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Phytoequivalency of *Ginkgo biloba* products: Pharmacopoeial method

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Abstract

Ginkgo biloba L., maidenhair tree, is the only living species in the Ginkgophyta division. Standardized *G. biloba* extracts is used within the pharmaceutical preparations for the treatment of Alzheimer's and Parkinson's diseases, as well as vascular dementia, vascular tinnitus, and toxicological properties. Plant extract contains three different active chemical classes: Flavonoids (kaempferol, meletin, isorhamnetin, etc.), terpenoids (ginkgolides A, B, and C), and ginkgolic acid. In this study, the commercial preparations obtained from the pharmacy were analysed and compared with standard medicine according to their chemical compositions. Chemical analyses were performed by reverse phase High Performance Liquid Chromatography (HPLC). Seven commercial products were analyzed and compared with standardized extract contained medicine Tebokan® (Abdi Ibrahim, Turkey) by HPLC. While none of the commercial product was found to be equal to the Tebokan®, only one product was investigated similarly according to the important chemical compounds. Quercetin and Isorhamnetin were evaluated in all samples and the standard as well.

Keywords

Ginkgo biloba, HPLC, isorhamnetin, quercetin.

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INTRODUCTION

Medicinal plants play an important role in healthcare and have long been used as the primary remedy for almost all types of ailments. They have been shown to be effective cures for a variety of bacterial, viral, and inflammatory diseases in both conventional and modern medicine, and they are also used to relieve many side effects associated with illnesses or drug-induced side effects (Lorenzo *et al.*, 2019). Chemical drug discovery ushered in a new age of medicine, but a large number of cases of drug resistance, as well as unfavorable side effects, prompted scientists to search for safer alternatives to synthetic medicines. (Thomford *et al.*, 2018).

The *Ginkgo biloba* L., fossil tree, is over 250 million years old and the oldest living tree in the world. Ginkgo has been used in Chinese medicine to treat many ailments since ancient times (Dziwenk and Coppock, 2021). *G. biloba* is one of the widely used medicinal herbs all over the world. Neurological diseases are the main target area for the plant, but it is also used for the treatment of amnesia, forgetfulness, tinnitus, hearing loss (deafness), and vertigo (Yoshikawa *et al.*, 1999). It's antiaging, antioxidant, anti-inflammatory activities, as well as, promotion of circulation and neuroprotective effects against diabetes, hypertension, peripheral and cerebral ischemia, eye problems and dementia were tested (Hasler, 2000; Chan *et al.,* 2007; Cheng *et al.,* 2013; Mohanta *et al.*, 2014; Xiong *et al.,* 2014).

Flavonols and terpene lactones are known as two active groups of compounds found in *G. biloba* leaf extracts. Free radical scavenging and antioxidant activity of *G. biloba* preparations were attributed to flavonols such as isorhamnetin, kaempferol, quercetin, and their derivatives. Terpene lactones are known as platelet activating factor antagonists that are both selective and potent (Xie *et al.,* 2014; Dziwenk and Coppock, 2021). Ginkgolides A, B, C, J, and M are the main diterpene lactones of Ginkgo leaves and they are responsible for their pharmacological effects (Scholtyssek *et al.,* 1997; Jaracz *et al.,* 2004).

Inflammation is currently being linked to a variety of diseases, including obesity, diabetes, cardiovascular disease, asthma, bowel disease, cancer, and autoimmune diseases. (Medzhitov, 2008). In some of the researches, *G. biloba* extract has shown anti-inflammatory activity *in vitro* and *in vivo* by modulating proinflammatory cytokines (Wadsworth *et al.,* 2001; Biddlestone *et al.,* 2007). Alzheimer's disease is a cognitive syndrome that affects

the central nervous system. It is diagnosed by memory loss and impairments in other cognitive areas. This condition is linked to the behavior analysis and laboratory testing (Sasaki *et al.,* 2003). Two clinical studies have confirmed the therapeutic effects of *G. biloba* extract on cognitive dysfunction in Alzheimer's disease patients (Ihl *et al.,* 2011; Ihl *et al.,* 2012). Patients with anxiety were given 80 mg or 160 mg of standardized extract of *G. biloba* (EGb 761) for three times a day within four weeks in a clinical trial (Dubber and Kanfer, 2006). For dementia, a daily dosage of 60-480 mg of comminuted herbal substance as an infusion, split into two or three doses, has been given for up to a year. As a result, the most widely studied dose ranges were from 120 to 240 mg per day, with 240 mg possibly being the most effective. Lower doses of *Ginkgo* must be used for all uses (not more than 120 mg per day) and work our way up to a higher dose for all *Ginkgo* uses (Chermat *et al.,* 1997).

In the present research, commercial Ginkgo products were investigated and compared with standard medicine according to their chemical compositions via HPLC.

MATERIALS AND METHODS

Plant Materials and Chemicals

Standard medicine Tebokan® and commercial *Ginkgo biloba* products contained standardized *G. biloba* extract were purchased from local pharmacies from Iran and North Cyprus. The HPLC standards, reagents and all of the solvents were purchased from Sigma as analytical grade.

Preparation of Extracts

All of the products and standard medicine were extracted according to the European Pharmacopoeia method for *G. biloba*. Briefly, an amount of sample containing 80 mg of standardized extract was taken from each product and was mixed with extraction solvent mixture containing water/methanol/diluted HCl (1/6/3) and then it was extracted in the sonicator for 15 min at the room temperature. After 15 min, 10 mL of the extract was transferred to the brown-glass vial and closed with aluminum crimped lid. This solution was hydrolyzed in a water bath for 25 min and then was cooled to 20 °C before HPLC analysis.

Samples and Standards

For HPLC tests, isorhamnetin and quercetin were used as standards. Stock isorhamnetin and quercetin solutions were prepared in 1 mg/ml concentration with methanol and diluted to appropriate concentrations for the calibration curve. After linear regression of peak areas vs

concentrations, the calibration graphs were plotted.

All of the reference standard solutions were held at a temperature of 20 °C.

HPLC Analysis

Chemical profiles of the extracts were investigated using the HPLC method with photodiode array (PDA) detector (Agilent 1260 infinity). Samples were eluted using C18 reverse phase column (150x0.46 mm, 5□m) with aqueous H_3PO_4 (pH 3.5) (A solution) and methanol (B solution) solutions as a mobile phase. Flow rate was 1 ml/min and the injection volume was 20 microliters. Quercetin and isorhamnetin were identified at 370 nm and the results were expressed according to calibration curves of standards.

RESULTS AND DISCUSSION

In this study, seven commercial *Ginkgo biloba* solid form products were investigated with Tebokan® medication. Standard medicine Tebokan® and commercial *G. biloba* products contained standardized *G. biloba* extract (EGb 761) were extracted with the same procedure given in the extraction part. Standard medicine Tebokan® contains 80 mg of standardized *G. biloba* leaf extract. For this reason, all of the products were extracted in an amount to contain 80 mg of standardized extract.

The extracts were analyzed qualitatively and quantitatively using an HPLC with a PDA detector, and calibration curves of quercetin and isorhamnetin which are active compounds of *G. biloba* leaf extract were prepared at 370 nm.

All of the extracted samples were injected to the HPLC under the same conditions and Tebokan® as well. All of the samples and standards were evaluated according to the quercetin and isorhamnetin constitutions. Calibration curves of quercetin and isorhamnetin were prepared using an external standard dilution method and calibration equations/calibration coefficients were calculated using these curves (Figure 1). The calibration curves of isorhamnetin and quercetin showed good linearity ($r^2 > 0.999$) within relatively wide concentration ranges.

Each extract was injected three times and mean values were calculated with standard deviations (mean ± SD) as shown in Table 1 (Figure 2). According to the Table 1, none of the samples were exactly the same as standard medication due to the amounts of quercetin and isorhamnetin which are active phenolics for *G. biloba* standardized extract.

Table 1: Quercetin and isorhamnetin amounts within the *G. biloba* products and Tebokan®.

Sample	Quercetin	Isorhamnetin
	Mean ± SD (% in extract)	**Mean ± SD (% in extract)**
G1	7.520 ± 0.157	0.497 ± 0.009
G2	0.304 ± 0.034	0.353 ± 0.041
G3	4.650 ± 0.125	2.644 ± 0.119
G4	12.180 ± 0.227	1.409 ± 0.096
G5	3.774 ± 0.311	0.390 ± 0.034
G6	0.867 ± 0.030	0.076 ± 0.003
G7	0.297 ± 0.012	0.013 ± 0.003
Tebokan®	0.404 ± 0.029	0.212 ± 0.001

According to the fingerprint analysis within the Figure 2, flavonoids detected in the G2 extract were similar with the Tebokan® extract. On the other hand, as seen in Table 1, the amounts of quercetin and isorhamnetin in G2 were found to be close to the standard drug Tebokan®. While the G3 and G4 extracts contained very high levels of both compounds, the amount of quercetin in the G1 extract was found to be quite high but isorhamnetin amounts in the G6 and G7 extracts were calculated to be very low according to the Tebokan®. When both Table 1 and Figure 2 were examined, it was thought that the extracts with very high amounts of quercetin and isorhamnetin may be adulterated with pure substance.

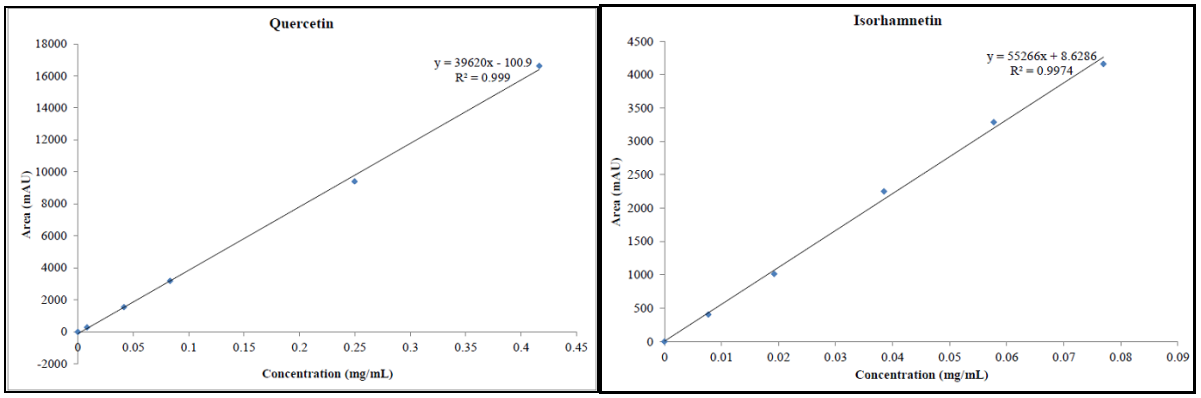

Figure 1: Calibration curves of quercetin and isorhamnetin.

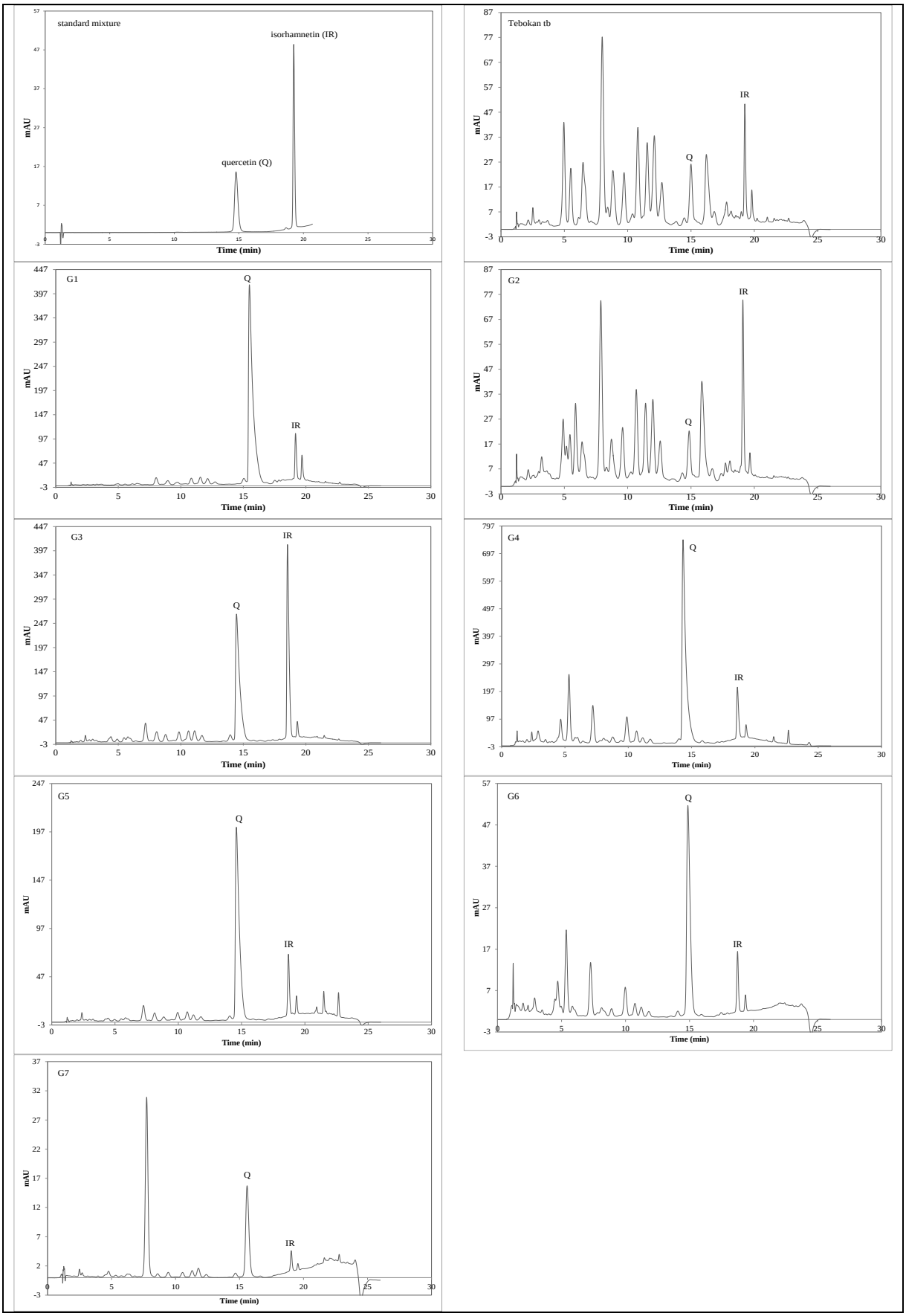

Figure 2: HPLC chromatograms of samples and standards.

Demirezer *et al*. (2014) published an article related to the adulteration of 13 pharmaceutical dosage forms of *G. biloba* and they found a broad range of active constituents. In this paper, flavonoids and ginkgolides were investigated by HPLC-DAD and none of the pharmaceutical forms were found to be accurate according to declaration quantities of the compounds on the label. When looking at the results of both previous studies and the present study, it is clear that many *G. biloba* products on the market do not contain standardized leaf extract and therefore may not have the same effect as the standard drug Tebokan. The present study has once again showed the importance of standardized extract utilization, standardization and quality controls of the pharmaceutical products.

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