

Izv. Prof. dr.sc. Bojan Šarkanj

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Iva Jurčević Šangut

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