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Phytoequivalency of *Ginkgo biloba* products: Pharmacopoeial method

Haleh Piri, Beste Atli, Nesrin Oztinen, Ezgi Ak-Sakalli, Muberra Kosar*

Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, T.R North Cyprus, Mersin 10 Turkey.

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, 5μm) 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 Mean ± SD (% in extract)	Isorhamnetin 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.

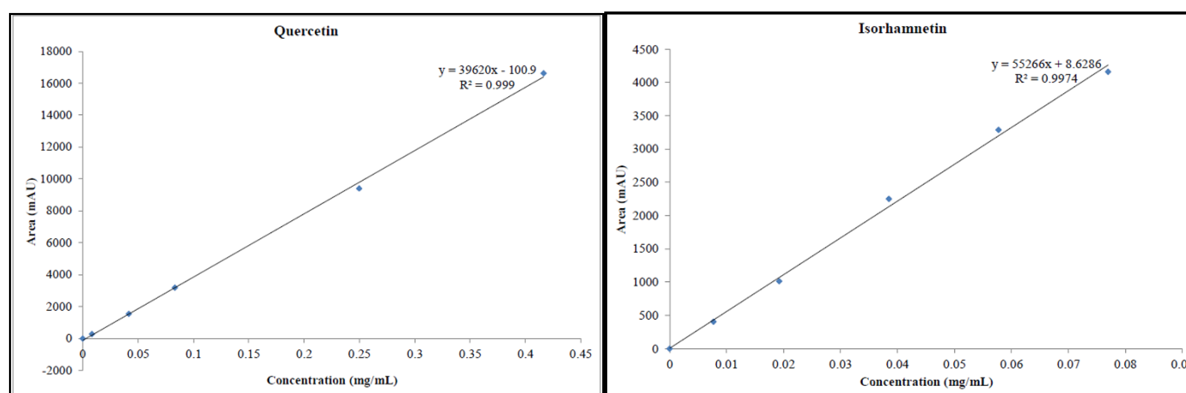

Figure 1: Calibration curves of quercetin and isorhamnetin.

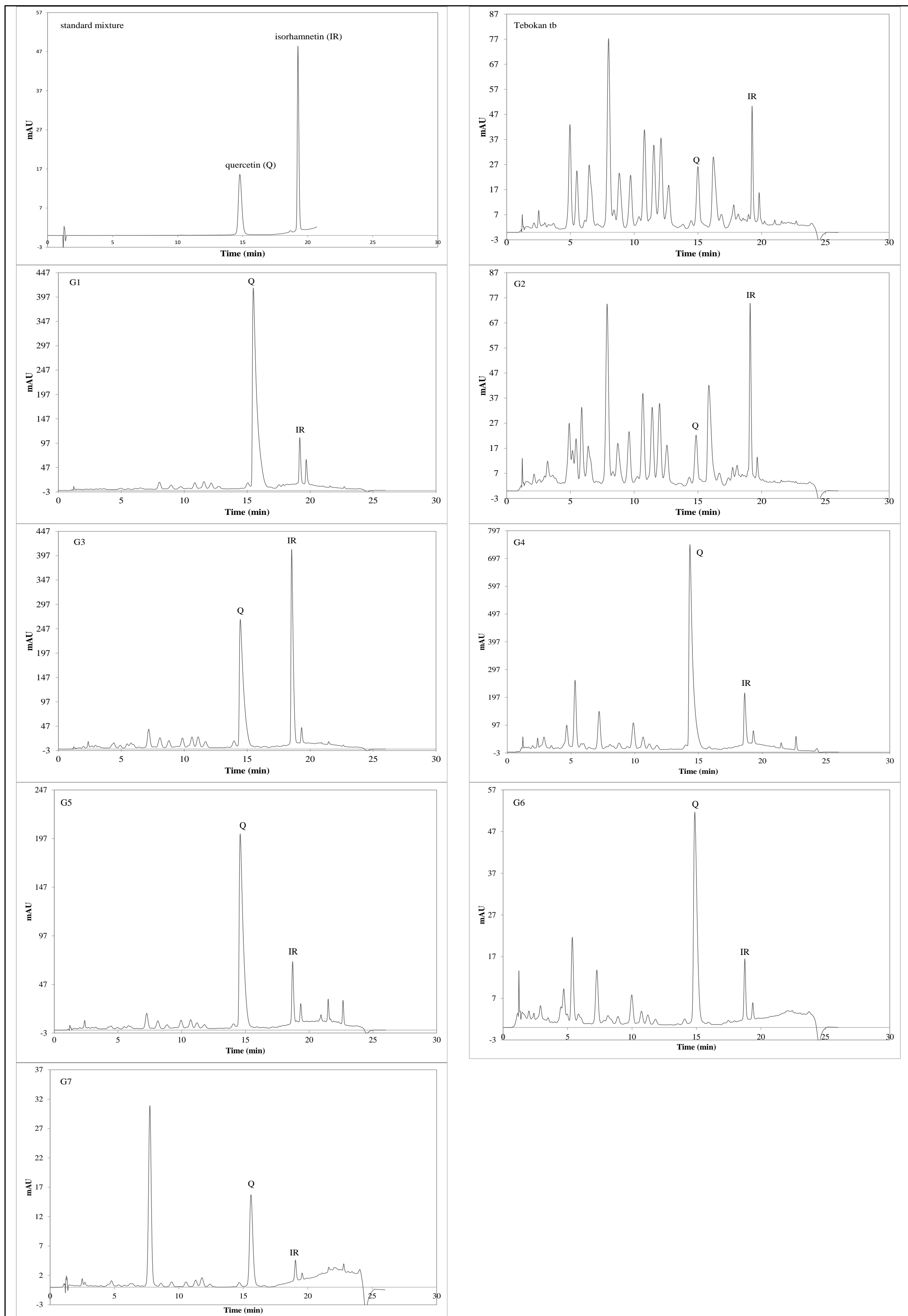

Figure 2: HPLC chromatograms of samples and standards.

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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Honey plants of Tepebaşı (Diorios) in North Cyprus

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Abstract

Tepebaşı village (Diorios) is the one the most beautiful sides of the Northern Cyprus. It is located within the borders of Kyrenia district. The area covered by a dense pine forest. In this study, the research was conducted in two beehives of Tepebaşı village. Flowering plant specimens were collected, dried according to the herbarium regulations and kept in the Herbarium of Faculty of Pharmacy, Eastern Mediterranean University. Total of 29 wild and medicinal flowering plant species were determined by their pollen grains and nectar. In the article, photographs of honey plants, which were taken in their natural habitats, are given with their scientific names and with common names in Turkish and English as well.

Keywords

Honey plants, local names, North Cyprus species photos, Tepebasi.

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INTRODUCTION

Beekeeping is a traditional agricultural activity performed nearly every region in Cyprus due to its rich flora. In 2013, 112 honeys from 25 countries participated at the World Beekeeping Awards, competing for their quality, taste and aroma. Cyprus was involved in the competition with two types of honeys, the Cypriot flower honey and the Cypriot thyme honey. Both honeys won the United Nations- gold medal in their category (Βραβεία στο Τζιβέρτι, n.d.).

Cyprus is ranked as the third largest mediterranean island with an area of 9251 sq.km after Sicily and Sardinia. The island is located in the south of Turkey, west of Syria and Lebanon, north of Israel and Egypt, and southeast of Greece (Ilseven *et al.,* 2006).

Due to the political reasons, the island is subdivided into four main segments. I. The Republic of Cyprus occupies the southern two-third of the island (59.56%). II.The Turkish Republic of Northern Cyprus occupies the northern one-third (35.04%) of the island, and III.the controlled Green Line provides a buffer zone that separates the two and covers 2.64% of the island. Lastly, two bases under British sovereignty are located on the island called Akrotiri and Dhekelia,IV: covering the remaining 2.76% (Ilseven *et al.,* 2006).

Cyprus enjoys an intense Mediterranean climate, with long dry summers from mid–May to mid–October, and mild winters from December to February, which are separated by short autumn and spring seasons.

One of the best studies about the flora of Cyprus was performed by R. D. Meikle. This study divides the island into 8 botanical divisions as shown in Figure 1 (Meikle, 1977, 1985).

Figure 1: 8 Botanical divisions of Cyprus.

Division 1 is very heterogenous area topographically, geologically, and floristically. This region is mostly hilly with deep narrow valleys. The costs are generally low, and sandy or rocky, except for a limited range of steep cliffs just Northwest of the Baths of Aphrodite. Special plants of division 1 includes; *Alyssum akamasicum* Burtt, *Arenaria rhodia* Boiss .subsp.*cypria* (Holmboo) McNeill, *Cistus monspeliensis* L., *Cistus ladanifer* L., *Pistacia* x *saportae* Burnot, *Trifolium argutum* Sol., *Arbutus unedo*L., *Phlomis lunariifolia* Sm., *Tulipa cypria* Stapf ex Turrill , *Scilla cilicica* Siehe, *Phleum crypsoides* (d'Urv.)Hack .

Division 2 includes a region called Troodos Mountains. The highest peak of the Troodos Mountains is the Olympus with the height of 1952 m. This division has rich endemic flora. There are many endemic plants to list but the two most important ones are Golden Oak (*Quercus alnifolia*) and Cedar (*Cedrus libani* ssp.brevifolia) (Meikle, 1977).

Division 3 consists of mainly vineyards and the costal belt is extensively cultivated. Important plants of this division include *Alyssum chondrogynum* Butt , *Linum maritimum* L., *Fagonia cretica* L., *Erodium crassifolium* L'He'r ex Aiton, *Astragalus pehuenches* Niederl. subsp. *lefkarensis*, *Neurada procumbens*L., *Artedia squamata* L., *Centaurea veneris*(Sommier))Beg *Cionura erecta* (L.) Griseb. *Convolvulus cyprius* Boiss. *Ipomoea sagittata* Poir. *Ephorbia thompsonii* Holmboe , *and Cladium mariscus* (L.) Pohl

Division 4 is mostly cultivated or heavily grazed, with typical Mesaoria cornfields in the north and numerous barrens, eroded chalk or limestone hills in the south. Larnaca Salt Lake provides a habitat for interesting *Limonium* species and other halophytes. Important plants of this division include; *Horningiaprocumbens*(L.)Hayek *Matthiola fruticulosa* L. *Erodium crassifolium* L'Her. ex Aiton , and *Drimia undula* Stearn *Crambe hispanica* L., *Galium pisiferum* Boiss. and *Scilla hyacinthoides* L., have been recorded from Ayia Napa, and *Ipomoea imparati* (Vahl) Griseb. Syn. of *I.stolonifera* from sandhills at Famagusta

Division 5 is mostly occupied by cereal fields of Mesaoria with interesting weed communities due to the extensive use of herbicides, it is almost weed free and the region is uninvitingly monotonous for the botanists. Kyrenia range in the north of the division has a rich and characteristic flora .

Division 6 is heavily cultivated, with cornfields in the centre and east and extensive *Citrus* groves of Morphou. Botanically, the most important regions in this division are Kormakiti and Ayia Irini.

Important plants of this division include; *Argyrolobium uniflorum* (Decne)Jaub.ex Spach,*Cyclamengraecum*Linksubsp.*anatol icum* Ietsw., *Achillea santolina* Sibth.et Sm. *Convolvulus oleifolius* Desr. and other rare plants. The *Tulipa cypria* is locally abundant in fields of Diorios and Mrytou

Division 7 has the richest flora among all island. The number of endemics and the rarities are too many to list. This division is mainly uncultivated with extensive areas of *Pinus brutia* and *Cupressus sempervirens* forests on the upper slopes. Important plants of this division include; *Iberis odorata* L., *Pteranthus dichotomus* Forssk., *Daucus durieua* Lange,*Chlamydophora tridentata*, (Delile) Ehrenb. ex Less. *Salvia barrelieri* Ete syn .*S. crassifolia* and others

Division 8 is an area with low hills and sand or rocky shores. This division includes many rare plant species such as *Fumaria gaillardotii* Boiss. *Enarthrocarpus arcuatus*, Labill., *Helianthemum ledifolium* (L.) Mill and *Trifolium globosum* L., *Rosmarinus officinalis* L. is a very rare wild plant which grows in some abundance on rocky shores between Yialousa and Rizokarpaso .

There is no up-to-date floristic records of vascular wild plants of Northern Cyprus. The region's most important and reference source for all concerned.prepared with great effort and based on specimens collected from the region between 1988-1993 by Viney (Viney, 1994, 1996 and 2011)

An illustrated flora of North Cyprus " it is only a book listed the wild plant species based on the collected specimens between 1988-1993 , published with line drawing.It has been provento be invaluable guide to the flora for nature lover and botanists.

Also a booklet published by Viney (Viney 1992) to introduce the 19 endemi species of North Cyprus with line drawings.

Sami Tamson's book is an essential resource for all orchid lovers, with its extraordinary photographs introducing the Orchidaceae family species (Tamson, 2014)

The Dynamic Checklist of Cyprus is a website with up-to-date information. Vascular flora of Island comprises 1650 indigenous taxa (Hand R., Hadjikyriakou & Christodoulou 2011) The studies conducted by a number of researches revealed the presence of 1257 species in the Flora of North Cyprus . It is not an exact number, a more precise number can be obtained if studies on the flora are concentrated. With a recent study, a new species from the Poaceae family has published (Hand, Chrysostomou & McLoughlin 2021) Thus, the number of endemic species of North Cyprus increased to 20.

Tepebaşı (Diorios) is a village which is located within the borders of Kyrenia district of The Turkish Republic of Northern Cyprus. (Figure 2) The village is

located 300 m above the sea level on the top of the hill (Özersoy, 2019).

Most of the administrative area of the village is covered by a dense pine forest, while in other areas the villagers cultivate mainly cereals, carob and olive trees. Tepebaşı forest occupies an area of 8,400 acres, and the village covers an area of 27,587 acres (36.9 square miles). The village is also main habitat for the endemic tulip of Cyprus which is called '*Tulipa cypria'* (Figure 3).

Figure 2: Location of Tepebaşı (Diorios) on map of Cyprus.

Figure 3: Endemic tulip of Cyprus (*Tulipa cypria*) and Natural vegetation of Tepebaşı (Diorios).

MATERIALS AND METHODS

Field studies

First of all, the beekeepers of the Tepebaşı (Diorios) village were used as a good source for gathering information about the plants that contain pollen and nectar. Two main apiaries which are away from each other was selected for collecting plants (Figure 3 and 4). The plant collecting studies was performed for 9 months. Totally 29 plant species were collected during the field studies which were carried between March and November 2020. While collecting the

plants, the photograph of each specimen was taken for further identification process. The collected plant specimens were pressed and dried. Dry specimens were identified scientifically and placed in the herbarium.

Pressing and drying plant specimens

Plant specimens were collected according to the plant collection regulations. Each specimen should consist of a stem with attached leaves and if it is possible flowers or fruits as well. All necessary information about the collected plant specimens such as collection date, location, collectors name and etc. were noted to hand register book. Specimens were placed between the individual newspapers containing the specimens for allowing the airflow and for absorbing moisture. The newspapers and cardboards were placed between two wooden frames for rigidity. The plant press was tightened using F-clamp bar. The plant pressing process was continued until the specimen was completely dry. During the drying process the wetting paper was changed continously in each two days. Dried samples were placed into freezer at regular intervals to protect them from insects. Dried specimens were attached onto cartoons and their identification was done with the aid of the experts and the books; An Illustrated Flora of North Cyprus by D.E. Viney, Wild Flowers of Cyprus by George Sfikas and A Photagrapher's Eye View of the Flowers of Northern Cyprus by Laura Lushington and Sonia Halliday. Each specimen was labelled with a card containing the scientific name, common name, location, date of collection, name of collector and the name of the person who identified the sample.

Figure 4: Apiary B.

Figure 5: Pressing plant specimens.

RESULTS

Table 1: List of the plant species visited by honey bees in Tepebaşı**.**

Family	Scientific name	Common name	Turkish name
Amaryllidaceae	*Allium neapolitanum* Cirillo	Neapolitan garlic	Napoli soğanı
Apiaceae	*Ferula communis* L.	Giant fennel	Çakşır otu
	Foeniculum vulgare Mill.	Common fennel	Rezene
Asparagaceae	*Bellevalia trifoliata* (Ten.) Kunth	Purple roman squill	Öküz sümbülü
	Ornithagalum pedicellare Boiss.	Star of Bethlehem	Tükürük otu
	Urginea maritima Baker	Sea squill	Ada soğanı
Asphodelaceae	*Asphodelus aestivus* Brot.	Asphodel	Çiriş out
Asteraceae	*Anthemis palaestina* Reut. ex Boiss.	Israel's chamomile	Mayıs papatyası
	Calendula arvensis M.Bieb.	Field marigold	Portakal nergisi
	Chrysanthemum coronarium L.	Crown daisy	Sarı papatya
	Inula viscosa (L.) Aiton	False yellowhead	Yapışkan anduz otu
	Pallenis spinosa (L.) Cass.	Spiny starwort	Dikenotu
	Taraxacum cyprium H.Lindb.	Dandelion	Karahindiba
Boraginaceae	*Heliotropium europaeum* L.	European turn-sole	Beyaz bambul
	Lithodora hispidula (Sm.) Griseb.	Shrubby gromwell	Ebruliçalı
Brassicaceae	*Sinapis arvensis* L.	Field mustard	Lapsana
Capparaceae	*Capparis spinosa* L.	Caper bush	Gebre otu
Cistaceae	*Cistus parviflorus* Lam.	Rockrose	Küçük çiçekli laden
	Helianthemum obtusifolium Dunal	Yellow Cyprus Sun-rose	Kıbrıs güneş gülü
Cucurbitaceae	*Ecballium elaterium* (L.) A.Rich.	Squirting cucumber	Eşek hıyarı
Fabaceae	*Calicotome villosa* (Poir.) Link	Hairy thorny broom	Azgan
	Vicia sativa L.	Common vetch	Yabani fiğ
Lamiaceae	*Prasium majus* L.	White hedge-nettle	Çalıbaba
	Thymus capitatus Hoffmanns. & Link	Thyme	Tülümbe
Malvaceae	*Malva sylvestris* L.	Common mallow	Büyük ebegümeci
Oxalidaceae	*Oxalis pes-caprae* L.	Bermuda buttercup	Ekşilice
Ranunculaceae	*Anemone coronaria* L.	Poppy anemone	Taçlı dağ lalesi
	Ranunculus millefolius Vahl	Jerusalem buttercup	Düğünçiçeği
Rosaceae	*Sarcopoterium spinosum* Spach	Thorny burnet	Abdestbozan

Description of honey plants of Tepebaşı (arranged in family alphabeticial order)

1. ***Allium neapolitanum*** Cirillo

Photo 1: *A. neapolitanum* from natural habitat.

Flowering time: February- May

Collection number: 01

Bees collect both pollen and nectar.

2. ***Ferula communis*** L.

Photo 2: *F. communis* from natural habitat.

Flowering time: March-May

Collection number: 02

Bees collect both pollen and nectar.

3. ***Foeniculum vulgare*** Mill.

Photo 3: *F. vulgare* from natural habitat.

Flowering time: August- October

Collection number: 03

Bees collect both pollen and nectar.

4. ***Bellevalia trifoliata*** (Ten.) Kunth

Photo 4: *B. trifoliata* from natural habitat.

Flowering time: February- May

Collention number: 04

Bees collect pollen from this plant.

*5. **Ornithagalum pedicellare*** Boiss. & Kotschy

Photo 5: *O. pedicellare* from natural habitat.

Flowering time: March- April

Collection number: 05

Bees collect pollen.

*6. **Urginea maritima*** (L.) Barker

Photo 6: *U. maritima* from natural habitat.

Flowering time: July- September

Collection number:06

Bees collect both pollen and nectar.

*7. **Asphodelus aestivus*** Brot.

Photo 7: *A. aestivus* from natural habitat.

Flowering time: April- June

Collection number:07

Bees collect both pollen and nectar.

8. ***Anthemis palaestina*** (Reut. ex Kotschy) Reut. ex Boiss.

Photo 8: *A. palaestina* from natural habitat.

Flowering time: March-June

Collection number: 08

Bees collect both pollen and nectar.

9. ***Calendula arvensis*** M. Bieb

Photo 9: *C.arvensis* from natural habitat.

Flowering time: January- May

Collection number: 09

Bees collect both pollen and nectar.

10. ***Chrysanthemum coronarium*** L.

Photo 10: *C. coronrium* from natural habitat.

Flowering time: March- May

Collection number: 10

Bees collect both pollen and nectar.

11. ***Inula viscosa*** (L.) Aiton

Photo 11: *I.viscosa* from natural habitat.

Flowering time: August- November

Collection number: 11

Bees collect both pollen and nectar.

Nectar is too much.

12. ***Pallenis spinosa*** (L.) Cass.

Photo 12: *P. spinosa* from natural habitat.

Flowering time: May- July

Collection number: 12

Bees collect pollen from this plant.

13. ***Taraxacum cyprium*** H.Lindb.

Photo 13: *T. cyprium* from natural habitat.

Flowering time: April- May

Collection number: 13

Bees collect both pollen and nectar.

14. ***Heliotropium europaeum*** L.

Photo 14: *H. europaeum* from natural habitat.

Flowering time: May- November

Collection number: 14

Bees collect both pollen and nectar.

15. ***Lithodora hispidula*** (Sm.) Griseb.

Photo 15: *L. hispidula* from natural habitat.

Flowering time: February- May

Collection number: 15

Bees collect both pollen and nectar.

16. ***Sinapis arvensis*** L.

Photo 16: *S. arvensis* from natural habitat.

Flowering time: March- September

Collection number: 16

Bees collect both pollen and nectar.

17. ***Capparis spinosa*** L.

Photo 17: *C. spinosa* from natural habitat.

Flowering time: May- August

Collection number: 17

Bees collect both pollen and nectar.

18. ***Cistus parviflorus*** Lam.

Photo 18: *C. parviflorus* from natural habitat.

Flowering time: February- May

Collection number: 18

Bees collect both pollen and nectar.

19. ***Helianthemum obtusifollium*** Dunal.

Photo 19: *H. obtussifollium* from natural habitat.

Flowering time: February- May

Collection number: 19

Bees collect both pollen and nectar.

*20. **Ecballium elaterium*** (L.) A.Rich.

Photo 20: *E. elaterium* from natural habitat.

Flowering time: January- July

Collection number: 20

Bees collect pollen from this plant.

*21. **Calicotome villosa*** (Poir.) Link

Photo 21: *C. villosa* from natural habitat.

Flowering time: December- April

Collection number: 21

Bees collect both pollen and nectar.

*22. **Vicia sativa*** L.

Photo 22: *V. sativa* from natural habitat.

Flowering time: May- September

Collection number: 22

Bees collect both pollen and nectar.

23. ***Prasium majus*** L.

Photo 23: *P. majus* from natural habitat.

Flowering time: December- June

Collection number: 23

Bees collect both pollen and nectar.

24. ***Thymus integer*** Griseb.

Photo 24: *T. integer* from natural habitat.

Flowering time: June- August

Collection number: 24

Bees collect both pollen and nectar. Flowers are very rich in nectar.

25. ***Malva sylvestris*** L.

Photo 25: *M. sylvestris* from natural habitat.

Flowering time: March- September

Collection number: 25

Bees collect both pollen and nectar.

*26. **Oxalis pes-caprae*** L.

Photo 26: *O. pes-caprae* from natural habitat.

Flowering time: December-May

Collection number: 26

Bees collect both pollen and nectar.

*27. **Anemone coronaria*** L.

Photo 27: *A. coronaria* from natural habitat.

Flowering time: April- June

Collection number: 27

Bees collect pollen from this plant.

*28.**Ranunculus millefolius*** Vahl

Photo 28: *R. millefolius* from natural habitat.

Flowering time: April- May

Collection number: 28

Bees collect pollen and nectar from this plant.

*29. **Sarcopoterium spinosum*** (L.) Spach

Photo 29: *S. spinosum* from natural habitat.

Flowering time: February- April

Collection number: 29

Bees collect pollen from this plant.

CONCLUSION

In the field studies, total of 29 plant species belonging to 17 different families were collected around beehives. However, if this study can be repeated in the future, more plant species can be observed because the Tepebaşı village was faced with a great forest fire in May 2020 and 7,500 acres forest was burned in total. Nectar richness These studies were carried out in 3 regions as follows

1. Güzelyurt (Morphou) (Korkmazer, Çağan).
2. Boğaztepe (Monarga) (Aybenk, Abdullah).
3. Tepebaşı (Diorios).

Since these are thesis projects, they were done in a limited time and focused on early spring flowers. During the studies, flowering plant samples were collected from around selected beehives, dried according to the herbarium rules and stored of the plants was identified by the observations of beekeepers and pollen richness was identified by microscopic studies. During the graduation projects of three students at EMU Faculty of Pharmacy in Northern Cyprus, wild and medicinal flowering plant species visited by honey bees to produce honey were researched:

in the EMU herbarium. When the plant species were checked at the end of the three projects, it was seen that some medicinal species were present in all three studies. These species are common in the island: Bees from these species receive both pollen and nectar.

1. *Chrysanthemum coronarium,*
2. *Ferula communis*
3. *Malva sylvestris*
4. *Oxalis per-caprea*
5. *Pallenis spinose*

In this study, Tepebaşı species that were not included in other studies are listed below

1. *Allium neapolitanum*
2. *Bellevalia trifoliata*
3. *Ornithogalum pedicillare*
4. *Urgenia maritima*
5. *Litodora hispidula*
6. *Anemone coronaria*

These three studies are the first and preliminary studies within this direction in Northern Cyprus. Beekeeping is an important source of income in Northern Cyprus. These studies, should be taken into consideration in order to determine the origin of the honey and to preserve its extraordinarily rich flora.

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Antiradical activities and phenolic compositions of *Rosa canina* L. from Iran and Turkey

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Abstract

Rosa canina L. is a climbing wild rose plant species native to Europe, Northwest Africa, and Western Asia from the Rosaceae family. Antioxidants such as polyphenols and ascorbic acids, as well as carotenoids, Vitamin B and E, are abundant in the flesh of *Rosa canina* fruits. Plant phenolics, particularly flavonoids, have attracted a lot of attention in recent years because of their wide range of biological effects, including antiinflammatory, antiallergic, and antibacterial properties. Functions of flavonoids as antioxidants, free radical scavengers, and divalent cation chelators were recorded in the literature among others. Epidemiologic studies have shown a link between increased flavonoid antioxidant intake and a lower risk of cardiovascular disease and certain types of cancer. In this experiment, dry and fresh fruits of *Rosa canina* from two regions (Turkey and Iran) were investigated according to *in-vitro* antiradical assays such as (2,2′-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$). The phenolic composition of the extracts was also analyzed by HPLC and spectrophotometric methods.

Keywords

ABTS, DPPH, HPLC, phenolics, *Rosa canina*.

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INTRODUCTION

Rosaceae family, composed of over 100 species, is widely distributed in Europe, Asia, the Middle East, and North America (Nilsson, 1997). *Rosa canina* L. is a perennial shrub plant, which has a height of about 2-3 m with multiple arching stems. Fruits are smooth, deep pink and they ripen through September to October. Fruits can remain fresh on the plant for months and eventually turn black. Plants can reproduce sexually by seed, and vegetatively by suckering and layering (Nilsson, 1997; Pavek, 2012). Turkey is one of the most significant germplasm centers, accounting for approximately 25% of all rose species. These 25 species are native to Turkey and can be found anywhere from sea level to 3000 meters above sea level. *R. canina* is a hardy plant that can withstand harsh environments. It grows wildly in Turkey's numerous regions particularly in central and north-east Anatolia (Ercisli, 2005). Peasants have been gathering rose hips (fruits) from scattered sites in most parts of Anatolia since ancient times as a food source (Ercisli, 2007). In the North West of Iran, rose hips can be found as well because of its significant climate which is needed for the growth of the plant. The pseudo-fruit of *R. canina*, the rose hip (brier hip, brier, dogberry, dog rose, hip fruit, hop fruit, sweetbrier, wild brier), is an un-shaped receptacle containing various achenes. The hip is rich in flavonoids, pectin, vitamin A, B complex, C, and E, as well as minerals such as calcium, iron, selenium, and manganese. Mg, K, S, and Si have also been discovered in trace quantities. Because of its rich content, it is used as a functional food. It can be eaten raw, but there is only a thin layer of flesh and a layer of irritating hairs just below the flesh layer, making it difficult to eat. So it is preferred to be used as juice, wine, tea, jelly, jam, alcoholic beverages, marmalade, and herbal tea. Fruits can be used for cosmetic purposes as well (Yildiz and Alpaslan, 2012; Pavek, 2012).

Rose hips are very rich in phenolic compounds such as phenolic acids (protocatechuic acid, 4-hydroxy benzoic acid, syringic acid, caftaric acid, ferulic acid, 2.5-dihidroxy benzoic acid, vanillic acid, chlorogenic acid, p-coumaric acid, t-caffeic acid, and sinapic acid), flavonoids (catechin, procyanidin-B2, (−) epicatechin, 4-methyl catechol, epicatechin gallate, and t-resveratrol) and gallic acid. The most important chemical component of fruits is vitamin C (ascorbic acid) (Demir *et al.,* 2014).

Due to the high amount of phenolic compounds, fruit parts of *R. canina* are

being used for different medicinal purposes such as immunosuppressive, antioxidant (Rein *et al.,* 2004), anti-inflammatory (Winther *et al.,* 2005; Lattanzio *et al.,* 2011), anti-arthritic (Ameye and Chee, 2006), antiosteoartrit (Gruenwald *et al.,* 2019), analgesic, antidiabetic (Orhan *et al.,* 2009), cardioprotective, antimicrobial (Deliorman Orhan *et al.,* 2007), gastroprotective, and skin ameliorative activities. According to the German Commission E Monographs, the fruits can be used to treat kidney and lower urinary tract disorders, inflammatory disorders, fever, colds, and infectious diseases such as influenza, as well as gastrointestinal disorders (Blumenthal *et al.,* 1998). The large amount of phenolic compounds in the rose hip has made it a great treatment and prevention for acne, aging and other facial and beauty problems (Patel, 2013).

In the present study, it was aimed to investigate and compare the dry and fresh fruits of *R. canina* samples, collected from two different origins such as Turkey and Iran, for their antiradical activities and phenolic compositions *in vitro*.

MATERIAL AND METHODS

Plant material and reagents

Rosa canina L. fruits were collected from two different places: Ser Mountains of West Azarbayjan of Urmia city of Iran and Lake of Yayla Buldan/Denizli Turkey. All of the chemicals and solvents that were used in the assays were purchased from Sigma Aldrich as analytical grade.

Extracts preparation

5 gr of each sample was extracted with 70% methanol using sonication for 60 mins. After filtration, the samples were evaporated by rotatory evaporator at 45 °C under vacuum. Dried extracts were kept at -18 °C until analyses.

Total phenolics assay

The Folin-Ciocalteau method was used to calculate total phenols as gallic acid equivalents. First of all, 100 µL of the sample was transferred to a 10.0 mL volumetric flask containing 6.0 mL of H_2O, to which 500 µL of undiluted Folin-Ciocalteu reagent was applied. 1.5 mL of 20% aqueous Na_2CO_3 was added after 1 minute, and the amount was made up to 10.0 mL with H_2O. Except for the extract, all of the reaction reagents were present in the controls. The absorbance was estimated at 760 nm after 30 minutes of incubation at 25 °C and compared to a gallic acid calibration curve. Total phenolics were calculated as gallic acid equivalents and presented as the average of three independent studies (Singleton *et al.,* 1999).

High Performance Liquid Chromatography (HPLC) analysis

Phenolic 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, 5µm) with 2.5 % formic acid in water (A solution) and 2.5 % formic acid in acetonitrile (B solution) solutions as a mobile phase. Flow rate was 1 ml/min and the injection volume was 20 microliters. Compounds were identified at 280 nm, 320 nm, and 360 nm wavelengths according to phenolic groups such as benzoic acids, hydroxycinnamic acids, and flavonoids, respectively.

All of the standards and extracts were injected in triplicates, and mean values and standard deviation were calculated. The amount of ascorbic acid in the extracts was calculated using the benzoic acid calibration curve due to the lack of commercial standard (Ph. Eur., 2013).

DPPH radical scavenging activity

Gyamfi et al.'s method was used for assessing the ability of the extracts to scavenge DPPH radical. In Tris-HCl buffer (50 mM, pH 7.4), a 50 µL aliquot of each extract was combined with 450 µL of Tris-HCl buffer (50 mM, pH 7.4) and 1.0 mL of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl in methanol. Except for the extract or positive control substance, the controls included all of the reaction reagents. The absorbance was measured at 517 nm after 30 minutes of incubation in dark at room temperature (23°C). Eq 1 was used to calculate the percentage inhibition, and a nonlinear regression algorithm was used to estimate the half maximal inhibitory concentration (IC_{50}) values (SigmaPlot 2001 version 7.0, SPSS Inc., Chicago, IL). As a positive control, butylated hydroxytoluene (BHT) was used. The average of three measurements is used to calculate the results.

Percentage inhibition = [(Abscontrol - Abssample) / Abscontrol] × 100 (Eq. 1)

$ABTS^{+}$ radical scavenging activity

The extracts' ability to scavenge $ABTS^{+}$ radical was measured via spectrophotometry. 36 mg $ABTS^{+\bullet}$ and 6.6 mg $K_2O_8S_2$ were dissolved in 10 ml distilled water. The mixture was allowed to stand at room temperature for 12-16 hours in the dark before the analysis, and the absorbance was adjusted to 734 nm to 0.7 at ambient temperature. Extracts were diluted with 70% methanol. The absorbance was measured for 30 min with 1 minute intervals at 734 nm after adding 10 µL of extract to 990 µL of $ABTS^{+\bullet}$ solution. The total antioxidant activity was calculated using a prepared Trolox calibration curve (Re *et al.,* 1998).

RESULTS AND DISCUSSION

The samples were divided into two portions. One portion was kept at -18 ºC and the other portion was dried at the room temperature. Fresh and dry fruits from two different locations were extracted with 70% methanol. The extraction yields are given in Table 1. According to the Table 1, the yield of the Turkey sample was higher than Iran's yield. Total phenolics within the extracts were measured by Folin Ciocalteau reagent and the calculated gallic acid equivalent total phenolics are shown in Table 1. The order of total phenolics amounts of the extracts can be given as IF > TF > TD > ID. In the Folin Ciocalteau colorimetric assay, the gallic acid calibration curve was used for the calculation of total phenolics amounts of the extracts.

Table 1: Extraction yield and total phenolics data for *Rosa canina* samples.

Sample *	Extraction Yield (%)	Total phenolics ($mg_{GAE}/g_{extract}$)	DPPH• (IC_{50} mg/mL)
IF	9.27	16.241 ± 0.016**	0.210 ± 0.008
ID	17.03	3.654 ± 0.006	2.095 ± 0.232
TF	21.60	8.552 ± 0.021	0.344 ± 0.016
TD	57.76	6.161 ± 0.013	0.813 ± 0.074

*IF, Iran Fresh fruit; ID, Iran Dry fruit; TF, Turkey Fresh Fruit; TD, Turkey Dry fruit. **mean ± SD (n=3)

The chemical composition of the extracts of both samples was investigated using reverse phase HPLC. Due to HPLC analysis, three different substances could be identified such as benzoic acid, luteolin, and ascorbic acid, within the extracts (Table 2, Figure 2). Although ascorbic acid was the major chemical component in all of the extracts, only luteolin and benzoic acid could be detected in only one of the extract (Table 2). According to the Table 2, the amount of ascorbic acid was varied between 24.18 % and 151.89 %. The previous results within the literature for total ascorbic acid obtained from wild growing *R. canina* were calculated between 2189.7 mg/100g and 2404.0 mg/100g. The ascorbic acid amounts can be varied between species and due to the temperature, geographic area, cultivation and storage time (Taneva *et al.*, 2016).

Table 2: HPLC analysis of the extracts of *Rosa canina* samples

Samples*	Luteolin (%)	Benzoic acid (%)	Ascorbic acid (%BAE**)
ID	____	____	47.28 ± 0.33
IF	1.20 ± 0.04***	____	24.18 ± 0.16
TD	____	____	151.89 ± 0.84
TF	____	4.26 ± 0.05	57.42 ± 0.24

*see Table 1; **BAE, benzoic acid equivalent;
***mean ± SD (n=3)

R. canina is a remarkable plant among other *Rosa* species due to its high phenolic content. Also, the concentration of ascorbic acid in *R. canina* is higher than other species (*Czyzowska et al.,* 2015). Furthermore, the comparison between *R. canina, R. sempervirnes, R. coccinea* in an experiment showed that the amount of bioactive compounds having the antioxidant activity was higher in the *R. canina* (Kerasioti *et al.,* 2019). In another experiment about the chemical composition of fruits of *R. canina*, *R. dumalis* subsp. *boissieri*, *R. dumalis* subsp. *antalyasis*, *R. villosa, R. pulverulenta* and *R. formis*, the highest phenolic content was observed in *R.canina* (96 $mg_{GAE}/g_{extract}$) (Ercisli, 2007).

DPPH radical scavenging activities of the extracts of both plant samples were tested at room temperature and the calculated IC_{50} values are given in Table 1.

A moderate correlation (r^2=0,61) was calculated between radical scavenging activities and total phenolics amounts in all of the extracts. According to the Table 1, the most active extract was fresh fruit extract of the Iran sample. These results correlated with the previous papers related with IC_{50} values of *Calendula* extracts (Kerasioti *et al.,* 2019).

On the other hand, the antiradical activities of the extracts were also investigated with $ABTS^+$ radical scavenging assay. Antiradical activities of *R. canina* extracts are shown in Figures 2 and 3 at two different concentrations (0.06 mg/ml and 0.03 mg/ml) due to time and absorbance as kinetically. According to the results, only ID and TD extracts showed reverse ranking at low and high concentrations. The antiradical activity orders of the extracts are as follows; TD>ID>TF>IF for 0.03 mg/mL and ID>TD>TF>IF for 0.06 mg/mL.

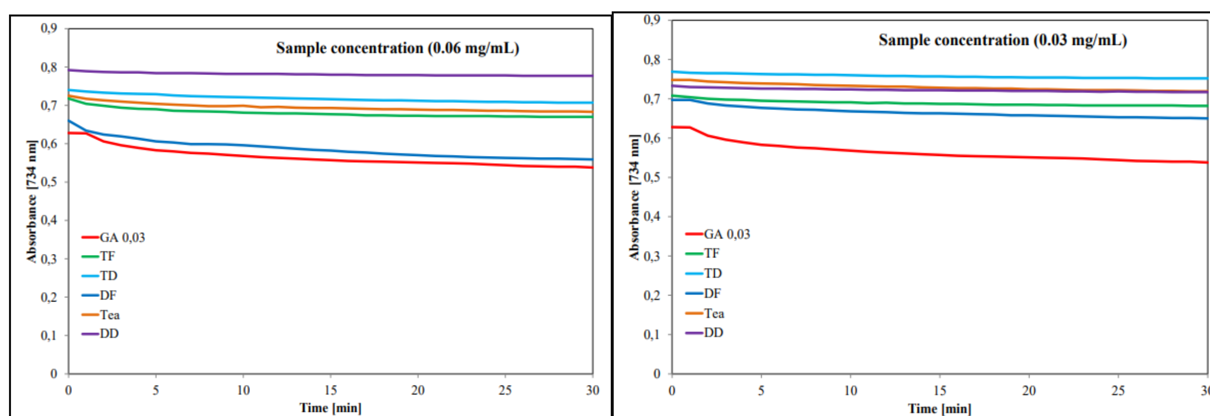

Figure 1: $ABTS^{+\bullet}$ scavenging activity of *R. canina* extracts in 0.03 and 0.06 mg/mL concentrations.

The high radical scavenging ability, established by using $ABTS^{+\bullet}$ assay, could be explained due to the high content of ascorbic acid. The antioxidant activity of 50% ethanol extract was mainly due to the high level of total phenolic content, which is similar outcome in comparison with another research that was conducted to evaluate the radical scavenging ability (Montazeri *et al.,* 2011).

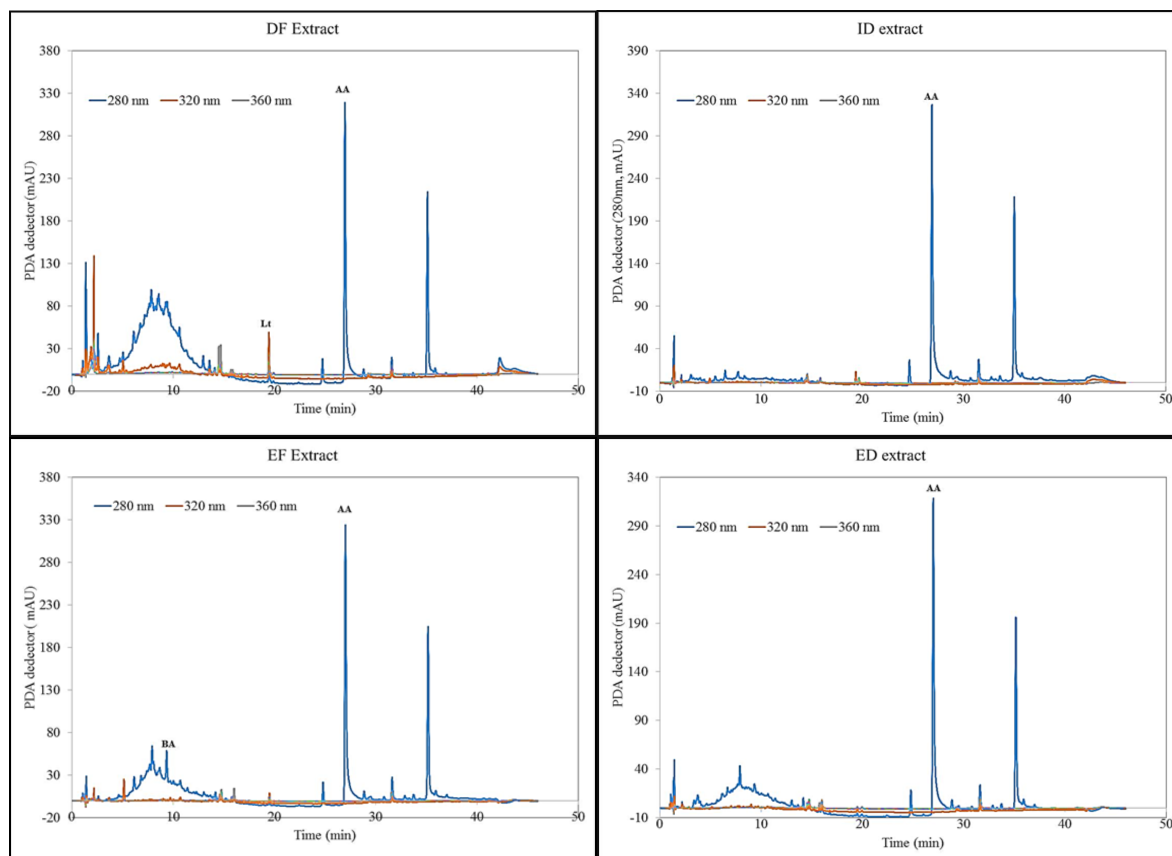

Figure 2: HPLC chromatograms of extracts.

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Short Review: A new promising technology to ensure drug safety

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Abstract

With the recent technological developments and the declaration of an individual or a community known as Satoshi Nakamoto in 2008, it can be assumed that the blockchain technology is mainly used for cryptocurrency, and it is the beginning of a disruptive transition in every area of existence. However, this technology has attracted attention towards providing security in the pharmaceutical supply chain in recent years as well.

Benefits and drawbacks of good manufacturing practies (GMP) and supply chain tracking in pharmaceutical manufacturing can be determined via blockchain and sensor (Radio Frequency Identification Technology) technologies and these technologies can ensure the protection and health of the chain all the way up to the patients.

Furthermore, the capacity can be tracked back to the raw material and pharmaceutical supply chain, which essentially ensures the drug's protection by gathering, transferring, and exchanging drug data in the manufacturing, storage, delivery, and sales connections. After a precise study conducted by the experts in this particular area, it was determined that the concept of counterfeit drugs can be fully eliminated.

Keywords

Blockchain technology, counterfeit drugs, drug safety, good manufacturing practices (GMP).

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INTRODUCTION

Drug formulations are used to treat common illnesses such as headaches, colds, coughs, and runny noses, as well as more severe conditions such as stomach ulcers, cardiovascular disorders, infection removal, and even life-threatening diseases. However, if drug formulations are not produced, stored, and distributed on a regular basis under adequate manufacturing, storage, and distribution conditions, they may pose a threat to life. As a result, pharmaceutical manufacturers must be able to ensure that drug formulations are tested using validated testing methods and that calibration, equipment, and instruments are carried out in accordance with regulations and laws. Furthermore, the quality control and quality assurance departments in the facilities should provide appropriate quality under predetermined parameters (Bocek *et al.,* 2017).

Furthermore, it is the responsibility of the drug company to ensure that each drug product is faultlessly produced and delivered to the patients. Many of these commitments have been expressly stated by the authorities in the standards of GMP. In this case, GMP, which is essential in drug production, can be interpreted as a set of rules that ensures safe and efficient production in the pharmaceutical industry (Rabah, 2017). Nonetheless, counterfeit drugs continue to be a major problem in drug markets around the world, resulting in the deaths of nearly one million people per year.

Despite the fact that the drug manufacturing is strictly regulated by authorities, the prevalence of counterfeit products demonstrates that transparency, identity authentication, and visibility which play essential roles in the drug supply chain, are not entirely feasible under the current system (Sadouskaya, 2017). It is unknown whether the required conditions are established or not in the drug storage and shipping conditions that should be used in the current drug tracking systems, especially in cold chain conditions. As a consequence of this scenario, the safety of vaccines and protein-based drugs has been jeopardized. (Nugent *et al.,* 2016). Because of the complexities of traditional systems and fragmented technical solutions, it is becoming increasingly difficult to monitor a single drug package from point of development to point of consumption, and it is much more difficult for parties to account for it when the issues occur (Sadouskaya, 2017; Rabah, 2017). Exploration of these new technologies to eradicate all of these negative circumstances and protect human health is

as inevitable as the generation of creativity in the drug formulation production and distribution processes (Roberts *et al.,* 2018). The present review article point out that the blockchain technology, which has developed rapidly, can be used to resolve the problem of confidence in the pharmaceutical supply chain.

Blockchain technology

In the early 1990s, the concept of blockchain was debated. However, this technology became more relevant after a paper posted on a website (metzdowd.com) by an individual or a group known as Satoshi Nakamoto in October, 2008. The first sign of blockchain technology was the Bitcoin: A Peer-to-Peer Electronic Cash System. This technology saves records as thousands of data on the server, and the blocks form a structure (Bocek *et al.,* 2017). There is not a way to alter any documents in this manner. Since each record has a timestamp, when it wishes to update a record, the records on thousands of servers must be updated entirely (Peters and Panayi, 2019).

As a result, blockchain technology can be described as a highly reliable infrastructure technology in which encrypted records in the system can be controlled by a stable, distributed structure and the approved individuals can track all of the movements.

Advantages of Blockchain technology

Blockchain technology has many features that can be useful in different areas. Moreover, it can bring benefits for all of the components that takes place in production, especially for customers. Such benefits and key factors have been listed in the following:

1- *Compliance and transparency*. These are the most important advantages of blockchain. These properties will aid in the elimination of organizational silos within current sections of the supply chain, as well as the leadership's understanding of how to make the supply chain more competitive on various levels.

2- *Tracking and Tracing*. Companies can obtain and provide better knowledge about the product life cycle as a result of these features, which provide all forms of detailed information.

3- *Reduction of errors in auditing*. Because of this blockchain function, auditing reports can be submitted to the digital ledger and easily reviewed and confirmed.

4- *Fraud security.* Blockchain technology is “unhackable.” It reduces the likelihood of some kind of fraud. Furthermore, it does not depend on patches, making blockchain the most stable cybersecurity project in the market.

5- *Increase of customers' trust.* Blockchain allows patient to find all necessary information about the origins of the products, way of transportation and packaging, which consequently increases their trust to the company. Even only this feature demonstrates the necessity of using blockchain in drug production.
6- *Real-time feedback from consumers.* Patients may react in real time to items they have received. It will assist different parties in the supply chain in analyzing their work and avoiding many mistakes (Sadouskaya, 2017; Nugent, 2016; Prisco, 2019).

GMP and Blockchain technologies in drug safety

GMP covers all stages of production in pharmaceutical production which includes starting materials, production site, equipment, training and hygiene of each personnel. Detailed procedures for each process that may affect the quality of the complete product in GMP are essential. There should be systems that provide documented evidence that the correct procedures are followed consistently in every step of the production process. Nowadays, to ensure this, manufacturers provide records by keeping standart operation procedure (SOP) documents (Peters and Panayi, 2019). However, there are many drawbacks to it. In this system, the fact that these records reflect the truth when it is the human factor is a condition of the record holder. At this point, blockchain technology, which is an unchangeable recording system, finds solutions to the problems that will arise from the human factor. However, it will provide a database that auditors can rely on when reviewing all these records. In addition, the manufacturing companies will be able to review their production records in an instant. Thus, they will play an important role in increasing their quality and saving time (Li *et al.,* 2006; Apte and Petrovsky, 2016).

GMP- Blockchain and sensor technologies in drug safety

All of the digital records created with the sensors has no possibility to change after these records registered on blockchain basis. This will show whether the products are in compliance with the SOP standards. Thus, whether or not the manufacturer makes production in accordance with GMP standards, the quality of pharmaceutical production will be able to monitored and checked. In addition to all of these, auditors will be able to make continuous follow-up. Thus, it will be an important step in securing human health (Radanović and Likić, 2018; Bocek *et al.,* 2017)

The utility of blockchain technology in the safe drug supply system has been shown in detail (Figure 1).

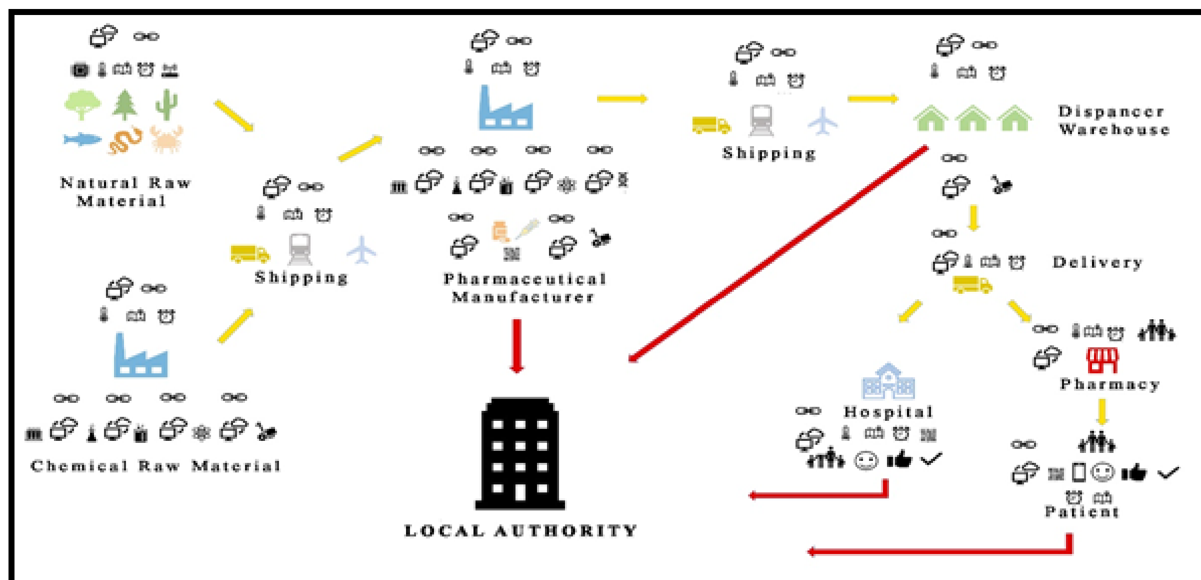

Figure 1: Original and safe drug supply system with blockchain technology.

CONCLUSION

Except drug manufacturing technology, there are various applications of blockchain technology for example, smart contracts, operational risks in financial markets and payment systems. However, pharmaceutical production is the most important area for safe production. Drug formulations should be produced within appropriate and safe conditions and delivered to the patients.

Drug packages are authenticated, timestamped and placed on the blockchain at each delivery point through the drug production process. Therefore, the drug packages can be tracked. Blockchain makes the distribution of medicines transparent and secure, so it can prevent the drugs from thefts and reduce the possibility of price manipulation and delivery of expired drugs. In addition to the use of the blockchain to ensure the transparent safety of pharmaceutical production, sensor technology is also needed to assist it. Moreover, with all these technologies available, GMP rules should be strictly apply in pharmaceutical production. Another advantage of this technology is that the problems in process such as shipment are detected immediately and these problems are solved at the beginning of the process to prevent time and labor losses.

In addition, it will be possible to eliminate the market shortages of drugs by providing communication between independent points such as social security institution, retail point of sale, warehouses and producer. Furthermore, the blockchain technology will allow the fight against counterfeit drugs. Thus, social security institutions will not reimburse the price of

counterfeit drugs. In addition to the important advantages of blockchain in the drug supply chain, it is inevitable that sensor technology should be used with the blockchain in order to make the pharmaceutical production completely transparent.

In conclusion, considering all of the advantages and disadvantages of this new technology, which has been used in many fields in the recent years, has to be accepted as a promising technology in the production of safe drugs.

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