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LETTER TO EDITOR

A New and Different Perspective on Traditional and Complementary Medicine

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Dear Editor

I would like to present to you and your readers a different perspective on the classical medical education I received at the medical school like any physician and then on the art of routine patient treatment in the medical profession.

I was born in Izmir in 1970. After primary and secondary education, I graduated from Samsun Ondokuz Mayıs University Faculty of Medicine in 1994 as a medical doctor. When I was a student at the medical school, I started to think that there were some deficiencies in medical knowledge and treatment techniques in the lectures taught to us. As a matter of fact, a complete treatment of many diseases has not been performed yet. I realized that the treatment techniques in the current system are symptom-suppressing, and in fact I came to the conclusion that the underlying causes of the diseases should be eliminated. Even when I was a student in medical school, I tried to do some studies on physiopathology and developed a treatment technique I named "Remember-Regeneration Therapy Method" (RTM). After my researches and trials, I focused on my studies in the field of holistic medicine.

Throughout my more than 25 years of medical experience, my search for perfecting the treatment technique continued and in this direction, I received a master's degree in Pharmacognosy at Ankara University Faculty of Pharmacy in 1996. Since the 1990s, I have trained and provided trainining more than 40 in traditional and complementary medicine in nearly 30 different countries, including Russia, the USA, Germany, South Korea, India, Canada, Switzerland, Spain, Azerbaijan, France, Romania, and I attended seminars. Since the beginning of my medical adventure, I have been happy to contribute in helping patients recover in more than 140 thousand cases, almost all of which are considered difficult. Most of these diseases consist of psoriasis, urticaria, rheumatoid arthritis, gangrenous foot, celiac, MS and many types of cancer, and I have achieved great success in the treatment of these diseases. In our clinic, treatment success rates are between 75% and 95% in this and similar challenging diseases related to the immune system. As a result of our success both from Turkey and many parts of the world, there have been intensive patient applications; also I am proud of that the RTM system and some of the treatment achievements with the system are published in academic publications and presented to the scientific community. I currently have over 10 published academic articles on RTM treatment.

According to my personal opinion; today, the biggest problem in the field of traditional and complementary medicine is that most of the physicians who are interested in this field try to treat their patients with an approach that is far from holistic medicine perception. I think every detail of the treatment should be carried out in a systematic, method and goal. The patient and the patient's problems should be approached physiopathologically, especially a resource-oriented approach should be adopted rather than result-oriented. Each patient may have their own special circumstances. Two patients with the same disease may not have the same causes. For this reason, the perception of "there is no disease, there is a patient" is of vital importance in the way of healing. Unfortunately, the commercial approaches recently exhibited by some of our colleagues in this

field marginalize traditional and complementary medicine practices, which contain thousands of years of ancient knowledge, in the eyes of the public and authorities.

Another problem that we encounter in the field of traditional and complementary medicine is that these practices are not fully understood in the eyes of the public and are perceived as treatments that are far from scientific. In fact, the situation is on the contrary in developed countries. Many patients with scientific background prefer these methods in their treatment. To me reason for this negative perception in Turkey "stabbing" called "cupping" have been practiced in places unlicensed, made by people without medical competence and licenses, so, this is causing damage to the people of these applications. Traditional and Complementary Medicine Practices lose prestige in the eyes of the public and they experience a lack of trust in the eyes of the patients, even though it is forbidden to make such applications by unauthorized persons, because there is not effectively prevention. In my opinion, Traditional and Complementary Medicine Practices should only be applied by physicians trained in this field and authorized institutions should not allow the activities of these unlicensed enterprises.

It is a big problem that many products that can be called phytotherapeutic today are not produced under appropriate conditions, in the right technology and with the right method. Phytotherapeutics form the basis of the RTM system, which takes place in a holistic perception of medicine. In my professional journey, I founded the company "Naturin Nutraceuticals" in 2005 to provide the right phytotherapeutics to my patients, within the framework of the steps to perfect my treatment technique. Naturin Nutraceuticals moved to its new campus in 2014 with new investments. Today it made a distinguished name as a facility in the area of food supplements.

Health is a sacred area in the medical profession and in my perception. And as in every sacred area, medicine has responsibilities and rituals. Honesty is among the foremost rituals in this area. The RTM system has been shaped in line with this understanding of reality. Discourses that create a false perception that do not reflect the truth create a reaction against traditional and complementary medicine practices with a multiplying effect over time, which puts other physicians who carry out their business ethically under suspicion.

Studies and publications on "holistic medicine/ traditional and complementary medicine" are increasing day by day, especially in developed countries. The result of the perception of treating diseases with "methods of suppressing symptoms" is before us. Medical science has now entered a return journey to essence. Turkey itself must not be abstracted from this transformation and the leadership of the university in the true healing to reach this point in academic work should be done, these studies should be integrated into academic publications. I wholeheartedly congratulate your magazine and your team that served this purpose.

In today's world, nearly 70% of health expenditures are made for chronic diseases. According to data from 2015, in Turkey, the number of "Type 2 Diabetes" patients just using the drug at least 3 times a year has reached to 13 million. Modern medicine cannot treat these types of diseases, exhibiting a symptom-suppressing perspective due to its philosophy, and each new patient increases the total number of patients to higher figures. With this approach, healthcare systems are not sustainable either economically or in terms of public health and give alarm all over the world. Traditional and Complementary Medicine Practices, on the other hand, can give permanent positive results directly on chronic diseases, even if they are applied alone. The RTM system has achieved proven success in chronic diseases thanks to its revolutionary approach in Holistic Medicine perception.

My opinion is that; Traditional and Complementary Medicine Practices should be emphasized, disseminated and explained to the society correctly as a state policy in order to achieve a sustainable and healthier society. If we do this, a real transformation in health will occur and we will be counted among the countries that lead world medicine.

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ORIGINAL RESEARCH

Ethnomedicinal Herbal Knowledge and Practice among elders in Igalamela-Odolu Local Government Area of Kogi State, Nigeria

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Abstract

Objective: Documented Population based data on the use of herbal medicinal products and traditional knowledge among the younger generations is lacking in Nigeria and Africa at large and this is due to dearth of information passed across by the elderly ones. The aim of this study is to investigate and document the extent of use and general knowledge of herbal medicine among elders in Igalamela-Odolu Local Government of Nigeria for its use by the younger and future generation.

Methods: This study was carried out in Uwowo and Ajaka communities in Igalamela-Odolu Local Government Area of Kogi State in the year 2018, using a semi structured questionnaire/Interview and informal conversation with the respondents. Data collected were analyzed using Data analysis plus to generate frequencies.

Results: In total, sixty-eight (68) plant species distributed under forty (40) families with their ethnomedicinal uses were documented. Gastrointestinal tract disorders ranked highest among the categories of diseases cited by the respondents. It stood at 18.28% plant species cited by 20.15% respondents while Opthalmology and Venereal diseases ranked the lowest with 1.08% as mentioned by 0.85% respondents.

Conclusions: The study revealed that older generation are the major custodians of herbal knowledge. There is therefore the need for proper documentation of the use of herbal medicines and transfer of knowledge by the elderly population to younger and future generations for the management and treatment of human diseases.

Keywords: Herbal Medicine, Gastrointestinal Tract Disorder, Traditional Knowledge, Igalamela

INTRODUCTION

Traditional knowledge (TK) is a knowledge developed, sustained and passed on within a traditional community and between generations. It is the knowledge developed around a given conditions of the environment indigenous to a specific geographic zone[1]. It is cost effective, readily available, socially desirable and economically affordable.

Ethnomedicine is the use of plants in an unorganized medical system or formal training by members of an indigenous culture. It is also referred to as herbal medicine or native medicine in the traditional African curative system. Medicinal plants have been identified and used throughout human history and the documentation of traditional knowledge particularly on the medicinal uses of plants has offered many significant drugs of modern day[2,3]. According to the World Conservation Union (WCN), it has been estimated that one-quarter of all prescription drugs are developed from plants and several of these come from the humid tropical forests[4]. Over 80% of people living in developing countries rely on herbal medicines as their immediate choice in the treatment of diseases confirming its importance in Primary Health Care[5].

Herbal medicine use is widely popular because of the growing interest in health promotion and folk healing in the general population, dissatisfaction with some biomedical therapies and public recognition of herbal remedies due to advertising and media reports. However, the documentation of traditional knowledge on medicinal plants usage by the native people in Kogi State is still far from complete. Studies have been carried out on the ethnobotanical herbal knowledge and practice in

different local government areas and communities in Kogi state[6,7,8,9]. However, not much is known on the involvement of people on the uses of medicinal plant species and documentation of traditional knowledge in Igalamela-Odolu local government area of Kogi state. Hence, the aim of this study is to evaluate the level of use, and the general knowledge of ethnomedicine among the elders resident in Ajaka and Uwowo communities of Igalamela-Odolu Local Government Area of Kogi State in the North Central region of Nigeria.

MATERIALS AND METHODS

Description of study area

This study was conducted in Uwowo and Ajaka communities, Igalamela-Odolu Local Government Area of Kogi state (Figure 1) in October, 2018. Igalamela-Odolu Local Government Area is bordered by the Niger River in the West and Enugu State in the east. Its headquarters are located in the town of Ajaka in the north of the area at $7^0$10'16"N and $6^0$49'35"E. The northeasterly line of equal latitude and longitude passes through the Local Government Area. It is endowed with numerous forest reserves and community forests. It has a landmass of 2,175 km^2 and a population of 148,020 at the 2006 Nigeria Population Census[10]. The people depend mostly on the natural environment for their livelihood; they are involved in subsistence Agriculture. The climate of Igalamela-Odolu Local Government Area is divided into the tropical wet and dry climate. Government Area is divided into the tropical wet and dry climate. The rainy season lasts from April to October while the dry season lasts from November to March.

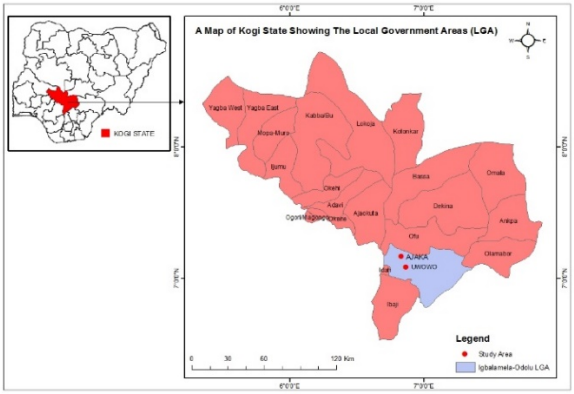

Figure 1. Map of study area

Sample procedure and data collection

The purpose of the study was explained to the respondents in the communities and informed consent was obtained from each of the respondents. The ethnobotanical data were collected through oral interview in native dialect and detailed discussions were conducted with elderly men and women knowledgeable and experienced in treating wide range of diseases with herbal preparations according to the method of Martin[11] and Betti[12]. Each plant recorded was mentioned by at least two respondents among a total of thirty-nine (39) consisting of 18 men and 21 women that were interviewed. Semi-structured questionnaire was used to collect data. The plants were identified and collected at the point of location. Data were collected to give the families, botanical names, common names and local names of the various plant species. Their use(s), specific plant part used and method of preparation were also recorded. Identification of some species was done by the authors while the difficult ones were done with assistance of an experienced taxonomist at the botanical garden, University of Ibadan. Standard literatures and floras such as Flora of West Tropical Africa[13] and medicinal plants from Nigeria[14] were also consulted.

Data analysis

Data collected were analyzed using Data analysis plus to generate frequencies. The results are presented in tables, bar chart and pie chart.

RESULTS AND DISCUSSION

The present work is based on the local knowledge of most commonly used plants by the elders within the area of study. Each plant species used is provided with its scientific name, family, common name, local name, habitat, plant parts mostly used, uses and method of preparation of the herbal remedies. The plant materials collected from the study area using the identification methods, and their medicinal information given, was examined and gathered which are described below.

Plant species

The study showed a total of sixty-eight (68) plant species distributed under forty (40) families.

Table 1. Ethnomedicinal plants of the Igala people with their uses

S/No	Family	Botanical Name	Common Name	Vernacular Name (Igala)	Plant parts used	Method of preparation	Ailment treated/Therapeutic effect
01	Amaranthaceae	*Amaranthus cruentus* L.	Purple amaranth	Etete-pupa	Leaves	Decoction with *Kigelia africana* leaves	Constipation, Fever & Anaemia
02	Anacardaceae	*Mangifera indica* L.	Mango	Umagolo	Stem- bark	Decoction	Anaemia
03	Anacardaceae	*Spondias mombin* L.	Hog plum	Ochikala	Stem-bark	Decoction	Malaria/Typhoid
04	Annonaceae	*Uvaria chamae* P. Beauv	Bush banana	Ailoko	Root-bark	Poultice	Swollen legs; Venomous bites/stings (snake, scorpion, etc)
05	Asclepiadaceae	*Calotropis procera* (Aiton) W.T Aiton	Apple of Sodom	Ebogu/Ugbabe	Latex	Extract the latex and apply	Wound healing; Toothache
06	Araceae	*Colocasia esculenta* L.	Cocoyam	Ikachi	Corm (Tuber)	Boil	Tonic
07	Arecaceae	*Cocos nucifera* L.	Coconut	Unoba	Seed	Liquid is collected from the fruit and drink	High body temperature
08	Asteraceae	*Ageratum conyzoides* L.	Goat weed	Iloji-anagbo	Leaves	Decoction with addition of Malt drink	Anaemia
09	Asteraceae	*Vernonia amygdalina* Delile	Bitter leaf	Illo	Leaves	Juice with *Ocimum gratissimum* to in water	Menstrual disorder
10	Bignoniaceae	*Kigelia africana* (Lam.) Benth.	Sausage tree	Ebie	Leaves	Juice with *Amaranthus cruentus* & *Mentha arvensis*; Cook as soup but no application of seasoning	Convulsion; Infertility in women
11	Bignoniaceae	*Newbouldia laevis* (P. Beauv.) Seeman ex Beauv.	African border tree	Ogishi	Stem-bark Leaves	Decoction; Juice with addition of a little salt; Juice	Induces labour; Toothache; Dysentery
12	Boraginaceae	*Heliotropicum indicum* L.	Indian heliotrope	Okogunu	Leaves	Decoction	Skin infection; Headache
13	Caesalpinaceae	*Burkea africana* Hook	Wild syringa	Ofo	Stem-bark	Juice	Swollen body
14	Caesalpinaceae	*Daniella oliveri* (Rolfe) Hutch & Dalziel	African copaiba balsam tree	Agba	Stem-bark	Decoction	Blood tonic
15	Caesalpinaceae	*Senna alata* (L.) Roxb.	Candle bush	Ogujeba	Leaves	Apply leaf sap or make a poultice	Skin infection, Itching

16	Caesalpinaceae	*Senna obtusifolia* (L.) Irwin & Barn.	Sicklepod	Idagbofifi	Leaves	Decoction	Sore throat, Laxative
17	Caricaceae	*Carica papaya* L.	Pawpaw	Echibakpa	Leaves; Fruit	Juice; Slice the unripe fruit & soak in water to ferment for 24 hours	Induces labour, venereal diseases; Typhoid/Malaria
18	Clusaceae	*Garcina kola* Heckel	Bitter kola	Egoligo	Seed	Crush 7 seeds with 2 garlic together & squeeze 2 lime oranges in water, then add natural honey	Asthma
19	Connaraceae	*Byrsocarpus coccineus* Schum. &Thonn.		Achamadele	Root-bark	Chew; Poultice	Stomachache; Venomous bite/sting
20	Convulvulaceae	*Ipomoea batatas* (L.) Lam.	Sweet potato	Odumu	Tuber	Boil	Tonic
21	Euphorbiaceae	*Alchornea cordifolia* (Schum. &Thonn.) Mull. Arg.	Christmas Bush	Oyi	Leaves	Decoction	Stomachache, Colic
22	Euphorbiaceae	*Bridelia ferruginea* Benth		Ede	Leaves:stem-bark (2:1)	Decoction	Fibroid, Laxative
23	Euphorbiaceae	*Euphorbia hirta* L.	Asthma herb	Omiaku-ikele	Whole plant	Decoction	Hemorrhoids
24	Euphorbiaceae	*Hymenocardia acida* Tul.	Wedding hat	Enache	Stem-bark	Chew; Decoction	Dysentery; Stomachache
25	Euphorbiaceae	*Jatropha curcus* L.	Physic nut	Ikekene	Leaves; Stem	Decoction; Poultice	Cough; Wound
26	Euphorbiaceae	*Manihot esculentus* Crantz	Cassava	Abacha	Tuber	Poultice	Venomous bites/stings
27	Euphorbiaceae	*Phyllanthus muellerianus* (Kuntze) Exell.		Oganana	Leaves	Decoction	Worm expellant, Migraine
28	Lamiaceae	*Mentha arvensis* L.	Wild mint	Ashefa	Leaves	Decoction/Infusion	Catarrh
29	Lamiaceae	*Ocimum africanum* Lour.	Lemon basil	Curry	Leaves	Condiment in soup	Flatulence, Colic
30	Lamiaceae	*Ocimum grattissimum* L.	Scent leaf/ African basil	Anyeba	Leaves	Condiment in soup	Dysentery, Tonic
31	Lauraceae	*Persea Americana* Mill	Pear	Pear	Fruit	Eaten fresh	Hypertension
32	Leguminosae	*Arachis hypogea* L.	Groundnut	Opa	Seed	Eat fresh uncooked	Ulcer
33	Leguminosae	*Pterocarpus erinaceus* Poir	African Kino	Ache	Leaves	Decoction	Infertility

34	Loganiaceae	*Anthocleista djalonensis* A. Chev.	Cabbage tree	Odogwu	Leaves	Decoction	Stomachache, Colic
35	Loranthaceae	*Tapinanthus dodoneifolius* (DC.) Danser	Mistletoe from *Parkia biglobosa*	Oche-oliugba	Leaves	Decoction	Cough, Painkiller
36	Loranthaceae	*Tapinanthus* spp.	Mistletoe from any plant	Oche-oli	Leaves	Dry and grind to powder form and add to pap	Miscarriage
37	Lythraceae	*Lawsonia inermis* L.	Henna tree	Oli-inale	Root-bark	Decoction	Weight reduction
38	Malvaceae	*Corchorus capsularis* L.	White Jute	Bolibo	Leaves	Condiment in soup	Laxative, stimulant
39	Malvaceae	*Gossypium hirsutum* L.	Cotton	Totowu	Leaves	Juice	Blood disorders
40	Malvaceae	*Sida acuta* Burm. f.	Common wirewood	Efa	Root	Crush and add potash as poultice for 24 hrs; Squeeze 4 lime oranges, 3 garlic and boil together. Leave to ferment for 24 hrs	Rheumatism; Waist pain, Heart problem
41	Meliaceae	*Azadirachta indica* A. Juss	Neem tree	Oli-neem	Leaves	Decoction & add Malt drink	Yellow fever
42	Meliaceae	*Khaya senegalensis* (Desr.) A. Juss	Dry-zone mahogany	Ago	Stem-bark	Decoction	Skin infection
43	Mimosaceae	*Prosopis africana* (Guill. & Perr.) Taub.	Iron tree	Ukpiye	Stem-bark	Chew; Decoction	Stomachache, worm expellant
44	Moraceae	*Ficus exasperate* Vahl.	Fig tree	Ogbaikolo	Leaves, Stem-bark	Decoction	Blood tonic
45	Moringaceae	*Moringa oleifera* Lam.	Moringa	Igeligedi	Leaves, Seed	Dry and grind to powder form; Use as condiment to soup	Low sperm count, High Blood pressure
46	Musaceae	*Musa* spp	Banana	Ogede	Root	Decoction with leaves of *Gossypium hirtum*	Blood tonic
47	Musaceae	*Musa* spp	Plantain	Ogede-agbo	Leaves	Decoction of yellowish leaves mixed with *Citrus aurantifolia* leaves &yellowish *Carica papaya* leaves	Typhoid, Yellow fever
48	Myrtaceae	*Psidium guajava* L.	Guava	Goba	Leaves	Decoction	Dysentery, Fever
49	Poaceae	*Bambusa vulgaris* Schrad. Ex J.C. Wendl.	Common Bamboo	Otacho	Leaves	Decoction	Cough
50	Poaceae	*Cymbopogon citratus* (DC.) Stapf	Lemon grass	Egbo-Oyibo	Leaves	Poultice and inhale; Decoction	Cattarh Headache

51	Poaceae	*Heteropogon contortus* (Linn.) P. Beauv.	Spear grass/wild oats	Elie/Abenedichi	Root; Whole plant	Maceration for 24 hours; Decoction	Vomiting; Stomachache
52	Papilionaceae	*Desmodium mauritianum* (Wild.)DC.	Stick tight	Igbaligba-okolo	Leaves	Decoction	Menstrual pain
53	Papilionaceae	*Desmodium velutinum* (Willd) DC.	Canelapreta	Umogaji	Leaves; Seed	Decoction; Roast , blend and add palm kernel oil	Headache; Migraine
54	Portulacaceae	*Portulaca oleraceae* L.	Common Purslane	Etikeleku	Whole	Poultice	Whitlow
55	Rosaceae	*Parinari curatellifolia* Planch. Ex Benth	Mobola plum	Ijakere	Leaves	Decoction	Blood tonic
56	Rubiaceae	*Crossopteryx febrifuga* (Afzel.) Benth.	English African Bark	Omukpakpa	Leaves; Stem-bark	Decoction and add to Pap; Decoction	Dysentery; Laxative, Hemorrhoid, Lactation
57	Rubiaceae	*Gardenia jasminoides* J. Ellis	Cape Jasmine	Ikaga	Root	Decoction	Rheumatism
58	Rubiaceae	*Sarcocephalus latifolius* (Sm.) E.A. Bruce	African peach	Ogbayi	Leaves	Juice 14 leaves in water	Sore throat
59	Rutaceae	*Citrus paradisi*Macfad.	Grapefruit	Alemu-iba	Fruit	Juice	Fever, lower cholesterol
60	Rutaceae	*Citrus sinensis* L.	Sweet orange	Alemu	Fruit	Juice	Constipation, boost immunity
61	Sapindaceae	*Paullinia pinnata* Linn.		Egwubiomekpa	Leaves	Decoction	Stomach upset, Laxative
62	Solanaceae	*Physalis angulata* L.	Cutleaf ground cherry	Ekpakpo	Leaves	Decoction	Skin infection
63	Solanaceae	*Solanum tovrum* SW.	Wild eggplant	Ika-ewe	Leaves	Cook as soup	Hypertension
64	Tiliaceae	*Corchorus olitorius* L.	Jute mallow	Otakiliko	Leaves	Cook as soup	Constipation, Tonic
65	Urticaceae	*Laportea aestuans* (L.) Chew	Wood nettle	Atewogboligboli/ Atewogbogbodo	Leaves	Decoction; Poultice	Stomachache, Headache; Swollen skin
66	Verbenaceae	*Stachytapheta jameicensis* (L.) Vahl.	Blue porterweed	Eneokaku	Leaves	Decoction	Headache
67	Verbenaceae	*Vitex doniana* Sweet	Black plum	Ejiji	Leaves	Decoction	Swollen body
68	Vitaceae	*Cissus populnea* Guill. &Perr.		Oro-okoyo	Leaves	Decoction	Infertility

Headache and Migraine was the main ailment treated with *Desmodium velutinum*, *Laportea aestuans* and *Stachytapheta jameicensis*. *Daniella oliveri* and *Ficus exasperata* are used as blood tonic while *Sennaalata* is a good remedy for skin infection (Table 1).

The most cited plant family was Euphorbiaceae (10.15%) followed by the family Caesalpinaceae (5.80%) as shown in Table 2.

Tropical plants have been used for medicinal purposes since the evolution of man. Many of these tropical plants are used to treat and cure a wide variety of diseases. The accumulation of knowledge of plant used is passed on from generation to generation. It is the ancient people of all ages that were having knowledge of medicinal plants, which they acquired as a result of trial and error[15].

Plant habits and parts used as medicines

Approximately 34% of the plants mentioned were from herbs, 32.4% were from trees, 27.9% from shrubs, 2.94% from climber and 2.94% from shrubby parasite (Figure 2). This could be attributed to the type of vegetation (derived savanna) of the study areas. Majority (55%) of the herbal medicines mentioned were obtained from leaf while latex produced the least (1%) (Figure 3). The rationale for the use of leaves could be the abundance of phytochemicals they contain. Furthermore, leaves are recognized as the major synthesis site of secondary metabolites in plants and are the most frequently used plant parts by traditional medicine practitioners[16,17]. This also constitutes an advantage as harvesting leaves on a sustainable manner ensures continuity of the plant[18].

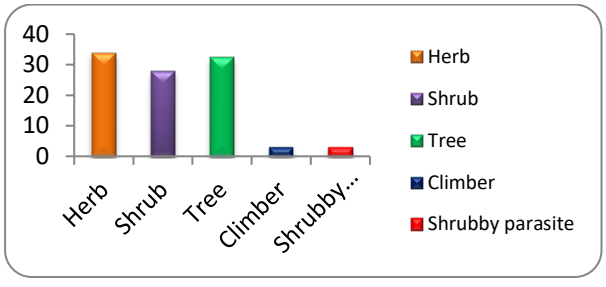

Figure 2. Plants habit of medicinal plants mentioned by respondents

Table 2. Medicinal plants families mentioned by the respondents

S/ NO	Family	Occurrence	% Occurrence
01	Amaranthaceae	1	1.45
02	Anacardaceae	2	2.90
03	Annonaceae	1	1.45
04	Asclepiadaceae	1	1.45
05	Araceae	1	1.45
06	Arecaceae	1	1.45
07	Asteraceae	2	2.90
08	Bignoniaceae	2	2.90
09	Boraginaceae	1	1.45
10	Caesalpinaceae	4	5.80
11	Caricaceae	1	1.45
12	Clusaceae	1	1.45
13	Connaraceae	1	1.45
14	Convulvulaceae	1	1.45
15	Euphorbiaceae	7	10.15
16	Lamiaceae	3	4.34
17	Lauraceae	1	1.45
18	Leguminosae	2	2.90
19	Loganiaceae	1	1.45
20	Loranthaceae	2	2.90
21	Lythraceae	1	1.45
22	Malvaceae	3	4.34
23	Meliaceae	2	2.90
24	Mimosaceae	1	1.45
25	Moraceae	1	1.45
26	Moringaceae	1	1.45
27	Musaceae	2	2.90
28	Myrtaceae	1	1.45
29	Poaceae	3	4.34
30	Papilionaceae	2	2.90
31	Portulacaceae	1	1.45
32	Rosaceae	1	1.45
33	Rubiaceae	3	4.34
34	Rutaceae	2	2.90
35	Sapindaceae	1	1.45
36	Solanaceae	2	2.90
37	Tiliaceae	1	1.45
38	Urticaceae	1	1.45
39	Verbenaceae	2	2.90
40	Vitaceae	1	1.45

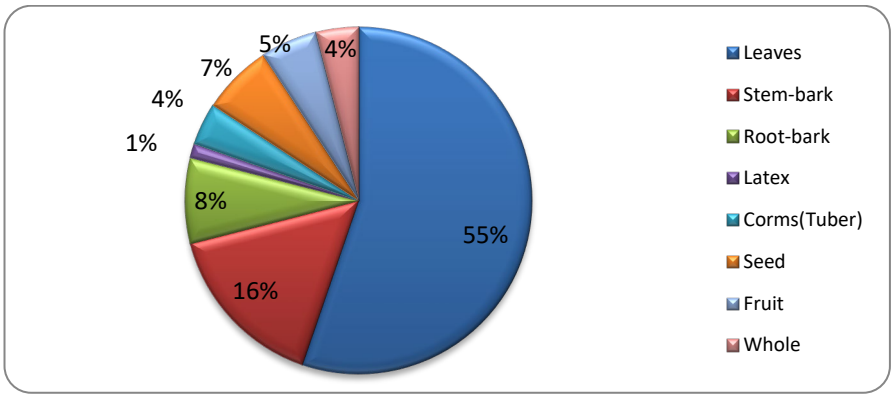

Figure 3. Plants parts of the medicinal plants mentioned by respondents

Disease category

When the diseases were categorized, gastrointestinal tract (GIT) disorders ranked highest among the categories of diseases cited by the respondents (20.51%) and 18.28% of plant species were mentioned for GIT. Next to it was Fever with 15.38% citation and 15.05% plant species mentioned for it. Others were metabolic disorders (12.82%), haematology (11.11%), dermatology (11.11%) and obstetrics and gynaecology (9.40%) with 9.68%, 12.90%, 10.75% and 10.75% number of species of plants cited (Table 3).

Table 3. Disease category in the study area

S/NO	Disease category	Citation	% Citation	Number of species	% Number of species
1.0	Cardiovascular diseases	6	5.13	5	5.38
2.0	Dermatology	13	11.11	10	10.75
3.0	Dental disorders	2	1.71	2	2.15
4.0	Fever	18	15.38	14	15.05
5.0	Gastrointestinal tract	24	20.51	17	18.28
6.0	Haematology	13	11.11	12	12.90
7.0	Metabolic disorders	15	12.82	9	9.68
8.0	Musculoskeletal	4	3.42	3	3.23
9.0	Obsterics&Gynaecology	11	9.40	10	10.75
10	Opthalmology	1	0.85	1	1.08
11	Respiratory/Ear, Nose & Throat	9	7.69	9	9.68
12	Venereal diseases	1	0.85	1	1.08
		117		93	

The high prevalence of GIT disorders in the area could be attributed to feeding habit of the people, moreover, high occurrence of Fever could be attributed to closeness to river[19] and bushes, and the inability to adopt preventive measures like the use of mosquito nets and this could be due to the poverty level of the people in the communities. However, there was no confirmation to support the claims. This may also reflect the conditions of the study area being a rural setting. The health issues are common in rural areas as the finding agrees with the works of Betti[20] in his study of medicinal plants sold in Yaoundé markets Cameroon.

CONCLUSION

The study helps us to understand the ethnomedicinal uses of identified plants to the Igala people of Igalamela-Odolu local government area of Kogi State.

This suggests that ethnomedicinal knowledge can be best obtained from the indigenous people who

use plants, animals, and minerals or have something to do with the various biological resources constantly or more often. The documentation is essential to preserve the ethnomedicinal uses of plants. There is need to create awareness or enlightenment for the conservation of this biodiversity rich area and also the proper use of these floras that would protect the life of this generation and the future generation, as such the senseless destruction of flora that are useful for life maintenance would be curtailed. More so, specialist knowledge of the older practitioners should be transferred to the younger generation and proper documentation of this knowledge be done as this will help the younger and future generations keep the useful aspect of their tradition which is helpful to their life. The documentation of the herbal health remedies in the area under study does not prescribe or recommend for their use till it is been subjected to pharmaceutical analysis in other to validate their authenticity and future prospect.

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ORIGINAL RESEARCH

Evaluation of Traditional and Complementary Medicine Methods in Patients Undergoing Physical Therapy for Chronic Musculoskeletal Pain

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Abstract

Objective: Nowadays, due to the complaints of musculoskeletal pain, patients apply to traditional and complementary medicine (T&CM) methods in combination with conventional medical treatments. In our study, we aimed to determine the frequency and socio-demographic features of applying to T&CM in patients undergoing conventional physical therapy.

Material-Method: Our study is a survey study conducted between January 2020 and March 2020. Patients included in the study were divided into groups in terms of gender and age.

Results: 59 (12%) of 470 physiotherapy patients included in the study tried T&CM treatment at least once due to pain. Cupping therapy has been applied most frequently. It was found that the application to T&CM was significantly higher in patients who received physical therapy before ($p = 0.001$). T&CM applications of patients who received medical treatment for pain previously were found to be significantly higher ($p = 0.048$). When the patients were evaluated according to their gender, body mass indexes, ages, marital status, smoking, pain localizations, there was no significant relationship between T&CM applications.

Conclusion: T&CM therapies have started to reach all patient groups regardless of age and gender. Medical and physical therapy in conventional medicine is preferred for musculoskeletal pain, but patients have also begun choice T&CM methods. More information with should be provided about the effectiveness of T&CM methods, applied by physicians.

Keywords: Traditional and Complementary Medicine, Physical Therapy Modalities, Musculoskeletal Pain, Acupuncture

INTRODUCTION

The definition of Traditional and Complementary Medicine (T&CM) in the world and in our country is expressed as various health practices used for the protection and improvement of health, prevention and treatment of diseases. T&CM applications can be used alone or in combination with conventional medicine approaches[1]. Physical therapy means the use of various physical agents for medical treatment. It has found application in many diseases from the ancient history to the present day. Physical medicine and rehabilitation is used most often for conventional pain relief in musculoskeletal system pain. Apart from this, soft tissue rheumatism, muscle spasm, contractures, hematoma resolution, arthritis, chronic inflammation, peripheral painful neuropathies, joint stiffness, spasticity treatment, pre-exercise preparation are just some of the areas of use[2]. Physical agents are generally classified in three sections as thermal, mechanical and electromagnetic modalities. According to the clinician's indication, more than one physical therapy agent can be prescribed by a physical medicine and rehabilitation specialist. Physical

agents are very safe treatment options with very few side effects, when used in the correct indication.

Physiological effects of physical agents often occur when it helps to reduce and control pain. Effectiveness in treatment does not always mean eliminating the disease. For clinicians, the process of reducing symptoms and/or giving time to other treatments often means effectiveness in treatment. Patients who do not get a definitive result with physical therapy methods, evaluate the cessation of physical therapy or accompanying medical treatment and the application of T&CM methods.

Our aim in this study is to determine the socio-demographic characteristics of patients who received T&CM, due to chronic musculoskeletal pain that applied to the physical medicine and rehabilitation outpatient clinic and the frequency of admission to T&CM applications.

MATERIALS AND METHODS

Our study is a survey study conducted between January 2020 and March 2020. Patients who applied to the physical medicine and rehabilitation outpatient clinic due to musculoskeletal pain and diagnosed with knee, hip, shoulder, foot osteoarthritis, cervical and lumbar discopathy, cervical and lumbar spinal stenosis, lateral epicondylitis, myofascial pain syndrome, and fibromyalgia were examined. Patients aged 18 and over who were treated in the physical therapy unit with complaints of pain were included in the study. In the study, the demographic data like gender, age, BMI and marital status of the participants, habit of smoking, concomitant diseases, duration of pain complaints, pain relief uses, side effects of the pain relievers and exercise status were asked.

In addition, it was questioned whether they had previously received physical therapy and whether they had traditional medicine or not. Patients included in the study were divided into groups as genders and ages.

Approval was obtained from the ethics committee of Bakirkoy Dr. Sadi Konuk Training and Research Hospital for our study. Ethics committee approval was received with the number 2020-387.

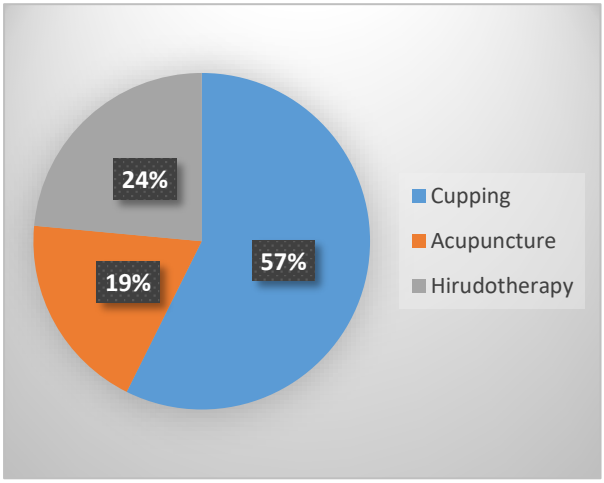
Statistical analysis

Statistical study was done with SPSS program version 24.0. Descriptive data are expressed as mean ± standard deviation and / or percentage. For continuous variables, the difference between the two groups was done using Mann-Whitney U test and independent variable t test, chi-square Pearson test for categorical variables. $P < 0.05$ was considered statistically significant.

RESULTS

Data obtained from 470 patients who received physical therapy in the physical therapy unit of our hospital due to chronic musculoskeletal pain were evaluated. It was seen that 410 of the patients were female and 60 were male. The mean age of the patients was calculated as 55.6 (± 14.2). The youngest patient was 18 years old, while our oldest patient was 91 years old. Considering the marital status of the patients, 377 were married and 93 were single. Body mass indices (BMI) were found to be 29.3 (± 5.3) on average. It was observed that there were 104 patients with BMI between 18-25, 173 patients with BMI between 25-30, 123 patients with BMI between 30-35, 60 patients with BMI between 35-40 and 15 patients with BMI of over 40. It was determined that 399 of the patients had never smoked before. When the duration of pain was evaluated, the mean was 64.6 (± 92.8) months, the shortest duration of pain was 6 months, and the longest was 60 years. Foot pain was present in 10 patients, 156 patients low back pain, 88 patients neck pain, 3 patients elbow pain, 97 patients knee pain, 16 patients hand pain, 11 patients hip pain, 76 patients shoulder pain, 9 patients applied to the outpatient clinic due to back pain. 294 patients said that they had received medical treatment due to similar complaints before. In 49 of these patients, drug-related side effects were observed. They had to discontinue the drug most often due to stomach pain. 59 (12%) of the patients tried T&CM treatment at least once due to pain. The most common was cupping therapy and then they received acupuncture and hirudotherapy, respectively (Graphic 1). Our patients did not prefer other T&CM methods.

When the patients were asked whether they had received physical therapy before, it was observed that 45 patients received 4 or more times, 34 patients received 3 times, 64 patients received 2 times, and 105 patients received once before. 222 patients were receiving treatment for the first time. When patients' complaints were compared with T&CM applications, it was seen that the application to T&CM increased statistically significantly as the duration of complaints increased (p value <0.001).



Graphic 1. T&CM methods that patients have previously applied with pain complaints

Comparing those who received physical therapy before and those who did not, it was seen that the application to T&CM was significantly higher in patients who received physical therapy before (p value < 0.001). T&CM applications of patients who received medical treatment for pain before were found to be significantly higher (p value is 0.048). When the patients were evaluated according to their gender, BMI, age, marital status, smoking, and pain localizations, there was no significant relationship between T&CM applications (Table1). Pearson test used in Gender, Maritial Status, Smoking, Medical treatment before. Mann-Whitney U test and independent variable t test used in age, BMI, Physical therapy before, pain location.

DISCUSSION

Today patients with musculoskeletal pain complaints, use traditional and complementary medicine methods along with conventional medical treatments. According to World Health Organization 2000 data, the frequency of T&CM applications is 80% in Africa, 70% in Canada, 48% in Australia, 42% in the USA, 38% in Belgium and 49% in France. In our country, the frequency of T&CM applications is reported as 42% -70%[3]. The first legal regulation on T&CM in our country is the acupuncture treatment legislation enacted in May 1991 and was revised in 2002. The Ministry of Health published the "Traditional and Complementary Medicine Practices Regulation" in the official newspaper on October 27, 2014[4]. The regulations include acupuncture, ozone, mesotherapy, prolotherapy, hypnosis, hirudotherapy, reflexology, homeopathy, phytotherapy, osteopathy, chiropraxia, maggot applications, apitherapy, cupping and music therapy methods. Evidence-based data on efficacy, safety and mechanisms of action are not yet sufficient. However, there is a growing interest in the community and among healthcare professionals[5].

In our study, 59 (12%) patients tried T&CM at least once due to pain complaints. The most common cupping therapy was tried, followed by acupuncture and hirudotherapy, respectively. In the literature, there is a wide range in the frequency of T&CM use in studies from different countries, and the most important indication for use is muscular and skeletal system pain [2,6]. In a study conducted with 219 patients diagnosed with degenerative osteoarthritis, it was reported that the rate of T&CM application was 35.6%, the rate of benefit was 43.6%, and the frequency of side effects was 3.8%[7]. In two studies conducted in our country on rheumatological diseases, it was reported that the rate of using T&CM methods in patients with rheumatoid arthritis and ankylosing spondylitis was 30.8% and 46.2%, respectively [8,9]. We think that the number of professionals who will apply methods that have indications for musculoskeletal pain such as mesotherapy, osteopathy or prolotherapy is insufficient. Therefore, these therapy methods were not encountered in our study. The reason for the low number of patients applying for T&CM in our study compared to the

Table 1. Demographic data of patients, smoking, duration of complaints, medical treatment uses and pain localizations are available. The results of the p values are shown.

			T&CM* applications		
Gender (n(%))			Yes(n(%))	No(n(%))	*p* value
	Female	410(%87,2)	51(%12,4)	359(%87,6)	*0,589*
	Male	60(%12,8)	8(%13,3)	52(%86,7)	
Age (mean(±stan. Dev.))		55,6(±14,2)	59(%12,6)	411(%87,4)	*0,236*
BMI (mean(±stan. Dev.))		29,3(±5,3)	59(%12,6)	411(%87,4)	*0,18*
Marital Status (n(%))					
	Married	377(%80,2)	44(%11,7)	333(%88,3)	*0,542*
	Single	93(%19,8)	15(%16,1)	78(%83,9)	
Smoking (n(%))					
	Yes	71(%15,1)	7(%9,6)	64(%90,4)	*0,303*
	No	399(%84,9)	52(%13)	347(%87)	
Complaint period (month) (Mean)		64,6(±92,8)	59(%12,6)	411(%87,4)	*0,001*
Physical Therapy before (n(%))					
	No	222(%47,2)	15(%6,8)	207(%93,2)	*0,001*
	1 time	105(%22,3)	24(%22,9)	81(%77,1)	
	2 times	64(%13,6)	4(%6,3)	60(%93,7)	
	3 times	34(%7,2)	7(%20,6)	27(%79,4)	
	≥4 times	45(%9,6)	9(%20)	36(%%80)	
Medical treatment before (n(%))					
	Yes	294(%62,6)	44(%15)	250(%%85)	*0,048*
	No	176(%37,4)	15(%8,5)	161(%91,5)	
Pain Location (n(%))					
	Low Back	156(%33,2)	24(%15,4)	132(%84,6)	*0,768*
	Neck	88(%18,7)	7(%7,8)	81(%92,2)	
	Shoulder	76(%16,2)	12(%15,7)	64(%84,3)	
	Foot	10(%2,1)	1(%10)	9(%90)	
	Back	9(%1,9)	1(%11,1)	8(%88,9)	
	Knee	97(%20,6)	13(%13,3)	84(%86,7)	
	Hand	16(%3,4)	1(%6,3)	15(%93,7)	
	Others	18(%3,8)	2(%11,1)	16(%88,9)	
* T&CM: Traditional and Complementary Medicine					

literature may be that the patients are currently receiving physical therapy and they want to continue the treatment with conventional methods. In our study, it was observed that 45 patients received 4 or more treatments, 34 patients 3 times, 64 patients 2 times, and 105 patients received 1 treatment before. 222 patients were receiving treatment for the first time. When the patients who had received physical therapy before and those who did not, it was observed that the application to T&CM was significantly higher in patients who received physical therapy before (p=0.001). Among the reasons for T&CM application in the literature, diseases with back and neck region pain are in the first place[6]. In our study, patients most frequently received physical therapy for low back pain (33%). Among these patients, T&CM application was also the most common in patients with low back pain. Shoulder area follows the low back area. There was no statistically significant difference between the areas where physical therapy was applied and T&CM treatment. In some of the studies in the literature, they stated that women and patients with a high level of education,

use T&CM more frequently, because of the lower pain threshold of women and the high interest in new treatment methods[8,10,11]. On the other hand, there are studies in the literature showing that the application to T&CM methods and age, gender and education level are not related[7,12].

In our study, when the gender, BMI, age, marital status and smoking were evaluated, no significant relationship was found between T&CM applications. T&CM methods applied by physicians have started to reach all patient groups regardless of age and gender. Some patients use T&CM methods to avoid or delay surgical and invasive treatments, while others consider it as a treatment option they can try if their doctor recommends[13] .

CONCLUSION

In this study, due to reasons such as musculoskeletal pain, which increases in direct proportion to the increase in the life span of individuals, fear of the undesirable effects of conventional medicine drugs, the public's interest has been directed to T&CM and it is thought that this interest will increase even more in the future. In our study, we saw that both physical therapy and traditional and complementary medicine methods were tried many times to relieve patients' pain. With the high evidence-based researches about these methods that have been used in recent years, we should increase the knowledge about their effectiveness and direct the patients to the right methods when necessary.

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ORIGINAL RESEARCH

Traditional Baby Care Practices in Artvin, Turkey

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Abstract

Objective: This descriptive study was planned to examine mothers' traditional practices in baby care.

Material-Method: The study sample consisted of 172 mothers whose babies were treated in the neonatal unit and pediatric ward of a hospital. Data were collected through face-to-face interviews with mothers using "Sociodemographic characteristics form", "Traditional practices form" and "Care practices form".

Results: Mean age of the included mothers was 29.4±0.4 years and first pregnancy mean age was 23.9±0.3 years. Mean number of pregnancies was 2.4±0.1. Mean number of children was 2.1±0.1. Common traditional baby care practices include "forty days" or "half-forty days" ritual (the mother and the neonate bathing together as a ritual on the 20th day or 40th day after birth, with a view to protect the baby from diseases), swaddling, "crowding" (a sudden surprise visit of a crowd of relatives to baby's house in case the baby cannot stand upright or raise her head as expected, at a certain stage of development), using cheesecloth wraps over the baby's head to protect the newborn from jaundice, giving blessed sugar as the first food, waiting for the Azan (call to prayer) before breastfeeding. Education status, working status, income level and residence of the mother were not effective in practicing neonatal traditions.

Conclusion: Based on the results of this study, it was recommended to conduct larger scale and repetitive studies on this topic, in order to clarify the underlying reasons for mothers to adopt and implement such traditional baby care practices.

Keywords: Baby Care, Traditional Practices, Mother

INTRODUCTION

Traditional health care practices are essentially medical practices based on the beliefs, traditions, values and cultures. Each society has its own beliefs and practices regarding health and illness. Female productivity, birth, stopping breastfeeding, sex, death, illness, and suffering are not just private experiences, but all have a social basis. Therefore, health conditions involving aforementioned facts are usually determined by cultural practices, in addition to biological and environmental factors [1].

Traditions are practiced in almost every part of the world with some variations in different regions, families and people [2]. According to Maden, traditional knowledge and practices are recognized by all members of the society and they are anonymous in essence [3]. Furthermore, traditional health and illness practices are perfectly integrated with the other components of culture [4].

Although there are no large scale studies conducted in Turkey about traditional beliefs and practices, several studies are available examining local traditional medical practices. These studies show that some of traditional practices which may risk human health, are still in use today, such as bathing the baby in salt water to prevent her/his sweat smelling bad at later ages. Traditional practices especially for health care purposes are widely used in children [2]. Unfavorable or unhealthful traditional practices may prolong the healing processes, seriously inhibit effective treatment, and even result in death [5,6].

Whatever learned from previous generations and everything acquired and contributed to the culture are transferred to the next generations Therefore, culture is a heritage of one's own society. In other words, each individual inherits culture as a product

of the efforts and experiences of previous generations [7]. It is clear that practices are transferred from generation to generation with the help of cultural traditions [8].

Cultural differences identify societies. Each society has its own inherent traditions, customs, art, music, architecture, social norms, values, rituals, and health beliefs and practices, which do not emerge suddenly, but develops over time as a result of a deep heritage passing through the filters of history. These cultural components penetrate into all aspects of individuals' lives and cause them to develop certain behavioral patterns. Health behavior is one of the most essential ones of these behavioral patterns. Cultural factors affect health behaviors so deeply that they can influence the entire life of the individual. Therefore, this study was planned in order to examine the reflections of traditional neonatal care practices on baby health care and contribute to the literature.

MATERIALS AND METHODS

Type of the study

This is a descriptive type of study.

Time and place of the study

The study was conducted in the period between May-November 2017, in the State Hospital of Artvin, in north-east Turkey.

Target population and sample

The mothers of the infants or children who were under treatment at the neonatal unit or pediatric ward of Artvin State Hospital in the period between May and November 2017 constituted the target population of the study. Mothers with neonates aged 0-28 days in the neonatal unit and with babies aged 0-2 months in the pediatric service were included in the study. A total of 204 mothers were contacted within the specified period, but the sample consisted of 172 mothers who agreed to participate in the study.

Data collection

Data collection was performed through face-to-face interviews of the researchers with the mothers. Mean time to complete the data collection forms was 6 minutes.

Data collection tools

Study data were collected by the researchers using three forms, which were considered to be related to mothers' baby care practices and were created as a result of a detailed literature review [9–11]. These forms are "Sociodemographic characteristics form", "Traditional practices form" and "Care practices form".

-Sociodemographic characteristics form

It is a form for questioning demographic characteristics of the participants such as age, education level, and such demographic data.

-Traditional practices form

This form is prepared to identify common traditional practices in baby care.

-Care practices form

This form consisted of open-ended questions to determine whether the fifteen common baby care practices were practiced by mothers and, if so, in which way.

Data evaluation

Data were evaluated by SPSS 23.0 program. Numbers, mean values and percentages were used for descriptive data. Variance analysis and Student's t test were used as parametric tests, Kuruskal Wallis and Mann Whitney U tests as nonparametric tests in searching for differences.

Ethical aspect of the study

Ethical approval was obtained from Artvin Coruh University Ethics Committee (2017/3 session and decision no: 11), written permission from the institution where the study was conducted and verbal consent from all participants.

RESULTS

Age of the mothers varied between 18 and 43 (mean age 29.4 ± 0.4) years. Mothers' first-time pregnancy ages varied between 15 and 38 (mean age 23.9 ± 0.3) years. Number of pregnancies was between 1 and 8 (mean 2.4 ± 0.1). Number of children was between 1 and 5 (mean 2.1 ± 0.1) (Table 1).

Table 1. Some demographic data of the mothers

Variable	Min	Max	Mean	SD
Age	18	43	29.4	0.39
Age of first pregnancy	15	38	23.9	0.31
Number of pregnancy	1	8	2.4	0.11
Number of children	1	5	2.1	0.07

When the traditional baby care practices of mothers are examined, 10.5% of the mothers celebrated half-forty days ritual (20 days), while 68% practice forty days ritual (40 days). While 41.3% of the mothers were swaddling the baby, 58.7% were not. Approximately half of the sample (54.1%) used a yellow cheesecloth wrap over the head of their babies in order to prevent the baby from getting jaundice. The rate of those who gave blessed sugared water to the baby as the first food was 22.7%. The mothers who preferred to wait for 3-5 prayer times at the first feeding of the baby was determined as 2.9% (Table 2).

Table 2. Some traditional baby care practices and their frequency in the sample (n=172)

	Yes		No	
Traditional Baby Care Practices	**Number**	**%**	**Number**	**%**
Half-forty days ritual	18	10.5	154	89.5
Forty days ritual	117	68.0	55	32.0
Swaddling	71	41.3	101	58.7
Putting yellow cheesecloth wraps over the head against newborn jaundice	93	54.1	79	45.9
Giving blessed sugar as the first food	39	22.7	133	77.3
Waiting for 3-5 Azan before first breastfeeding	5	2.9	167	97.1

The scores obtained in the Traditional Practices Form lined up from highest to lowest respectively as mothers had secondary education, primary education and higher education, but there was no statistically significant difference between the scores (P> 0.05). Although housewife mothers were involved more in traditional practices than working mothers, there was no statistically significant difference in between, in terms of frequency of traditional practices (P> 0.05). The low-income group was performing traditional practices at highest frequency, followed by the middle-income group, and then the high-income group, but the difference between the groups was not statistically significant (p> 0.05). The frequency of traditional practices was the highest for those living in the village, followed by those in the town and in the city, respectively, but no statistically significant difference was determined between them (p> 0.05) (Table 3).

Table 3. Comparison of mothers' mean scores of traditional practices form with respect to demographic characteristics

	n	%	Mean	SD	Significance
Educational status					
Primary education	77	44.8	2.96	0.168	F:1.347 p>0.05
Secondary education	36	20.9	3.25	0.265	
Higher education	59	34.3	2.71	0.215	
Working status					
Working	35	20.3	2.91	0.291	t:0.92 p>0.05
Housewife	137	79.7	2.94	0.131	
Income level					
Low-income	12	7.0	3.92	0.570	Kw:2.849 p>0.05
Middle-income	152	88.4	2.86	0.125	
High-income	8	4.7	3.00	0.267	
Family type					
Nuclear family	137	79.7	2.85	0.135	MW U:2900.5 p>0.05
Extended family	35	20.3	3.29	0.248	
Residence					
Village	28	16.3	3.14	0.312	Kw:1.802 p>0.05
Town	49	28.5	3.08	0.212	
City	95	55.2	2.80	0.162	

Various traditional practices related to first breastfeeding were reported by 25% of the included mothers, such as giving *Zamzam* water to the baby before the first breastfeeding, applying date syrup to the mother's breast before breastfeeding, giving sherbet to the baby before breastfeeding and squeezing the mother's breast. Umbilical cord stump related practices were reported by 17.4% of the mothers, including putting walnuts or applying breast milk, olive oil or various creams around the baby's umbilical area, throwing or burying the fallen umbilical cord stump at religious or cultural or special places such as mosques, schools, hospitals or three-way junctions. Various practices with a view to protect the babies from getting newborn jaundice were reported by 64% of the mothers. They include covering baby's head with a yellow cheesecloth, breastfeeding frequently, pinning gold coins on the baby, dressing the baby in yellow clothes, putting gold objects into the baby's bath water, covering the face of the baby with a blue scarf and placing the baby under the light. As for abdominal gas problems, 63.4% of the mothers stated that they performed various applications to burp the baby, such as patting on baby's back, giving medicine, giving cumin water, massaging the baby's abdomen, putting a hot towel on the baby's feet, giving Zamzam water or putting the baby in a noisy environment. Practices related with baby's diaper rash were performed by 86% of the mothers, such as applying cream, powder, olive oil, starch or mother's milk, wiping with warm water or changing the diaper frequently. Various applications regarding moniliasis such as wiping with carbonated water, wiping with sugar syrup, giving water following feeding with formula or rubbing mother's hair to the baby's mouth were reported by 43.6% of the mothers. In case of diarrhea, 39.5% of the mothers practiced frequent breastfeeding or giving sugary water to baby and consuming constipating foods, e.g. banana, in addition to mothers themselves being more attentive to what they eat. In case of constipation however, 47.1% of the mothers reported various practices such as giving olive oil to the baby or rubbing baby's anus with olive oil, massaging baby's abdomen, frequent breastfeeding, and using laxative drugs. In case of cough, 57% of the mothers applied a hot towel on the baby's back and chest, gave the baby herbal tea, rubbed baby's forehead, rubbed the baby's chest by olive oil or applied hot administration.

Table 4. Frequencies of some traditional baby care practices

Care practices	Yes		No	
	Number	%	Number	%
First breastfeeding practices	43	25.0	129	75.0
Practices for fall off the umbilical cord stump	30	17.4	142	82.6
Practices for avoiding newborn jaundice	110	64.0	62	36.0
Practices for treating newborn jaundice	88	51.2	84	48.8
Practices for easy burping the baby	109	63.4	63	36.6
Practices related with diaper rash	148	86.0	24	14.0
Practices related with moniliasis	75	43.6	97	56.4
Practices for treating diarrhea	68	39.5	104	60.5
Practices for treating constipation	81	47.1	91	52.9
Practices for treating cough	98	57.0	74	43.0

DISCUSSION

Half-forty (completing 20 days) and forty days (completing 40 days) rituals can be mentioned among the common traditional practices in our country. In a study conducted in Adana, it is stated that women in puerperal period and newborns are not left alone at home during first forty days in the postpartum period, with a common belief to avoid puerperal fever (albasması). Accordingly, at the 20th day of birth half-forty days ritual is practiced and 20 pebbles are cleaned and put into a boiling cauldron along with prayers, and the mother and baby are washed together with this water. At the 40th day of birth, forty-days ritual is practiced, similarly 40 pebbles are cleaned and put into a boiling cauldron along with prayers, and the mother and baby are washed together with this water [12]. In this present study too, 10.5% of the mothers practiced half-forty days ritual and 68%

practiced forty days ritual.

In this study, we determined that 41.3% of the mothers swaddled their babies. Swaddling is one of the common traditional practices in Turkish society, with an intention for the baby to sleep comfortably and to have straight legs. However, swaddling is known to be an important risk factor for developmental hip dysplasia. Similar to our sample, mothers included in other studies were also determined to apply swaddling frequently. Mothers who swaddled their babies constituted 79% of the sample in the study by Biltekin et al. (2004), while 60% in the study by Uğurlu et al. (2004) [2,13]. In another study conducted in Erzurum region, the rate of mothers who swaddled their babies was 71.2% [14].

Another traditional practice as common as swaddling crowding around the baby. This can be described as the situation that the baby can not stand still firmly on her/his own feet and cannot keep her/his head upright. In such a case, a crowd of guests visit the house and surprise the baby; where all the guests take the baby in their hands and naps, and the baby is gently pressed on the gifts brought by the guests, in particular on meat. Another relevant practice is holding the baby on her/his feet, when a crowd or a funeral passes through the street. In this present study, 48.8% of the mothers reported to follow a traditional practice regarding crowding around the baby.

Another common traditional practice related with neonatal care in our country is to cover baby's face with a yellow cheesecloth in order to avoid getting newborn jaundice. In our study, about half of the mothers (54.1%) used a yellow cheesecloth for this purpose. Similar practices were also observed in other studies, at similar rates. Studies show that mothers frequently follow this tradition of using yellow cheesecloth over their babies' faces to prevent jaundice [2].

In this study, 22.7% of the mothers gave blessed sugar water as the first food to their babies. This is a traditional practice aiming to bring up the child as a kind and nicely talking person. Similar practices are also seen in other regions. In the study conducted by Özyazıcıoğlu and Polat (2005) in Erzurum region, 7.8% of the mothers were giving blessed sugar water or *dadak* (a mixture of biscuit and tea) as the first food to their baby [10].

In our study, 2.9% of the mothers followed the tradition of waiting for 3-5 azan, before the first breastfeeding. In a similar study conducted in Erzurum, the rate of mothers waiting for 3-5 azan to breastfeed their babies was 64%. In various studies conducted in different provinces of our country, the rate of such mothers varied between 9.3% and 58.5% [12,15,16].

The rates of various traditional baby care practices are given in Table 4. The rate of mothers varied between 17.4% and 86% in our study with respect to various baby care practices. Among these practices, the lowest rate was related to the fall of the umbilical cord, whereas the highest rate was related to diaper rash. Various traditional practices related to first breastfeeding, breastfeeding, burping, diarrhea, moniliasis, and avoiding neonatal jaundice of the baby were widely used by the mothers included in our study. In relevant studies on the subject, mothers frequently use traditional baby care practices at similar rates to those in this study [4,17]. In their studies for determining traditional methods used by mothers during their pregnancy and postpartum periods in Erzurum region, Çelik, Çapık, Engin (2012) observed that mothers follow traditional baby care practices at different rates, but very commonly [14]. In addition, similar results were observed in several studies conducted in different regions of our country. Traditional baby care practices were determined to be commonly used by Biltekin et al. (2013) in İzmir Ödemiş, Bölükbaş et al. (2009) in Ordu, Ayaz and Yaman Efe (2008) in Ankara, Çetinkaya, Özmen, Canbaz (2008) in Manisa, Yalçın (2007) in Konya Karaman, Dinç (2005) in Şanlıurfa, Biltekin et al. (2004) in Bornova, İzmir [2,5,6,18–20].

CONCLUSION AND RECOMMENDATIONS

This present study tried to determine traditional baby care practices used by women in the province of Artvin. It was observed that some traditional practices that mothers follow, do not give any harm to babies, but some practices may cause serious health problems for the babies in the short term or long term. For example, there are harmless practices such as burying the umbilical cord strump in schools, mosques, hospitals, etc., with a belief that the baby will be a respectable, beneficial and religious individual in the society in the future. On the other hand, there are some harmful and wrong traditional practices such as swaddling, which may cause problems in the anatomical structure of the baby, or waiting 3-5 azan for the first breastfeeding.

According to the findings of this study; traditional baby care practices are generally followed by mothers to a certain extent, but variables such as

mothers' educational status, employment status, income level, family type, and place of residence are not related to such practices. We suggest traditional baby care practices and the influencing factors should be further studied in larger samples. Traditional practices related to baby care are spiritual elements that develop in the society after passing through the filter of a long time period. It should not be forgotten that these spiritual elements are functioning as social support systems as well as playing significant roles in keeping society members together. The researchers involved in this study are not actually opposed to traditional baby care practices, in fact they are in favor of sorting out harmful or harmless practices and eliminating those harmful ones, and supporting the maintenance of the medically beneficial ones, which are important elements for the society.

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ORIGINAL RESEARCH

Investigation of the Knowledge and Attitude of Physicians about Traditional and Complementary Medicine

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Abstract

Objective: Traditional and complementary medicine (TACM), which is increasing all over the world, has become more common in our country. On the date of 27.10.2014, regulation on TACM practices which includes 15 different methods was published in our country. Although TACM methods have become legal in our country, they have not yet been included in the curriculum of the medical faculties and most physicians do not have enough knowledge about them. In this study, we aimed to increase the awareness of physicians about TACM, working at various levels of the medical faculty and to evaluate the knowledge, attitudes and behaviors of physicians about TACM methods.

Material-Method: This cross-sectional and descriptive study was conducted between May and July, 2018, among physicians working at Düzce University Faculty of Medicine. Questionnaires were administered to physicians participating in the survey, which includes descriptive questions and 25 questions evaluating the knowledge attitudes and behaviors of the participants about TACM methods.

Results: 50 physicians participated in our study. Acupuncture (86%), cupping (68%) and leech therapy (68%) were the most well-informed practices, respectively. The number of physicians with certificates participating in our study was quite low. 58% of the participants wanted to know more about TACM methods. The rate of physicians recommending TACM to their patients was found to be 38.3%. Also, 77.6% of the participants thought that TACM methods should be used as complementary. While 44.7% of the participants who participated in our study wanted TACM methods to be included in the curriculum, 21.3% did not. All of the participants think that the studies in the field of TACM are insufficient and as the reason, 56.4% believe that there is no scientific basis and 33.3% believe it is the bias of physicians.

Conclusion: As physicians' awareness and knowledge of TACM methods increase, more scientific studies will be conducted on this subject and their acceptability in modern medicine will increase.

Keywords: Traditional Medicine, Complementary Medicine, Physicians, Knowledge Evaluation

INTRODUCTION

Traditional and complementary medicine, which is increasing all over the world, has become more common in our country. Complementary medicine refers to practices that are used together with modern medical practices in the treatment of diseases but cannot be fully integrated into general health services. On the other hand, traditional medicine is all of the knowledge, skills and practices - which can be explained or not - based on beliefs and experiences fed by different cultures for centuries, which are used to protect from mental and physical diseases, to diagnose, heal or treat them, as well as to maintain general well-being [1,2]. Department of Traditional, Complementary and Alternative Medicine was established under the roof of the Ministry of Health in 2012 in our country. In 2014, its name was changed to the Department of Traditional and Complementary Medicine (TACM). In the same year, the Traditional and Complementary Medicine Practices Regulation was published. Fifteen TACM methods are defined in this regulation:

Acupuncture, Apitherapy, Phytotherapy, Hypnosis, Leech therapy (Hirudotherapy), Homeopathy, Caryopractic, Cupping therapy, Prolotherapy, Maggot therapy, Mesotherapy, Osteopathy, Ozone therapy, Reflexology and Music therapy. In addition, indication/ contraindication, the personnel and the materials required to be available in the application center are explained in detail in this regulation that is aimed to prevent inappropriate TACM methods and to increase the inspections[3]. Although TACM methods have become legal in our country, they have not yet been included in the curriculum of the medical faculties.

In recent years, although the research done with TACM methods has increased, it is still limited. With the increasing of elderly population, the increase of chronic diseases such as diabetes, cancer, obesity and hypertension has become an important health problem. In order to stay healthy while aging, many people now prefer complementary therapies in addition to modern medicine because of their low side effects and less invasive procedures. Therefore, the demands and questions of the patients regarding TACM are also increasing.

In this study, we aimed to increase the awareness of physicians about TACM, working at various levels of the medical faculty and to evaluate the knowledge, attitudes and behaviors of physicians about TACM methods. There are a limited number of studies on this subject in our country.

MATERIALS AND METHODS

Materials

The study is a descriptive cross-sectional study and it was conducted between May and July 2018, among physicians working at Düzce University Faculty of Medicine, who wanted to participate in the study voluntarily. Written permission was obtained from the Faculty of Medicine and the Scientific Research and Publication Ethics Committee in order to conduct the study.

Methods

As a data collection tool; the questionnaire form created by the researchers as a result of the literature review and experiences was applied through face-to-face interviews. The questionnaire includes descriptive questions about the gender and branch of the participant, and 25 questions evaluating the knowledge attitudes and behaviors of the participants about TACM methods.

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 statistical package program. Suitable descriptive statistics were calculated according to the types of all data included in the study (mean, standard deviation, minimum, maximum, percentage values).

RESULTS

50 participants were included in the study (29 female, 21 male) and their distribution in terms of gender is homogeneous. The average age of the participants is 34.14 ± 8.12 (24-52) and 62% of them are research assistants (Table 1).

Table 1. Demographic characteristics

		NUMBER	%
GENDER	Female	21	42.0
	Male	29	58.0
PROFESSION	Forensic Medicine	3	6.8
	Family Medicine	9	20.5
	Pediatric surgery	1	2.3
	Brain and Nerve Surgery	1	2.3
	Internal Medicine	3	6.8
	İnfectious diseases and clinical microbiology	3	6.8
	Medical Pharmacology	2	4.5
	Physical medicine and rehabilitation	2	4.5
	General surgery	2	4.5
	Chess Diseases	3	6.8
	Ophthalmology	1	2.3
	Obstetrics and Gynecology	2	4.5
	Otolaryngology	1	2.3
	Medical Microbiology	4	8.0
	Neurology	1	2.3
	Medical Pathology	3	6.8
	Pediatry	3	6.8
DEGREE	Research assistant	31	62.0
	Doctor lecturer	11	22.0
	Associate professor	4	8.0
	Professor	4	8.0

The most common TACM methods among the participants were: acupuncture (86%), cupping (68%) and leech therapy (68%); least known were:

prolotherapy (20%), osteopathy (22%) and homeopathy (24%) (Table 2).

Table 2. 'Which of the following TACM methods do you know about? (You can mark more than one option)' Distribution of the answers to the question

TACM METHODS		NUMBER	%
Maggot therapy	No	32	64.0
	Yes	18	36.0
Prolotherapy	No	40	80.0
	Yes	10	20.0
Music therapy	No	34	68.0
	Yes	16	32.0
Mesotherapy,	No	30	60.0
	Yes	20	40.0
Osteopathy	No	39	78.0
	Yes	11	22.0
Caryopractic	No	38	76.0
	Yes	12	24.0
Homeopathy	No	39	78.0
	Yes	11	22.0
Ozone therapy	No	24	48.0
	Yes	26	52.0
Reflexology	No	33	66.0
	Yes	17	34.0
Cupping therapy	No	16	32.0
	Yes	34	68.0
Leech therapy	No	16	32.0
	Yes	34	68.0
Phytotherapy	No	22	44.0
	Yes	28	56.0
Apitherapy,	No	35	70.0
	Yes	15	30.0
Hypnosis	No	24	48.0
	Yes	26	52.0
Acupuncture	No	7	14.0
	Yes	43	86.0

To the question "Do you think to get response-based medicine or evidence-based medicine?", 89.8% of the participants answered that it is evidence-based. When asked to give a score from 1 to 10 on the knowledge level of the participants about TACM, 6.1% thought they had a very good knowledge level (10 points), while 22.4% had very little knowledge (1 point). Also, 58% of the participants wanted to know more about TACM methods.42 participants (84%) knew that they could get a certificate and practice TACM as a physician, but only 28 participants (56%) wanted to get a certificate. Most of the participants (92%) did not have a certificate and 81.6% were undecided about whether TACM education was sufficient. 33.3% of the participants stated that TACM methods were placebo, and 78% stated that it should only be done by certified physicians. Also, 77.6% of the participants thought that TACM methods should be used as complementary. 79.6% of them had not applied for any TACM method before. Most of the participants (84%) answered yes to the question "Do you have any questions about TACM methods from your patients or your environment?"

While 73.5% of the physicians participating in our study do not question whether their patients apply for TACM methods, 12 participants (26.1%) think that approximately 10% of their patients apply TACM methods. 39 participants (84.8%) think that there is information pollution about TACM and 27 participants (57.4%) want to make scientific research about TACM, but most of them (91.3%) do not have any scientific studies. To the question "Do you think that the treatment costs of TACM applications should be covered by the state?", 21 participants (46.7%) answered no, 13 participants (28.9%) answered yes, 11 participants (24.4%) were undecided. Most of them (95.7%) believe that more scientific evidence is required before TACM can be used. To the question "Would you refer the patients to TACM centers?" 38.3% answered yes, 34.4% was undecided and 27.7% answered no. 42.6% answered yes, 34% no, and 23.4% no, to the question of "Do TACM applications delay the correct treatment?"

While 21 participants (44.7%) wanted TACM methods to be included in the curriculum, 10 participants (21.3%) did not want it, and 16 participants (34%) were undecided.

All of the participants think that the studies in the field of TACM are insufficient and as the reason, 56.4% believe that there is no scientific basis and 33.3% believe it is the bias of physicians (Table 3).

DISCUSSION

Studies to examine knowledge, attitudes and behaviors about TACM were mostly conducted with patients or students in our country. However, there are a limited number of studies examining the perspectives of physicians, who are in the most important position in this regard [4,5,6,7].

50 physicians participated in our study.

Table 3. Distribution of the answers given to the questions about TACM

		NUMBER	%
Do you think to get response-based medicine or evidence-based medicine?	Evidence-based	44	89.8
	Response-based	5	10.2
How many points would you give to your knowledge level about TACM applications from 1 to 10?	1	11	22.4
	2	6	12.2
	3	4	8.2
	4	3	6.1
	5	6	12.2
	6	10	20.4
	7	4	8.2
	8	2	4.1
	10	3	6.1
Would you like to know more about TACM applications?	Yes	29	58.0
	No	13	26.0
	Neutral	8	16.0
Did you know that you can get a certificate and apply TACM applications as a physician?	Yes	42	84.0
	No	7	14.0
	Neutral	1	2.0
As a physician, would you like to get a certificate for TACM applications?	Yes	28	56.0
	No	16	32.0
	Neutral	6	12.0
Have you ever been certified on TACM applications?	Yes	4	8.0
	No	46	92.0
How sufficient do you think the trainings given by TACM application centers are sufficient?	Enough	2	4.1
	Insufficient	7	14.3
	Neutral	40	81.6
Do you think TACM applications are placebo?	Yes	16	33.3
	No	15	31.3
	Neutral	17	35.4
Who do you think TACM applications should be done for?	1. Certified pysicians	39	78.0
	2. All physicians	2	4.0
	3. All healthcare professionals	4	8.0
	4. Physicians and nurses	1	2.0
	5. other	4	8.0
In which situations do you think TACM applications should be used?	In every situation	2	4.1
	Complementary as needed	38	77.6
	In cases of no treatment	2	4.1
	Should never be used	7	14.3
Have you ever applied for TACM applications?	Yes	9	18.4
	No	39	79.6
	Neutral	1	2.0
Do you have any questions about TACM applications from your patients or your environment?	Yes very	13	26.0
	Yes little	29	58.0
	No	8	16.0
Do you ask your patients whether they apply for TACM applications?	Yes	10	20.4
	No	36	73.5
	Neutral	3	6.1
In your opinion, what percentages of your patients apply to TACM applications?	%10	12	26.1
	%20	6	13.0
	%30	8	17.4
	%40	8	17.4
	%50	8	17.4
	%60	2	4.3

	%70	1	2.2
	%90	1	2.2
Is there any information pollution about TACM?	Yes	39	84.8
	No	2	4.3
	Neutral	5	10.9
Do you have a study to clean up information pollution about TACM?	Yes	3	6.5
	No	42	91.3
	Neutral	1	2.2
Do you think the treatment costs of TACM applications should be covered by the state?	Yes	13	28.9
	No	21	46.7
	Neutral	11	24.4
Could you refer the patients you deem appropriate as a physician to TACM centers?	Yes	18	38.3
	No	13	27.7
	Neutral	16	34.0
Do TACM applications delay correct intervention?	Yes	20	42.6
	No	11	23.4
	Neutral	16	34.0
Is more scientific evidence required before TACM applications come into use??	Yes	45	95.7
	No	1	2.1
	Neutral	1	2.1
Would you like to research TACM applications and bring them to modern medicine?	Yes	27	57.4
	No	10	21.3
	Neutral	10	21.3
Do you think TACM methods should be included in the medical school course curriculum?	Yes	21	44.7
	No	10	21.3
	Neutral	16	34.0
Do you think scientific studies in the field of TACM are sufficient?	No	47	100.0
If not enough, what is the reason?	Inadequacy of the Ministry of Health	1	2.6
	Insufficient medical education	2	5.1
	Physicians' bias	13	33.3
	The influence of pharmaceutical companies	1	2.6
	Having no scientific basis	22	56.4

Acupuncture (86%), cupping (68%) and leech therapy (68%) were the most well-informed practices in our study, respectively. In a study among medical faculty students studying at our hospital, the most commonly known TACM methods were; acupuncture (77.5%), cupping (75.3%), phytotherapy (67.3%). In the study of Özçakır et al., in which general practitioners participated, it was determined that acupuncture, vitamin / mineral supplements and herbs, massage were most known TACM methods [6]. In another study, physicians stated that they mostly heard about acupuncture and leech therapy [4]. In a study conducted with anesthesiologists, the most known TACM method was acupuncture (71.9%) and ozone therapy was in the second place with a rate of 45.9%. 75.3% of anesthesiologists did not use any of the TACM methods [7]. 11 participants (22.4%) in our study thought that they had very little knowledge about general TACM applications and 3 participants (6.1%) thought they had a very good level of knowledge. In addition, the number of physicians with certificates participating in our study was quite low. In another study in Turkey the low level of knowledge of participants on TACM method was reported [8]. In a study conducted in Sweden in 2012, 95.7% of the physicians stated that they had no or low level of knowledge about TACM [9]. In a study conducted with general practitioners in Hungary, 82.5% claimed that they did not have sufficient knowledge about complementary medicine [10].

44 participants (89.8%) thought that they believed in evidence-based medicine, 33.3% of them thought that TACM methods were placebo. Leach et al. emphasize that evidence-based medicine practices should be available in TACM [11].

In our study, the rate of physicians recommending TACM to their patients was found to be 38.3%. Orhan et al. and Yüksel et al. recommended TACM methods with a rate of 47.2% and 16% respectively [4,12]. When the publications in different countries were examined, 57.9% of physicians in Italy, 48% in the USA and 41.0% in England recommended TACM methods to their patients [13,14,15]. In our study, 12 participants (26.1%) think that only 10% of the patients applied to TACM. In a study conducted with 5,882 individuals in seven geographical regions in Turkey, TACM using rate was found to be 60.5% [16]. The reason for the lower rate in our study may be due to the patients not sharing their experiences about these practices with their physicians or the workload of the physicians participating in our study. TACM applications are still not entering the general health insurance coverage in Turkey and 21 participants (46.7%) in our study also support this notion.

While 44.7% of the participants who participated in our study wanted TACM methods to be included in the curriculum, 21.3% did not. Similar results were found in other studies [7,17]. In medical education, it can be included as an elective course in the curriculum in order to inform students about these practices. Education standards, which are already given in the form of postgraduate education in our country, can be arranged and made more efficient and effective.

One of the limitations of this study is the low participation. In addition, only certified methods that provide by the Ministry of Health were considered in our study. Other limitations are that it is not determined which TACM method doctors refer their patients to and why.

CONCLUSION

As a result, due to the position of TACM, it has become a necessity for any physician to have the correct information even if they are not practitioners. In our country, TACM education standards, which are given as postgraduate education, can be made more efficient and effective.

As the awareness and knowledge of physicians about TACM methods increase, scientific studies on this subject will increase and some TACM methods can be included in modern medicine. In addition, patients' accessibility to TACM in professional healthcare providers will increase and irregular applications will decrease.

We think that evidence-based regulation, more research and education are needed to ensure patient safety, to set standards and to make the right decision on patient basis in terms of TACM.

Multi-center studies with more physician participation are needed on this subject.

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ORIGINAL RESEARCH

Use of Sunflower Seed Lecithin as an Emulsifier in Herbal Cream Preparation

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Abstract

Objective: Lecithin is a commercially used emulsifier. Generally, lecithin used in foodstuffs is obtained from soybean oil. In recent years, lecithin obtained from sunflower seeds is also used as an emulsifier in the food industry. In this study, the use of lecithin obtained from sunflower seeds was tested the cosmetics industry.

Material-Method: Considering the widespread production of sunflower oil, together with lecithin obtained from this source the aim of this study is a) To produce a cream containing sunflower lecithin and St. John's Wort oil b) To bring a new product to the market by performing microbiological tests of the produced cream. Lecithin can be separated from crude oil by the method of hydration. In addition to antimicrobial efficacy test, the artificial contamination created on the sample, the logarithmic calculation of the number of viable microorganisms remaining in the product is performed on the 7th, 14th and 28th days and the antimicrobial effectiveness of the sample is determined. The products obtained have been dermatologically tested in accredited laboratories whether they cause irritation or not.

Results: Dermatological, antimicrobial and microbiological tests of the herbal cream that the products have been tested and its reliability and authenticity have been proved. As a result of the evaluations and calculations made at the end of the 72th hour, it was evaluated as "not an irritant/not a cause of irritation" dermatologically. According to the results of the antimicrobial analysis, the sample was determined to be protected against microbial growth.

Conclusion: A commercial emulsifier was used in the herbal cream formulation with St. John's Wort, which was previously developed by our group. In this study, lecithin obtained from sunflower seeds was used instead of commercial emulsifiers. The dermatological tests and the antimicrobial efficacy tests of the obtained herbal cream were carried out in accredited laboratories. It has been proven by tests that the resulting cream formulation meets the necessary criteria.

Keywords: Lecithin, Sunflower Oil, Cream, Formulation/Stability

INTRODUCTION

Lecithin is a compound commonly derived from egg yolk, soybeans and recently sunflower, with another name being phosphatidylcholine (Figure 1). Lecithin is essentially a phospholipid, and phospholipids are generally substances that form, protect, and maintain cell building blocks and keep them healthy[1,2]. It is also reported that they prevent hardening of the cell membrane and protect cells against oxidation. Sunflower is a good source of lecithin in countries where it is grown[3,4]. Lecithin released during degumming is one of the important industrial wastes in the oil industry during the production of refined oils. The adhesive materials separated from the crude oil by degumming process are treated as 'process waste'. The most important component in this waste is lecithin. Lecithin is widely used in the food, textile, paint and cosmetic industries. Lecithin has been recognized by the FAO in "GRAS" (generally recognized as safe) status, meaning no limitation has been imposed for the amount of lecithin to be used in foods[5-7]. Because of its molecular structure, commercial lecithin has both lipophilic and hydrophilic properties, and its use as an emulsifier depends on this property[8]. The most important function of lecithin, which is emulsification, is holding two different liquids together and forming oil in water or water emulsions in oil. With these amphoteric

properties, lecithins are indispensable additives in food and cosmetic systems[5,9]. The first function that comes to mind of emulsifiers is their function in the emulsions formed by oil – in water, water – in oil. Oil in water can be expressed by "o/w", water in oil by "W/O" [10].

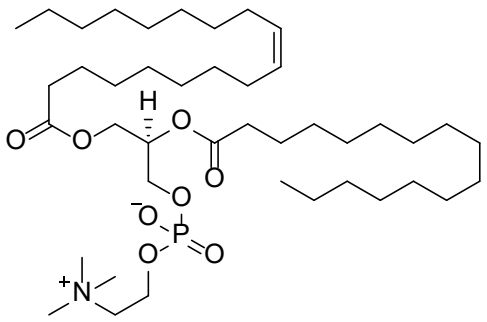

Figure 1. Lecithin molecule

The surface activity of sunflower lecithin is similar to that of soybean, but the amount of phosphatidylcholine of sunflower is higher than that of soy lecithin. Sunflower lecithin is more viscous and difficult to use than soybean lecithin. Sunflower lecithin can be added to the food or used as an additive for animal feed[11]. Sunflower lecithin is used in many commercial products thanks to its emulsifying properties and viscosity reducing effect. In recent years, the sunflower lecithin industry has grown in many countries. Special crushing plants are used for the production of sunflower lecithin because its seeds are smaller than soybeans. Sunflower lecithin is preferred as an alternative to soybean in Europe due to its good hazelnut flavor, suitable fatty acid content and emulsifying properties[12]. The emulsifying properties of modified sunflower lecithins are determined in O / W emulsions. The backscatter evolution, particle size distribution and mean particle diameters were examined for stability[13]. The PC enriched fractions of lecithins, alcohol fractions and enzymatically modified forms showed their best emulsifier properties in the main cream and destabilization processes.

The process of incorporating lecithin into cream formulations dates back to a long time. Among the products that use the product, creams, cleansers, moisturizing liquid make-up materials, beauty lotions and skin penetration enhancers can be listed[14]. The functions of lecithin in cosmetics can be listed as the ease of application in lipsticks, adding to creams to maintain softness, protecting hair, adding synergist against shampoos phenolic antioxidants and using it as emulsifier in prosthetic pastes[15,16]. The inclusion rate of lecithin in products is in the range of 0.5-1 %.

When lecithin is added to oil-based suspensions, it causes adsorption on the surface of the particles used in surfactant components, thus reducing surface roughness. With the addition of lecithin concentration, it causes an increase in flowing stress and does not cause further decrease in viscosity[17]. Lecithin is added to the formulations in small amounts as an emulsifier, which does not create an effect on the product in terms of color, smell and taste[18].

Considering the widespread production of sunflower oil, together with lecithin obtained from this source the aim of this study is a) To produce a cream oil phase containing sunflower lecithin and St. John's Wort oil b) To bring a new product to the market by performing microbiological tests of the produced cream.

MATERIALS AND METHODS

Cream formulation

St. John's Wort and sunflower extract lecithin cream was prepared according to the formulation given in Table 1[19].

Table 1. Cream formulation

St. John's Wort and sunflower extract herbal cream (400 g)	
Ingredients	**Amount (g)**
Aqua	290-350
Sodium Polyacrylate (and) Dicaprylyl Carbonate (and) Polyglyceryl-3 Caprate	6-10
Glycerin	8-16
Sorbitan Caprylate (and) Propanediol (and) Benzoic Acid	2.5-6.8
Coco-Caprylate	20-30
Hypericum perforatum Oil	11.6-17.5
Parfume	5-10.5
Helianthus annuus Seed Oil	11.6-17.5

Sunflower oil production

The oil extraction of 300 grams of sunflower seeds was carried out by cold squeezing and then crude oil was obtained by straining with vacuum. The resulting crude oil is 25 grams. Sunflower oil was obtained by cold tightening with 8.33% yield.

Production of lecithin

Lecithin can be separated from crude oil by the method of hydration. For this purpose, after the crude oil is heated for 2 hours at 60 ºC, 3% of the crude oil is added to the distillated water. Then it is mixed violently on the magnetic mixer. Lecithin swells and collapses with water. With the simple centrifuge process, the collapsed part is separated and heated at 80 ºC and spread as a thin film layer and the heating is continued and drying process is carried out. Lecithin is maintained at room temperature. It was determined by gravimetric analysis that lecithin content was obtained with 0.5% yield[20].

Antimicrobial activity test

In the antimicrobial efficacy test, after the artificial contamination created on the sample, the logarithmic calculation of the number of viable microorganisms remaining in the product is performed on the 7th, 14th and 28th days and the antimicrobial effectiveness of the sample is determined. In this analysis, ISO11930:2012 standard[21] was taken as reference. Microorganisms used in the test; *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 15404. Briefly, the preparation of the experiment; Working cultures were created by making subculture from stock cultures. Microorganisms were suspended in diluent and calibrated to 10^6-10^7 cfu/ml. Tenfold dilutions were made and bacteria were incubated at TSA, yeast SDA and mold PDA at 32.5 ± 2.5 C for 24-48 hours. 20 grams of cream samples containing lecithin were placed in sterile containers, and 0.2 ml of different microorganisms were added to each container to ensure homogeneity. The inoculated containers were incubated at 22.5 ± 2.5 C. For the 7th day enumeration; 1 g cream sample was taken from the containers and homogenized in 9 ml neutralizer. The mixtures, which were kept at room temperature for 30 ± 15 minutes, were diluted to correspond to 1/10 and 1/100. These dilutions are planted on TSA for bacteria, SDA for yeast and PDA for mold. The colonies in the petri dishes incubated under appropriate conditions were counted and logarithmic evaluation was done and interpreted. The same procedures were carried out on the 14th and 28th days.

Dermatological analysis

The aim of the study was to evaluate the skin compatibility of cosmetic products or raw materials on humans. Check whether cosmetic products or raw materials cause irritation on the skin. A sample of 0.02 mL of cosmetic product applied to the Volunteers ' back area is removed after remaining on the back for 48 hours. After half an hour, the first evaluation is done. Under the same conditions, a second evaluation is made at the end of 72 hours. Results are evaluated by dermatologists and responsible researchers. After calculations are made according to the results, the result is interpreted between "not irritating / not cause of irritation" and "very irritating/cause of high irritation". Deionized water is used as negative control in the study.

RESULTS AND DISCUSSIONS

In this study, a natural emulsifier used in herbal creams produced and which can be used instead of a commercially purchased emulsifier was used. Lecithin, which we want to benefit from the emulsifier feature, has been obtained in abundance from Sunflowers. St. John's Wort oil[22], used in the cream we produced as a cosmetic product, was taken from naturally grown plants in Düzce region. By making use of the thickener feature of lecithin, the thickener additive is also removed in the cream formulation. Thanks to the herbal cream produced, the effects of lecithin protecting the moisture of the skin, anti-blemish and strengthening the cell wall are also benefited. Dermatological, antimicrobial and microbiological tests of the herbal cream that we produced have been tested and its reliability and authenticity have been proved.

Dermatological analysis

Sunflower and St. John's Wort herbal cream is a product that is applied directly. The patches were closed for 48 hours so volunteers remained in the ridge area. The first measurements were made half an hour after the patches were removed. The area left open for 24 hours was reassessed at the 72nd hour. Test results at the end of 48 hours showed that the cream "was not an irritant / cause of irritation". As a result of the evaluations and calculations made at the end of the 72 hour, it was evaluated as "not an irritant/not a cause of irritation". In line with the above, the required parameters are shown in the tables below (Table 2-5). The tests were conducted within the scope of the Human Skin Compatibility Assessment dated European Cosmetics Association (COLIPA)-1997.

Table 2. Irritation classification criteria

Cosmetic Product Irritation Score	Classification
0-0.08	Not an irritant
0.08-0.16	Very mild irritation
0.16-0.56	Little irritation
0.56-1.0	Moderate to mild irritation
1.0-1.6	Cause of irritation
>1.6	Cause of high irritation

Table 3. Cosmetic product irritation test results in volunteers

No	Volunteer ID	Gender	Age	Skin Structure	Situations Encountered During the Study
1	CL401	M	34	Normal Skin	No reaction, No retreat
2	CL402	F	38	Normal Skin	No reaction, No retreat
3	CL403	M	27	Normal Skin	No reaction, No retreat
4	CL408	F	33	Normal Skin	No reaction, No retreat
5	CL413	F	36	Normal Skin	No reaction, No retreat
6	CL414	F	23	Normal Skin	No reaction, No retreat
7	CL415	F	22	Normal Skin	No reaction, No retreat
8	CL417	F	25	Normal Skin	No reaction, No retreat
9	CL423	M	23	Normal Skin	No reaction, No retreat
10	CL429	M	44	Normal Skin	No reaction, No retreat
11	CL440	M	30	Normal Skin	No reaction, No retreat
12	CL442	F	34	Normal Skin	No reaction, No retreat

Table 4. Calculations of dermatological patch test data

	48th Hour	72th Hour
Total Volunteer Data	0	0
Number of Reading	2	2
Total Volunteer Data / Number of Reading	0	0
Irritation Index	0	0
Results	Not irritating Not the cause of irritation	Not irritating Not the cause of irritation

Table 5. Dermatological patch test measurement results at 48 and 72 hours

No	Volunteer ID	Gender	Age	Negative Control	48. Hour Ratings			72. Hour Ratings		
					Erythema	Dryness	Edema	Erythema	Dryness	Edema
1	CL401	M	34	-	0	0	-	0	0	-
2	CL402	F	38	-	0	0	-	0	0	-
3	CL403	M	27	-	0	0	-	0	0	-
4	CL408	F	33	-	0	0	-	0	0	-
5	CL413	F	36	-	0	0	-	0	0	-
6	CL414	F	23	-	0	0	-	0	0	-
7	CL415	F	22	-	0	0	-	0	0	-
8	CL417	F	25	-	0	0	-	0	0	-
9	CL423	M	23	-	0	0	-	0	0	-
10	CL429	M	44	-	0	0	-	0	0	-
11	CL440	M	30	-	0	0	-	0	0	-
12	CL442	F	34	-	0	0	-	0	0	-

Antimicrobial efficacy

For antimicrobial effectiveness of samples; logarithmic reduction rates were calculated from the reduction equation ($R_x = lgN_0 - lgN_x$). The lg cfu / g value, which had been between 7.38 and 5.28 on day 0, was measured between 3.7 and 2.5 lg cfu / g on day 7 by making logarithmic calculations It was <10 on the 14th and 28th days (Table 6). The analysis made corresponds to Criterion A in ISO11930: 2012 standard and according to the results of the analysis, the sample was determined to be protected against microbial growth.

Table 6. Antimicrobial efficacy test logarithmic results

Microorganisms	0 hours		7th day			14th day	28th day
	cfu/g	lg cfu/g	cfu/g	lg cfu/g	lg reduction	cfu/g	cfu/g
Staphylococcus aureus ATCC 6538	2.41E+07	7.38	5.00E+03	3.7	3.68	<10	<10
Pseudomonas aeruginosa ATCC 9027	2.35E+07	7.37	4.00E+03	3.6	3.77	<10	<10
Escherichia coli ATCC 8739	2.28E+07	7.36	4.00E+03	3.6	3.76	<10	<10
Candida albicans ATCC 10231	2.60E+06	6.41	1.00E+03	3.0	3.41	<10	<10
Aspergillus brasiliensis ATCC 15404	1.90E+05	5.28	3.00E+02	2.5	2.80	<10	<10

CONCLUSION

Natural emulsifier used in herbal creams produced and which can be used instead of a commercially purchased emulsifier was used in this study. Lecithin, which we want to benefit from the emulsifier feature, has been obtained in abundance from Sunflower. The dermatological test results were showed that the cream was “not an irritant/not a cause of irritation". Antimicrobial efficacy test results indicate the sample was determined to be protected against microbial growth.

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ORIGINAL RESEARCH

Cytotoxicity of Some Retail Food Supplements in the Market

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Abstract

Objective: Medicinal plants and products obtained from medicinal plants are widely used in the treatment of various diseases in our country as well as all over the world. However, scientific data on the biological effects and mechanisms of action of most extracts from medicinal plants are still inadequate. Because of that, interest in scientific research of the biological effects of medicinal plants and products obtained from medicinal plants is increasing day by day. In addition, the reliability of these products in terms of use apart from their effectiveness is very important for public health. In this study, we aimed to investigate the cytotoxicity of commercially marketed natural herbal food supplements in L929 (mouse fibroblast) cells.

Material-Method: In this study, the WST-1 cell proliferation test protocol was applied to examine the cytotoxicity of 19 different products on the market in L929 cell lines and the results were evaluated according to Elisa microplate reading data. The products were also tested at concentrations of 0.02, 0.03, 0.04, 0.05, 0.06 µg / mL, taking into account the amounts of daily use.

Results: When the results were evaluated, 19 different herbal food supplement products sold on the market were cytotoxic in the L929 cell line, in the concentration range of 0.02-0.06 µg/mL, it was observed that the products were not toxic at these doses.

Conclusion: Herbal food supplement products in the market consist of many components. Therefore, the first thing to look at is the safety of the product. In this study, the cytotoxicity of 19 different products sold in the market was examined by considering the daily usage amounts in terms of reliability. Further studies are needed to determine their effectiveness.

Keywords: L929, Cell Culture, Cytotoxicity, Food Supplement, Herbal Mixture

INTRODUCTION

Plants had been used in medical treatments until synthetic drugs were discovered in all cultures and all over the world since the existence of humanity. The first humans invented the method of herbal therapy by trial and error, by observing animals and plants. They have cured many diseases by transferring the information from generation to generation.

As civilization progressed, with the industrial revolution and the development of the pharmaceutical industry, people moved away from nature and synthetic drugs replaced the drugs in nature. Although medical science has developed tremendously, today the drugs are expensive and some drawbacks of synthetic products have emerged, as an alternative to the drugs produced by the pharmaceutical industry has started to return to nature.

Today, herbs and natural herbal products are used in industry, food industry, cosmetics and perfumery, and many industrial areas. In addition, essential oil and its components obtained from plants are muscle relaxants, antibacterial, antiviral, antifungal, etc. It is used as[1-4].

Looking at the world in general, the idea and practice of returning to nature, most people prefer at least one of the alternative supportive treatment methods such as clothing, cosmetics, nutrition or treatment. According to the data of the World Health Organization (WHO), 80% of people (approximately 3.3 billion people) use traditional treatment methods for reasons such as the high cost

of synthetic drugs, frequent side effects, and the fact that medicinal plants can be easily obtained from nature[5-6].

The most important issue in returning to the use of these traditional products is reliability. Herbal extracts or mixtures must first be proven to be safe for health. Then their effectiveness should be looked at. In this study, reliability was taken into consideration. Purpose of the research; Cytotoxicity in the L929 (mouse fibroblast) cell line was evaluated in vitro, depending on the daily use of the tested products.

MATERIALS AND METHODS

Materials

Food supplement products used in the study were supplied from AYM-net® Company (AYM-net Herbal and Cleaning Products Import Export Consulting Trade Limited Company). The products used in the experiment and their contents are shown in Table 1. Dosage ranges for cytotoxicity analysis were preferred over the dose ranges these herbal mixtures were known to be effective. The doses used in cytotoxicity analysis are 0.02, 0.03, 0.04, 0.05 and 0.06 µg/ml.

Methods

L929 mouse fibroblast cell line was used in the study. Cells were cultured in serum containing medium (10% inactivated fetal bovine serum and RPMI-1640 medium containing 1% penicillin + streptomycin as antibiotic) at 37 ° C in T25 cm^2 flasks in a medium containing 5% CO_2 and 95% moisture. Cells were used for cytotoxicity test when 70% of the cells were confluent in density.

Cell viability assay

Experimental studies were carried out in Duzce University Traditional and Complementary Medicine Application and Research Center, Cell Culture Laboratory. WST-1 (tetrazolium salt based test) proliferation test (Takara Bio Inc., Shiga, Japan) was used to evaluate the cytotoxicity of the food supplements to be tested[7]. When the cells reached the appropriate concentration, they were inoculated into 96-well culture dishes with $5x10^4$ cells per well. Solutions in the dose range of 0.02-0.06 µg / ml for each herbal food supplement product to be tested were prepared and added to the nutrient medium. Each dose was studied in triplicate. A negative group was created with no product applied. Cells and products were incubated for 24 hours.

WST-1 test results the absorbance value (OD) of each well was read with a microplate reader (Biotek BT 800) at wavelength of 490 nm and reference range of 630 nm. Percentage of cell viability was calculated by dividing the optical density value measured in each well by the control optical density value and multiplying by hundred.

RESULTS

With this study, it was evaluated whether some food supplement products of, which are currently sold in the markets of our country, show toxic properties. The cytotoxicity of the products in different dose ranges was evaluated in vitro, considering the usage doses specified in the package insert. In this study where 19 different products were evaluated, L929 was applied to the mouse fibroblast cell line at doses of 0.02-0.06 µg / ml. According to the results of this research; it was found that the products for these doses did not show toxic activity. The results are grouped according to the portfolio of products and shown on the chart (Figure 1).

According to the analysis results, efficacy up to 90% cell viability was not taken into account in terms of data safety range. No toxic effects were seen at concentrations between the 0.02-0.06 µg / mL dose applied to the L929 cell line. In other words, it is possible to use safely at these concentrations and below.

DISCUSSION

Today, it is quite common to consume food supplements of various ingredients as a protective or auxiliary product for many diseases. But it should be noted that the toxicity that medicinal plants can create in cases of overdose and when mixed. The scientific accuracy of the plants to be used in all respects must be determined, discussed and proven by research. Some of the organic and inorganic compounds, mycotoxins and medicinal plants used for therapeutic purposes can cause liver and kidney damage[8]. Mensah et al (2019) demonstrated that plants have a toxic effect in their

Table 1. Food supplement products and their ingredients

Group	Product Code	Name of The Product	Product Content
Treatment support group / food supplements viability % graphics	A03	Black Cumin	Black cumin oil
	A06	Joint Copmlex	Glikosamine Sulfate
			Chondroitine Sulfate
			MSM (Methylsulfonlylmetan)
			Hyaluronoic Acid
			Type 2 Collagen
			Boswellıa serrata
	A08	Reishi Mushroom	Reishi mushroom (*Ganoderma lucidum*)
	A09	Pilles Complex	*Aesculus hıpocassaltium*
			Asplenium ceterach
			Cassia sp.
			Urtica sp.
			Horse tail
	A10	Cohosh Complex	*Ginko Biloba*
			Green Tea
			Valerian
			Melissa officinalis
			Black Cohosh
	A11	Saccex Complex	Olive Extrakt
			Cinnamomum
			Citrus
			Urtica sp.
			Berberis vulgaris
	A12	Psa Complex	*Epilobium angustifolium*
			Cetreahi
			Equisetum arvense
			Achillea millefolium
			Urtica sp.
	A14	Curcuma Soft Gell	Curcuma (Curcumin)
Weightening group / food supplements viability % graphics	A01	Active Form Herb Tea	*Prunus armeniaca* (%10)
			Rosmarinus officinalis (%30)
			others (%5)
			Heather Leaf
	A07	Form Complex	Erica Extrakt
			L-Carnitine
			Chitosan
			Green Tea Extrakt
			Melissa officinalis
	A16	Aloe Vera Energy Drink	Aleovera
			L carnitin
			Camellia sinensis
	A17	Coffee Aktif Vitamin	Reishi Mushroom (*Ganoderma lucidum*)
			Vitamin A, B1, B2, B3, D3 (%2,17)
	A24	Chitosan	Chitosan
Vitamin group / food supplements viability % graphics	A02	Omega 3	Omega 3
	A04	Calcium Magnesium Zing	Calcium, Magnesium, Zinc, Vit D3
	A05	Red Ginseng	Red Corean Ginseng Root Red Corean Ginseng Ekstrakt
	A13	Multivitamin	Panax Ginseng,Ginkgo biloba ve diğerleri
	A15	C-Vit	Vit-C
	A22	Vitamin 5	Royal Jelly, Pollen, Red Corean Ginseng, Propolis, Vit E, Vit A, Vit K2, Vit D3, Vit B12

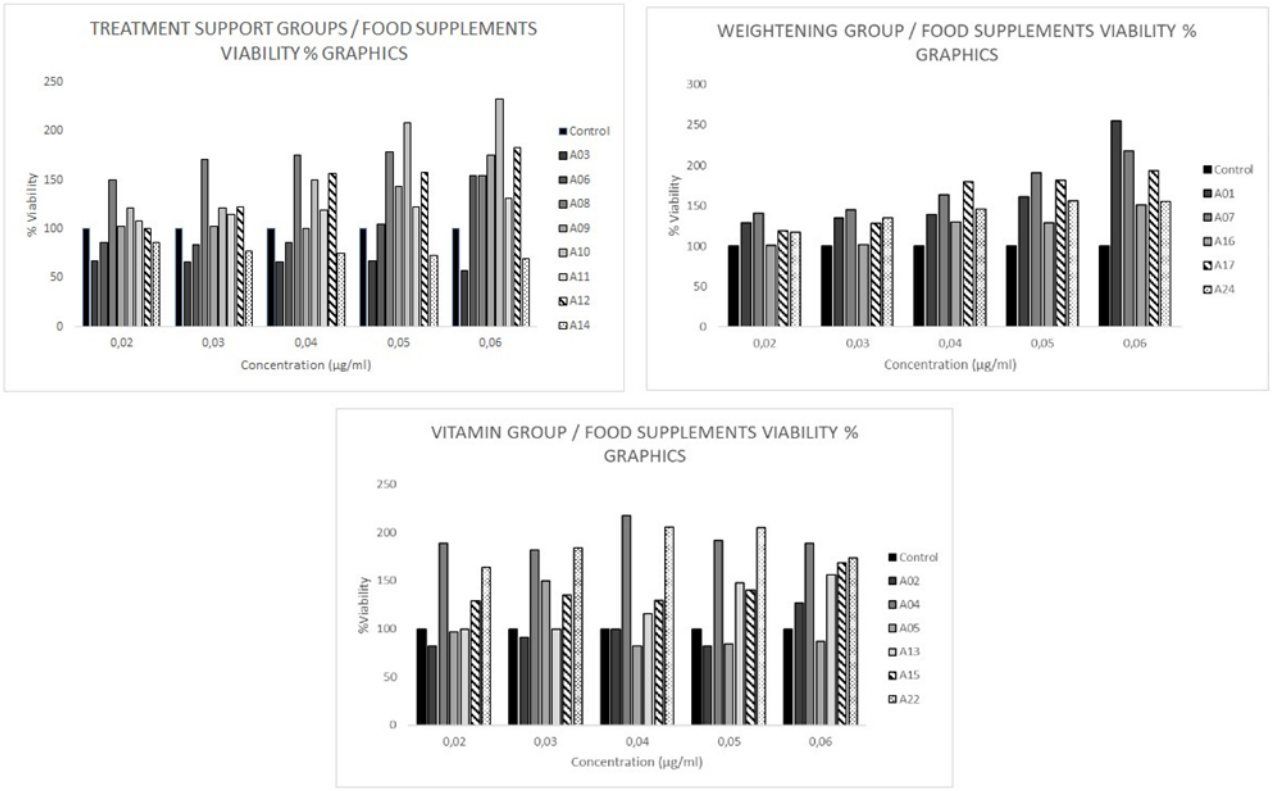

Figure 1. % Viability Plot of food supplements. The control group was accepted as 100%.

essence, which varies depending on the dose and the solvent used in the extract[9]. Although aristolochic acid isolated from Aristolochia has a positive effect on kidney function, it leads to rapid renal failure in overdose[10]. Glycyrrhiza spp. (liquorice) and Panax ginseng are among the most preferred plants in food supplements. They are often used as antimicrobial, expectorant, effective in colds, therapeutic in respiratory functions and also, the active substances they contain have been reported to cause adverse reactions such as edema, hypertension and electrolyte imbalances, insomnia, fatigue in some formulations (like herbal mixtures)[11]. In addition, there is a possibility that the beneficial effects of such medicinal plants and herbal products may turn into a harmful effect in long-term uses[12]. Food supplements containing minerals or vitamins, such as zinc and magnesium, provide osmotic balance, are a catalyst in biochemical reactions, and are a stabilizer in some protein structures. But on the other hand, essential elements have an accumulative property and seriously threaten human health in high doses[13].

In particular, the lack of a certain standardization and stable production techniques in the production of food supplements, which are often preferred in terms of their accessibility and easy use, suggests that the reliability of such products should be investigated. Even plants that have been involved in traditional medicine for centuries on the market today do not have standardized products that have been produced and analyzed with the exact concentration of substances known. So we tested the cytotoxicity of some food supplements, thinking that it is important to investigate the reliability of these products. In this study, it was found that the daily dose indicated in 19 different food supplements did not have a toxic effect. Albuz (2019) studied the determination of cytotoxic effects of ginger, turmeric and clove seeds on healthy cells, which are used as food supplements in daily life and have an important place in terms of Public Health. It was no found cytotoxicity at any concentration even the highest concentrations for ginger and turmeric extracts. On the other hand, the concentration of clove extract 1: 2 was reported

to be cytotoxic and the viability was 26.7 % [14]. In this study, no cytotoxic effect was found in the product containing turmeric. Yasar et al. (2020) found that the daily dose of a dietary supplement containing ginger and thyme does not cause acute and subacute toxicity in vivo[15]. The results in this study are in line with our findings. This has been found to be positive for the reliability and public health of products on the market.

A study using acute and subacute study models in Swiss albino mice showed that *Ganoderma lucidum* did not have significant toxicity and drew attention to the importance of its possible therapeutic uses[16]. Artıran et al. (2017), another study in spraque-Dawley type rats reported that daily supplementation of vitamin C in experimental animals did not pose a safety problem and even reduced the significant damage caused to the testes caused by gentamicin[17]. In this study, no toxic effects were observed in any of the reishi and vitamin-containing products.

One of the conditions in which food supplements are often used, which are predicted to be beneficial for health, is obesity. In cases of various diseases, such as obesity, the requirements of modern medicine should be met and licensed drugs should be used under the supervision of a doctor. But most of society resort to various natural ingredients to weaken, as with some health problems. For this purpose, many herbal products find a place in the market. The most innocent danger in many supplements with the thought that it can aid weight loss is that they don't work. The number of cases indicating that supplements and herbal mixtures used for this purpose lead to serious liver damage is quite large[18]. On the other hand, no toxic effects were found in any of the weight loss product group included in this study. This indicates the reliability of the products at the specified doses, but it cannot be claimed that in vitro studies are literally clinical equivalent. Because cytotoxicity tests provide basic information about the behavior of a substance and create a source for subsequent clinical trials[19].

CONCLUSION

In this study, the proliferative effectiveness of products used as a food supplement was studied in the L929 cell line at concentrations determined by taking into account the amount of daily use. According to the results of the study, products in the concentration range of 0.02-0.06 µg/mL do not show toxicity. This research supports that the tested food supplement products are non-toxic on the basis of concentrations created by taking into account the amounts of daily use. However, further studies are needed for their effectiveness.

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ORIGINAL RESEARCH

Comparison of Chemical Contents of Extracts in Different Solvents of Propolis Samples Produced in Duzce Province

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Abstract

Objective: Although the volatile components in the propolis composition are in very low concentration, they are extremely important for the characterization of propolis due to their aroma-giving properties and various biological activities. Since propolis is a product obtained from plants, its chemical composition depends on the local plant flora and the geographical and climatic characteristics of the region where the sample is collected. Therefore, different propolis samples can differ completely in terms of their chemistry and biological activities. Propolis extracts obtained by using different solvents have different contents. For this reason, the content of antioxidants varies, which causes differences in phenolic and flavonoid amounts. In the study, it was aimed to determine the efficacy of different propolis extracts produced as a result of beekeeping activities in Düzce province by comparing the chemical content.

Material-Method: The extracts of propolis collected from Düzce province were prepared using ethanol, water and PEG400 – water solvents. In the study carried out, the volatile components of three different extracts of propolis samples obtained from hives belonging to Yığılca Region were examined with LC-MS/MS, GC-MS UV. The determination of the total phenolic component (TPC) level was carried out with the Folin-Ciocalteu reagent, and the total flavonoid content (TFC) level with the AlCl3 based method.

Results: Major volatile constituents of Ethanolic extract; Diphenyl-1,2,5-oxadiazole (86.11%) and Benzenepropanoic acid ethyl ester (6.3%), Major volatile components of PEG400-water (50% - 50%) extract; 4-vinyl-2-methoxy-phenol (40.40%), Benzyl benzoate (17.16%), Methyl benzyl ketone (16.87%) ve Ethyl 3-methylnaphtho[1,2-c]pyrrole-1-carboxylate (14.32%), major volatile components of water extract; Benzyl Alcohol (79.91%) ve 4-vinyl-2-methoxy-phenol (8.86%). The highest TPC level was in ethanolic extract with 23,192.45 ± 396.54 mgGAE/100 g. Similarly, the highest TFC was found in ethanolic extract (7,190.12 ± 203.85 mgQE/100g). The water extract had the lowest levels at both TPC and TFC levels.

Conclusion: A It has been concluded that the highest phenolic content of propolis, which has recently begun to find an important area of use in the food and health sector, is obtained by ethanol extraction. When evaluated in terms of the obtained results from all methods, it is listed as Etanolic extract> PEG400> water extract. Further studies should be done using different solvents in order to extract as much of the components from propolis as possible.

Keywords: Propolis Extraction, LC-MS/MS, GC-MS, UV

INTRODUCTION

Apitherapy is among the traditional and complementary medicine practices and defined as "the way that bee and bee products are used as a protective and complementary application method in the treatment of some diseases" by the Ministry of Health [1]. One of the bee products used in apitherapy is propolis. Propolis has been used in traditional medicine both internally and externally since the early ages of humanity. It is one of them the products used as a food supplement in different parts of the world such as America, Europe, Brazil, Taiwan and Japan to support health and prevent

diseases such as aging, inflammation, heart diseases, diabetes and cancer [2–5].

In addition, there are many studies showing the antibacterial, antifungal, antiviral, local anesthetic, anti-inflammatory, antioxidant, hepatoprotective, immunostimulant and cytostatic activity of propolis [6].

Propolis is widely used in traditional medicine in many countries from Europe to East Asia due to all these features. This natural product, which is becoming increasingly important nowadays, also attracts a great deal of attention in the pharmaceutical, cosmetic and food industry [4,7,8].

The raw form of propolis cannot be used in the food and pharmaceutical industry. So, there are many studies such that the most effective content of propolis was investigated using different solvents. One of the most common solvents for propolis extraction is ethanol, because it contains more phenolic acid and polar compounds than water extract [9]. It is quite easy to extract the lipophilic components in propolis with ethanol. However, in the another study, it has also been reported that aqueous extract of propolis due its caffeeoilquinic acids to have a higher antioxidant activity and a high inhibitor/activator effect against some enzymes [10]. In addition, various solvents such as glycerin, propylene glycol and polyethylene glycol are also used in propolis extraction for pharmaceutical and cosmetic applications [11–14].

LC-MS/MS makes it possible to quantify the substance even at very low concentrations and provides a high sensitivity and precision for quantitative applications. GC-MS is also an excellent technique for identifying volatile substances [15,16]. GC provides a perfect separation, but to make flavonoids suitable for analysis, an extra derivatization step is often required prior to analysis [17]. Among all these methods, HPLC and accompanying MS, UV, DAD or PDA systems are undoubtedly still the most valid and reliable analytical technique for characterization of polyphenolic compounds [17–19].

In this study, it was aimed to determine the qualitative and quantitative content of ethanolic, aqueous and polyethylene glycol extracts of propolis produced in Düzce province using LC-MS/MS, GC-MS and spectrophotometric methods.

MATERIALS AND METHODS

The propolis samples used in the study were supplied from beekeepeers in Düzce province. The solvents used were provided in pharmacological purity. The extract preparation parts of our study were carried out at Düzce University, Traditional and Complementary Medicine Application and Research Center Laboratory, LC-MS/MS analyzes at Düzce University, Faculty of Medicine, Department of Pharmacology Research Laboratory, GC-MS and TPC and TFC analyzes were carried out in Düzce University Scientific and Technological Research Application and Research Center Laboratory.

Preparation of ethanolic extract

100 mL of 70% ethanol (70 ml ethanol+30ml water) was added to 10 grams of propolis sample. It was then left to stir for one week at 1100 rpm and room temperature in the dark. At the end of the period, the insoluble propolis sample was filtered using qualitative filter paper and filtrates were kept in the dark until analysis time.

Preparation of the water extract

100 mL of distilled water was added to 10 grams of propolis sample. It was then left to stir for one week at 1100 rpm and room temperature in the dark. At the end of the period, the insoluble propolis sample was filtered using qualitative filter paper and filtrates were kept in the dark until analysis time.

Preparation of the PEG400 - water extract

100 mL of 70% ethanol (70 ml ethanol+30ml water) was added to 10 grams of propolis sample. It was then left to stir for one week at 1100 rpm and room temperature in the dark. At the end of the period, the insoluble propolis sample was filtered using qualitative filter paper and filtrates were kept in the dark until analysis time.

The filtrate was evaporated to dryness. The obtained dry propolis extract was dissolved in 100 mL of 50% Polyethylene glycol 400 (PEG400)-water solvent mixture. The mixture was re-filtered through black band filter paper to remove insoluble. The filtrates were kept in the dark until analysis time.

LC-MS/MS analysis

Chemicals and instruments

Standards were supplied and MS grade methanol and formic acid solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA). High quality ultra-pure water was supplied by Human Zeneer Navi Power I Integrate (Human Corporation, Korea). The chemical content and composition of propolis were determined by using LC-ESI-MS/MS (Shimadzu, Kyoto, Japan).

Preparation of samples

The extract of samples (1 ml) were taken and added dilution solvent (9 ml) for each individual extract. The mixtures were vortexed for a minute and then diluted with the same solvents to 1 to 10. They were vortexed for a minute and filtered with 0.45 µm filters. Filtered solution was used for injection.

Analysis method

We used linear gradient LC-MS/MS method for all propolis analysis. Analysis was performed 100-mm x 4.6-mm, 5-mm particle C18 column.

Column oven set to 40 °C. Mobile phases (A) Ammonium formate (50 mM) +0.1% formic acid and (B) methanol. 0.3 ml/min flow and starting conditions with mobile phases %80/%20 respectively. From start to 5 min B was used %80; from 5 to 8 min B linear gradient remains %80; thereafter, a linear gradient back to %20 for 4 min to equilibrate column for next injection. Injection volume was 5 µl.

ESI-MS/MS analysis was performed using multiple reaction monitoring (MRM) to detect the major product ions from the protonated molecules of some phenolic and flavonoid contents (Table 1). The MS conditions were: nebulizer gas 2 ml/min and heat block temperature 450°C.

GC-MS analysis

The study was carried out on an Agilent 7890A GC System coupled to an Agilent 5975C inert MSD with Triple Axis Detector. Agilent HP5-MS (30 m × 0.25 mm × 0.25 µm) column was used as GC column.

The oven temperature was held at 40 ºC for 5 min., then ramped at 5 ºC / min. to 100 ºC for 5 min., then ramped at 20 ºC / min. to 225 ºC and held at this temperature for 8 min. The total run time was 33.25 min. The injector temperature was fixed at 200 ºC and splitless mode was used with helium carrier gas. The ion source was electron ionization and the MS source temperature was set at 230 ºC. The injection volume was 1.0 µL.

Total phenolic content (TPC)

The TPC of extracts was determined according to the Folin-Ciocalteau method with slight modifications [20]. All extracts were analyzed in triplicate. Briefly, the extracts were diluted with methanol (1:4). 800 µl 0.5 N Folin-Ciocalteau reagent was mixed with 40 µl extract solution and allowed to react for 5 min at room temperature in darkness. Then 800 µl of Na_2CO_3 (10%) was added and the volume of mixture was brought up to 3.0 ml with distillated water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm using spectrophotometer (Shimadzu, UV-1800). Gallic acid solution was used as a standard for constructing the calibration curve. TPC was expressed as mg of gallic acid equivalents (GAE) per g of propolis samples [21,22].

Total flavonoid content (TFC)

The TFC of extracts was measured by the colorimetric $AlCl_3$ method with few modifications [22]. Briefly, the extracts were diluted with methanol (1:4). 0.5 ml extract solution was mixed with equal volume of 2% $AlCl_3$ and 3.0 ml distillated water. The mixture incubated at room temperature in darkness for 1 h. Then the absorbance was measured at 415 nm with spectrophotometer (Shimadzu, UV-1800). Quercetin solution was used as a standard for constructing the calibration curve. TFC was expressed as mg of quercetin equivalents (QE) per g of propolis samples. All extracts were analyzed in triplicate [20,21,23,24].

Statistical analysis

Data were expressed as average ± standard error of the mean (SEM). The test results of TPC and TFC data were subjected to statistical analysis using SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA) for the analysis of variance (ANOVA) with comparison of means by Tukey test ($P < 0.05$).

RESULTS

In LC-MS/MS MRM (Multi Reaction Monitoring) mode was used for analysis. As seen in Table 2, the peaks of the generally expected phenolic and flavonoid groups are found in separate extractions. Depending on the type of solvent used in the extraction, the types of extractable substances differ. According to these results, it was observed that the highest component was in the ethanolic extract of propolis, and the lowest was in the aqueous extract of propolis.

GC-MS anaylsis

The volatile components of the extracts obtained from the propolis samples using three different extraction methods and the percentage distributions among these components are given in Table 3-5.

It was determined that the extract containing the most volatile component among the extracts was prepared with the ethanol solvent system. While the benzaldehyde compound is not included in the PEG400-water extract, it is included in the other two extracts. Although Methyl benzyl ketone compound is not present in water extract, it is included in the composition of the other two extracts and is one of the main volatile components of the PEG400-water extract with a ratio of 16.871%. While Ethyl benzoate compound is not found in PEG400-water extract, it is included in Ethanolic extract with a rate of 0.47% and in Water extract with a rate of 2.70%. While 4-vinyl-2-methoxy-phenol compound is the main volatile component of PEG400-water extract with a ratio of 40.40%, this ratio is reduced by 8.86% in water extract and none in Ethanolic extract. While the Benzyl Alcohol compound is the main volatile component of the water extract, it is not included in the other two extracts. Diphenyl-1,2,5-oxadiazole compound is the main volatile compound of Ethanolic extract.

Table 1. MRM parameters of compounds

	Compound	m/z	Ref. Ions	Ret. Time	Scan
1	Coumarin	147.30>91.10	147.30>103.10	6.902	(+)
2	Quercetin-3-o-rutinoside-7-o-glucoside	773.20>773.20	773.20>465.20-773.20>105.00-773.20>400.00-773.20>773.20	4.610	(+)
3	Robinin	741.20>287.20	741.20>595.20-741.20>433.20-741.20>257.30-741.20>741.20	5.988	(+)
4	Caffein	195.00>138.10	195.00>110.10	5.136	(+)
5	Chlorogenic acid	353.10>191.10		4.408	(-)
6	Cryptochlorogenic acide	353.20>173.20	353.20>135.20-353.20>179.10	4.282	(-)
7	EPGC	457.10>169.10	457.10>125.00-457.10>305.20	4.599	(-)
8	Vanillic acid	167.10>108.20	167.10>152.20	5.885	(-)
9	Catechin hydrate	288.90>109.10	288.90>245.10-288.90>271.20	4.302	(-)
10	Ellagic acid	301.10>301.10	301.10>145.10	6.673	(-)
11	Fisetin	284.90>135.10	284.90>121.00	6.850	(-)
12	Gallic acid	169.20>125.10	169.10>97.00	3.049	(-)
13	Kaemferol 3-glucoside	447.20>255.20	447.20>284.00	6.598	(-)
14	Procynadin B2	577.20>289.20	577.30>425.10	4.196	(-)
15	Quinic acid	190.90>173.20	190.90>127.00	1.755	(-)
16	Rosmarinic acid	359.10>161.10	359.10>179.00	5.883	(-)
17	Trans-ferulic acid	193.10>178.20	192.90>149.10-192.90>134.10	6.074	(-)
18	Quercetin-3-Galactoside	463.20>300.10	463.20>255.20	6.309	(-)
19	Cinamic acid	147.30>103.10	147.30>147.20	7.491	(-)
20	Quercetin-3-glucoside	463.00>463.00	463.00>300.00	6.374	(-)
21	Caffeic acid	179.00>135.00	179.00>179.00	5.374	(-)

Table 2. Contents of different extractions.

Compounds	Ethanolic extract	PEG400-water extract	Water extract
Coumarin	+		
Quercetin-3-o-rutinoside-7-o-glucoside	+	+	+
Robinin	+	+	+
Caffein			
Chlorogenic acid			
Cryptochlorogenic acide			
EPGC			
Vanillic acid			
Catechin hydrate			
Ellagic acid	+	+	+
Fisetin	+		
Gallic acid			
Kaemferol 3-glucoside	+		
Procynadin B2			
Quinic acid			
Rosmarinic acid			
Trans-ferulic acid	+	+	
Quercetin-3-Galactoside	+		
Cinamic acid	+	+	
Quercetin-3-glucoside	+	+	
Caffeic acid	+	+	

Total phenolic content and total flavonoid content analysis

Total phenolic and flavonoid content, which are known to be important factors that play a part in the antioxidant activities, were measured and results of propolis extracts obtained with three different solvents are given in Figure 1. Our results showed that the ethanolic extract has the highest total phenolic content (TPC, 23,192.45 ± 396.54 mgGAE/100g). PEG400-water extract followed it and water extract showed the lowest TPC value (6.191,03 ± 162.39 mgGAE/100g). Total flavonoid results (TFC) were also parallel to TPC results. Ethanolic extract showed the highest TFC value with 7190.12 ± 203.85 mgQE/100g. PEG400-water and water extracts followed respectively.

Table 3. Volatile components of ethanolic extract as determined by GC-MS

Compound	RT (min)	Compound Name	Distribution Ratio (%)
1	8.141	Benzaldehyde	0.64
2	8.457	Silicic acid tetraethyl ester	3.27
3	9.894	Methyl benzyl ketone	0.62
4	10.252	Benzoic acid ethyl ester (Ethyl benzoate)	0.47
5	10.346	[1'-(phenylsulfinyl)prop-2'-enyl]cyclohex-2-en-1-ol	0.34
6	11.472	2-Methoxy-4-vinylphenol (p-Vinylguaiacol)	6.39
7	11.638	Benzenepropanoic acid ethyl ester (Ethyl 3-phenylpropionate)	0.50
8	11.731	4-Phenyl-3-buten-2-one (Benzalacetone)	0.65
9	12.463	3-phenyl-ethyl ester -2-Propenoic acid (Ethyl cinnamate)	1.00
10	25.689	Diphenyl-1,2,5-oxadiazole (3,4-Diphenylfurazan)	86.12

Table 4. Volatile components of PEG400-water (50 % - 50 %) extract as determined by GC-MS

Compound	RT (min)	Compound Name	Distribution Ratio (%)
1	9.894	Methyl benzyl ketone	16.871
2	11.093	2H-1-benzopyran (1,2-Chromene)	5.254
3	11.555	4-vinyl-2-methoxy-phenol	40.401
4	11.736	4-Phenyl-3-buten-2-one (Benzalacetone)	5.978
5	15.166	Benzyl benzoate (Ascabin)	17.167
6	17.983	Ethyl 3-methylnaphtho[1,2-c]pyrrole-1-carboxylate	14.329

Table 5. Volatile components of water extract as determined by GC-MS

Compound	RT (min)	Compound Name	Distribution Ratio (%)
1	8.089	Benzaldehyde	3.10
2	9.147	Benzyl Alcohol	79.92
3	10.258	Benzoic acid ethyl ester (Ethyl benzoate)	2.70
4	10.564	N-3-chloro-2-methylpropyl)-N,N-Dimethylammoniumchloride	1.73
5	11.415	4-vinyl-2-methoxy-phenol	8.87
6	11.742	Tolpropamine	1.50
7	13.521	*o*-(2-(Methylamino)propyl)phenol	2.20

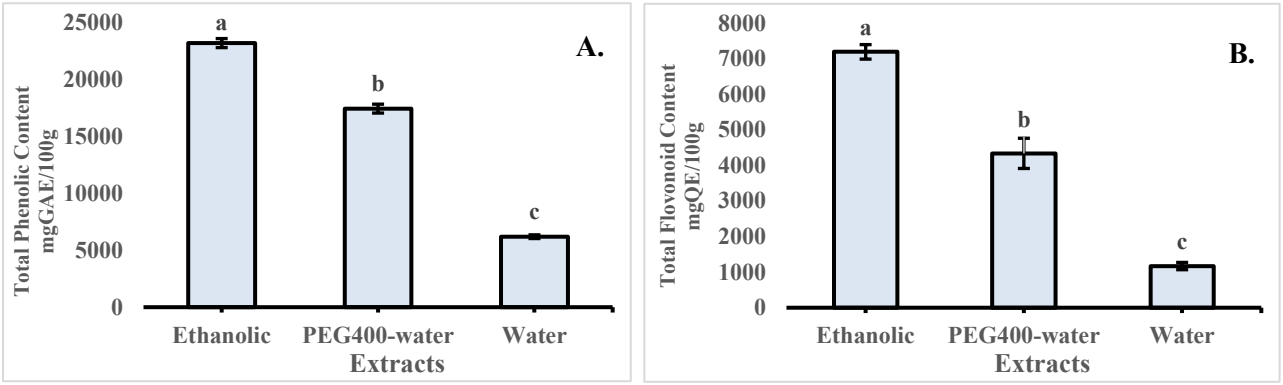

Figure 1. Total phenolics (A) and flavonoids (B) content of Düzce Propolis extracts. Different superscript letters (a–c) by each extraction method indicate significant differences according to Tukey's test at significance level $P < 0.05$.

DISCUSSION

There are many studies showing that the active ingredients of propolis easily dissolve in ethanol [25–32]. Despite the rich content of ethanolic extract, dissolution of the ethanolic extract in many products, especially in the pharmaceutical and food industry is a problem. The propolis aqueous extract cannot show the expected efficacy since the active ingredient content is very weak compared to the content of the ethanolic extract. Therefore, investigating the contents of propolis in different solvents is still an important issue that attracts the attention of many researchers. One of these solvents is polyethylene glycol (PEG). Water PEG solutions can also be used as suspending agents in topical ointments or to adjust the viscosity of other suspending agents [33]. PEG400 is known to be non-toxic if not taken in very high doses in one go [34]. Therefore, PEG400 is suitable for use in the pharmaceutical, food and cosmetic industries to increase the solubility of oil-based additives in water-based products. In addition, since PEG400 can be used as a thickener in pharmaceutical products, another advantage is that there is no need for extra thickening additives in products produced with the use of this extract.

There are many studies about the chemical composition of Turkish Propolis using different methods. However, the number of studies on propolis produced in Düzce is limited. Rasgele and Kekecoglu [35] investigated the ethanolic extraction of propolis produced in Yığılca district Düzce province by using another method (HPLC-DAD). But there are GC-MS studies made with propolis produced in the Black Sea Region. For example; Çelemli [36] reported that "benzoic acid" and "17-pentatriacontene" are determinant for the propolis samples collected from the Black Sea Region. Gençay and Salih [37] stated that propolis collected

from Rize, Bartın, Trabzon and Gümüşhane were rich in flavonoids and that, benzene,1-(1,1-dimethyl ethyl) and 28-norolean-17-en-3-one were the major component in Rize propolis. Popova et al. [38] indicated that Artvin propolis contained phenolic glicorides according to their GC-MS results. Sorkun et al. [39] determined that major flavonones in propolis were 43.55% and 50.55% Trabzon and Gümüşhane, respectively. Kocabas et al. [40] conducted with propolis samples collected from the Eastern Black Sea Region, volatile components of propolis samples were determined as; phenyl ethyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%) and cedrol (4.1%). These results reveal that geographical location, vegetation and climatic conditions have an important role on the volatile components of propolis when compared to the study carried out [40].

In this study, it was aimed to compare the contents of propolis in different solvents using different methods. According to obtained GC-MS results, major components were diphenyl-1,2,5-oxadiazole (3,4-diphenylfurazan) for ethanolic extracts, 4-vinyl-2-methoxy-phenol for PEG400-water and benzyl alcohol for water extracts of propolis. The 3,4-Diphenylfurazan derivatives, the major component of propolis ethanolic extract, have been found to be potent indolamine 2,3-dioxygenase inhibitors and are useful for the treatment of cancer cells. They are also useful as a new class of SENP2 inhibitors and can be used in the development of new therapeutic agents for various diseases targeting SENPs. Studies have revealed that 4-Amino-1,2,5-oxadiazole-2-oxide-3-carboxylic acid and azo derivatives, which are 3,4-diphenylfurazan derivatives, have vasodilating properties. The 3,4-Diphenylfurazan derivative 1,2,5-Oxadiazole-2-oxide and benzo [c] [1,2,5] oxadiazole-N-oxide derivatives show herbicidal activity [41].

2-Methoxy-4-vinylphenol, the major component of propolis PEG400-water extract, is an aromatic substance used as a sweetener and one of the compounds responsible for the natural aroma of buckwheat [42].

Benzyl alcohol, which is the major component of propolis water extract, is used as a bacteriostatic preservative in intravenous drugs, cosmetics and topical drugs [43]. In addition, the US Food and Drug Administration (FDA) permitted the use of a 5% solution of benzyl alcohol for head lice treatment in people six months and older in 2009 [44].

Total phenolic compounds and flavonoids have an important role in antioxidant activity by neutralizing free radicals. According to obtained UV results, total phenolic component values vary among different solvents in the amount of 6,191.03 ± 162.39 and 23,192.45 ± 396.54. In terms of total phenolic component, the highest amount was obtained from ethanol extract and the lowest amount was obtained from water extract. Asem et al.[45] stated that flavonoids from propolis exhibited a stronger antioxidant effect than vitamins C and E. Kubiliene et al.[14] used different solvents such as PEG400-water mixture, PEG400, olive oil, olive oil-water mixture and ethanol extract in their researches. They reported that total phenolic activity of nonethanolic solvents does not differ significantly from the concentration found in ethanolic extract and they have radical scavenging and antimicrobial activity. Similarly, the highest amount in terms of total flavonoid content was obtained with ethanol solvent. These results showed that the solvent with the highest amount of both phenolic compounds and flavonoids was ethanol. Although PEG400-water solvent was determined to be more successful than water, it was able to extract less phenolic compounds than ethanol. Our results have been found to be compatible with some previous studies. Mouhoubi et al.[46] stated that the optimum extraction solvent was 85% ethanol. It has been stated that 75% ethanol extract has wider polarity than water extract, making it a better solvent for propolis extraction. Fikri et al.[24] reported that the water extract can contain non-phenolic ingredients such as carbohydrates and terpenes. According to the results, extracts of propolis showed a phenolic composition significantly richer in phenolic compounds than flavonoids. Ozdal et al.[20] indicated that the TPC of the ethanolic extract was higher

than a previous study, which investigated propolis samples from different region of Turkey. The amount of flavonoid in the Düzce sample included in the same study was found approximately 3 times higher than our results. In addition, it was emphasized that the highest TPC and antioxidant capacity was obtained from the Marmara region, Kırklareli and Düzce samples [20]. It has been stated that the amount of polyphenols in propolis may vary depending on its geographical origin, and factors such as climatic conditions, vegetation around the hive, and harvest period affect the chemical composition of propolis [47]. In the light of this information, although the polyphenol content of propolis varies depending on various factors, ethanolic extract of Düzce propolis has been found to have higher phenolic and flavonoid content than the data in many studies in the literature [7,20,23,48–50]. According to the obtained LC-MS/MS results, the fact that the components expected to be found in the extracts are mostly in ethanol is a situation predicted from the literature. PEG was observed to be more effective than water in extracting these bioactive components. Since there are only so many MRMs in our method, it is not correct to make a complete generalization, but seeing the expected main phenolic components or derivatives can be considered sufficient [51,52]. Chong and Chua [53] reported the chemical composition of aqueous, PEG+aqueous, ethanolic and PEG+ethanol extracts of propolis using GC–MS and LC–MS/MS. In their study, they found that ethanolic extracts of propolis had higher yields than water extracts at different pH values without significant difference. Kubiliene et al.[14] analyzed the chemical content of aqueous, PEG-aqueous and ethanolic extracts of propolis using HPLC and UV. They reported that the ethanolic extract was 10-17 times higher in terms of phenolic component than PEG-aqueous and aqueous extracts, respectively. All these findings are in agreement with our studies which investigated chemical composition of propolis in different solvents.

CONCLUSION

As a result, according to the results obtained from our study in which three different extracts of propolis were analyzed using three different methods, the most suitable solvent is ethanolic extract. When evaluated in terms of the obtained results from all methods, it is listed as Etanolic extract> PEG400> water extract. PEG can be used as an alternative for sections that are sensitive to the use and consumption of alcoholic extract. However, it may not be considered sufficient since it is not close to the variety of substances that pass to ethanol. Further studies should be done using different solvents in order to extract as much of the components from propolis as possible.

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CASE REPORT

A Case with Not Relux Flow Detection with Leech Therapy for 6 Months Follow-Up in Venous Insufficiency

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Abstract
Chronic venous insufficiency is an important and frequent disease, characterized by the retrograde flow of blood in the lower extremity, is a common, debilitating disorder that is increasing in prevalence. Leech therapy is a traditional healing method for centuries and it has taken its place in modern medicine because of application areas with broad spectrum. Leech therapy is also used in the treatment of venous congestion. In this study, a case in which a patient with a high-level venous insufficiency was almost completely cured, is presented.
Keywords: Hirudotherapy, Venous insufficiency, Reflux flow in varices

INTRODUCTION

Venous diseases may occur in different forms up to painful varicose veins and even skin ulcers that are caused by serious venous insufficiency. Chronic venous insufficiency can cause loss of labor and life quality. Most of the lower extremity venous insufficiency originate from saphenous magna and parva veins[1,2]. The most important reasons of varices are insufficiency in valves of saphenous vein, weakness of vein walls and arteriovenous fistulas. Venous insufficiency in lower extremities can display themselves in various types; some are telangiectasias, appearance of varice packages by veins that become clear, feeling of pressure and weight rash on ankle, eczematous dermatitis and venous ulcers[3]. Leech therapy is a traditional healing method for centuries and it has taken its place in modern medicine because of application areas with broad spectrum. *Hirudo medicinalis* is the most frequently used leech type in medical practice. American Food and Drug Administration (FDA) labeled *H.medicinalis* for medical usage. Secretions of Leeches salivary gland have more than 100 different bioactive materials. It was reported that these secretions have vasodilator, bacteriostatic, analgesic anti-inflammatory and anti-coagulant effect. At the same time they were stated in various sources that they have antiedema effect, prevent microcirculation disorder, correct hypoxia and damaged vascular permeability of tissues, decrease blood pressure, increase immune response and cut the pain[4-6]. A case in which venous insufficiency is diagnosed by doppler usg and leech treatment was used is introduced in this article.

CASE

A 66-year- old female, diagnosed as high degree bilateral superficial venous insufficiency in lower extremity and middle degree deep venous insufficiency came to our clinic for leech treatment.

10 sessions of therapy were done collectively

including 3 times a week and in every session leeches were applied inboth lower extremities. Patient's venous insufficiency degree was observed both after 10 session and 6 months later from treatment by Ataturk University Medical Faculty radiology department lecturers.

Rising of calibration in main femoral vein (MFV) deep femoral vein (DFV), superficial femoral vein (SFV) and popliteal vein (PV) bilaterally, and high degree slowdown of flow were observed in doppler usg before treatment. Reflux was monitored along with valsalva maneuver in greater saphenous magna vein and saphenofemoral junction. And reflux in main femoral vein was observed 2-3 seconds with valsalv maneuver. These vessel's correct situation were healed in doppler usg after treatment. Minimally increased reflux degree was observed with valsalva only in left femoral vein. It was seen that any of abnormal condition or reflux was not observed with dopler usg 6 months later from treatment (Table 1).

Table 1. The results of changes of reflux flow during treatment and after treatment

Before leech therapy	It becomes visible due to high degree slowdown of flow and rising of calibration in main femoral vein deep femoral vein superficial femoral vein and politeal vein bilaterally. Reflux was observed in greater saphenous vein and saphenofemoral junction along with valsalva maneuver,and reflux in MFV was observed for 2-3 second with valsalva maneuver.
After leech therapy	Lumen, diameter and compressibility of MFV, DFV, SFV and PV were bilaterally normal. Reflux was minimally appeared in left femoral vein.
6 months later from leech therapy	Lumen diameter and compressibility of MFV, DFV, SFV and PV were bilaterally normal. Reflux was not detected with valsalva maneuver in observed veins.

DISCUSSION

In this study, a case in which a patient with a high level venous insufficiency was almost completely cured, is presented. There are lots of study where leech therapy was applied to varicose veins in the literature. In these studies meaningful results were obtained from patients who were treated with leeches[7,8]. A result in addition to medical treatment, hirudurotherapy is an alternative method in venous insufficiency.

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CASE REPORT

Leech Therapy in A Case with Arterial Embolism

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Abstract
Although there have been significant advances in the treatment of cardiovascular diseases, acute peripheral arterial blockage is still important due to limb-threatening ischemia and loss of function it causes in vital organs. Leeches have been used in the treatment of certain diseases since ancient times. US Food and Drug Administration (FDA) permitted the sale of leeches in United States of America and their use for general purposes, plastic surgery and micro-surgery in 2004. There are studies in the literature reporting leech therapy administration in post-surgical vascular permeability complications. In conclusion, hirudotherapy can be used as an alternative treatment in addition to medical treatment of arterial embolism. This article studied a case that had peripheral arterial embolism and recovered through leech treatment.
Keywords: Arterial Embolism, Hirudotherapy, Amputation

INTRODUCTION

Although there have been significant advances in the treatment of cardiovascular diseases, acute peripheral arterial blockage is still important due to limb-threatening ischemia and loss of function it causes in vital organs. The treatment can end up in the amputation of organs[1]. Leeches have been used in the treatment of certain diseases since ancient times, where *Hirudo medicinalis* and *Hirudo verbana* species have been mostly used in various treatments. The saliva of leeches contains over 100 different bioactive substances. Some of these secretions have been reported to have vasodilator, bacteriostatic, analgesic, anti-inflammatory and anticoagulant properties. In addition, various sources have stated that they have edema resolving effects and some other properties such as preventing microcirculation disorders, correcting damaged vascular permeability of organs and tissues and hypoxia, lowering blood pressure, increasing immunity and relieving pain. FDA permitted the sale of leeches in US and their use for general purposes, plastic surgery and micro-surgery in 2004. The use of hirudotherapy as a supportive treatment in modern medical practices is highly important[2,3]. This article studies a case in which leeches were used in the treatment of a patient with a diagnosis of Peripheral Arterial Embolism.

CASE

A 74-year-old male patient who had a history of coronary artery disease and diabetes mellitus. He had been visiting the cardiology service due to a chest pain and accompanying sweating complaints. The laboratory findings of the patient were as follows: hemoglobin, 13.9 g/dl; WBC, 16200/mm^3; C- Reactive Protein (CRP), 168 mg/L; erythrocyte sedimentation rate (ESR), 44mm/s; INR, 1.21. After the patient had coronary angiography, pain and redness developed starting at the second phalanx of the third finger on the right hand. Upon thinking that embolism increased, the case was consulted with cardiovascular surgery. The cardiovascular surgery reported that the flow in this area decreased as a result of the doppler examination. The case was also consulted with Infectious Diseases department due to high ESR and CRP. The high Sedim and CRP was thought to stem from the necrotic tissue in the finger as no agents of infection could be found. Upon this development, amputation decision was given as a

result of consultation with Orthopedics and Cardiovascular Surgery. The middle finger distal and middle phalanx were amputated. Following amputation, necrotic area and pain increased in the proximal phalanx of the same finger and hand as well. Amputation was considered at the proximal phalanx, too (Figure 1).

Figure 1. Limb before leech therapy

The patient refused this treatment, so the Family Medicine Leech Therapy Clinic was asked for consultation. So he was administered leech treatment for two sessions with two days interval. After the leech treatment, the existing redness, bruising and pain of the patient decreased on the first day of the treatment (Figure 2).

He had follow-ups for a week and the wound was controlled. The patient, who was also given anticoagulant therapy, had leak-like bleeding for 5 days in the area where leech treatment was administered. The laboratory findings of the patient began to improve after the treatment. The laboratory findings were as follows: hemoglobin, 12.3 g/dl; WBC, 14700/ mm^3; CRP, 51 mg/L; ESR, 24mm/s; INR, 1.22. The complaints of the patient were completely resolved in the follow-ups.

Figure 2. Limb after leech therapy

DISCUSSION

This article studied a case that had peripheral arterial embolism and recovered through leech treatment. There are studies in the literature reporting leech therapy administration in post-surgical vascular permeability complications[4,6]. Significant results have been obtained from the assessment of patients having leech treatment in these studies[3-5]. However, comprehensive studies are needed in this field. In conclusion, hirudotherapy can be used as an alternative treatment in addition to medical treatment of arterial embolism.

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MINI REVIEW

Hypnosis and Anesthesia

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Abstract
Hypnosis is the acceptance of a suggestion. Perception, memory, and voluntary movements can be changed with hypnotherapy. Rather than being an alternative to general or regional anesthesia in the field of anesthesia, hypnosis should be used as a complementary method that is integrated with these methods, increases the patient's compliance and comfort, and accelerates the healing process. Hypnosis is an important application in the reduction and control of both acute and chronic pain. In this text, we analyze the subject of hypnoanesthesia and discuss studies on hypnoanesthesia.
Keywords: Hypnosis, Pain, Anesthesia

INTRODUCTION

Hypnosis

Hypnosis is an abstract concept, which cannot be shown directly. It can be explained with the features it has. For these reasons, it is difficult to make a full description of hypnosis. According to the definition of the American hypnosis association, hypnosis is the bypassing of the critical factor of consciousness and placing an acceptable thought to the subconsciousness [1,2]. Hypnosis consists of the trance state and suggestion components. The trance state is a state in which the response to other stimuli is reduced, but the person is awake. The hypnotic trance state is a mental state in which both state and suggestion acceptability are present [1]. The process of creating a trance state in hypnosis is called hypnotic induction [2].

History

The Frenchman Anton Mesmer (1734-1815) was the first to use hypnosis medically. He believed there was a magnetic fluid in living things and thought that it had a healing effect. Marquis de Puysegur (1751-1825) called this situation somnambulism [3]. James Braid (1795-1860) is regarded as the descriptor of modern hypnosis and emphasized that a person's suggestibility is important. He used the word hypnosis, which comes from the Greek term for sleep [4]. James Esdaile (1805-1859) used hypnoanesthesia in India. He performed and published approximately 300 operations with only hypnosis [5]. John Elliotson performed 76 surgical cases using only hypnosis [6]. In 1955, the British Medical Association stated that hypnosis has a place to create anesthesia and analgesia in surgical and dental operations and that it can be used for analgesia in normal birth without affecting the course of the delivery [7]. In Turkey, for the first time in 1960, Doctor Recep Doksat announced hypnosis as a scientific study with his thesis on "Hypnotism" [2].

Studies on hypnoanesthesia

Many studies were done on the physiology of hypnosis. There are also many studies examining the effects of hypnosis on anesthesia, surgery, pain, and anxiety. However, there are still many mysteries waiting to be revealed in hypnosis.

Clinically, hypnosis has been used in anesthesia in a variety of settings. It has been studied as a complementary technique rather than an alternative

to general anesthesia. Scientific research methods limit the progression of hypnosis from experimental use to routine clinical practice. For example, it is challenging to find measurable physiological variables that define the hypnotic state. It is challenging to reliably and reproducibly measure a hypnotic trance, and a double-blind clinical trial involving hypnosis is impossible. However, recently, the interest in hypnosis has increased again due to conscious sedation seen more clearly in anesthesia.

Studies using positron emission tomography (PET) and functional magnetic resonance imaging (MRI) methods and potential studies evoked by painful stimuli have helped to better understand neural pain pathways.

According to the data obtained to date, large brain areas, including cortical and subcortical regions, are related to pain perception. Anterior cingulate cortex, insula, frontal cortex, 1st somatosensory cortex (S1), 2nd somatosensory cortex (S2), and amygdala are among the structures in the pain matrix. If the location and duration of pain are detected, high metabolic activity is observed in the lateral thalamus and at the S1 and S2 regions of the hemispheres.

On the other hand, in cases where emotional components of pain are emphasized, such as hypnosis, pain stimulation is mainly processed in the medial regions of the thalamus and reflected in the anterior cingulate gyrus [8].

Rainville and Faymonville tried to show the physiological correlations of hypnosis with PET. They stated that when there are changes in affective pain perception under hypnosis, there are metabolic changes in the anterior cingulate cortex, and there is no change under the same conditions in other cortex regions involved in pain perception. Functional MRI results were similar in healthy volunteers exposed to thermal pain with and without hypnosis [9].

In an experiment with 14 people, Li et al. revealed pain by stimulating the supraorbital nerve. This experiment showed that the pain threshold could be increased significantly when the hypnotic subjects were continuously suggested [10].

In a study conducted on preoperative anxiety, Ashton et al. showed that patients in the hypnosis group were more relaxed in the preoperative period than the control cases and displayed the therapeutic success of relaxation with postoperative self-hypnosis (autohypnosis) [11].

In a study conducted by Saadat et al. to reduce the preoperative anxiety of patients aged 18-65, compared with basal anxiety, a 56% decrease in the anxiety level of the hypnosis group at the entrance to the operating room was observed. On the other hand, a 47% increase in the anxiety of the control group was reported. It has been shown that patients in the hypnosis group were significantly less anxious after the intervention than those in the control group. As a result, a preoperative hypnosis session effectively reduces preoperative anxiety and fear [12].

Studies are showing the positive aspects of hypnosis in children as well. In a study, hypnosis was compared with a group given preoperative midazolam, and lower preoperative anxiety scores and postoperative behavioral disorders were observed in children in the hypnosis group [13].

In some studies, patients were given a 10-minute hypnotic induction session by a separate physician before sedation and local anesthesia for neck dissections and thyroid surgery. While patients in the hypnosis group had significantly lower pain scores, they needed less intraoperative opioid analgesics and sedatives. Postoperative nausea was also observed at a lower rate than the control group[14].

In another study of 171 people who underwent cataract surgery, the patients were divided into hypnosis (n=102) and a control group (n=69). The intraoperative drug use rate was significantly higher in the control group [15].

Sefiani et al. stated that hypnosis alone may be insufficient as an anesthetic. They reported that in the laparoscopic cholecystectomy and hernia repair operation series combined with local anesthesia, hypnosis, and sedation, 13 out of 35 cholecystectomy and 1 out of 15 hernia repairs required general anesthesia due to the discomfort of the patients [16].

CONCLUSION

Hypnosis is a safe practice that can be used in many areas and has no side effects. Hypnosis can be used safely to relieve anxiety before surgery, prepare the patient for the operation, postoperative analgesia, and discharge patients quickly. Case selection and patient compliance must be optimum to use hypnosis as an anesthetic method. We think that hypnosis should be used more widely to increase the psychological preparation and comfort of the patients who will undergo an operation and accelerate the recovery processes.

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REVIEW

Larva Treatment From Past to Present in Chronic Wounds

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Abstract
"Maggot Debridement Therapy" (MDT), known as larval therapy, is one of the traditional and complementary medicine practices with a history of application from ancient times to the present day. It is used in the treatment of chronic wounds that do not heal with conventional treatments. There are many case histories of successful treatment of stubborn wounds such as diabetic foot ulcers with MDT. Since it reduces the need for antibiotics and does not have any known significant side effects, it is an FDA approved treatment method that is now frequently used. In this review study, it is aimed to explain the development steps of MDT from past to present and its application methods and to discuss its medical importance in the light of the literature
Keywords: Larva, Maggot Therapy, Traditional Medicine, Wound, *Lucilia sericata*

INTRODUCTION

Wound is defined as the disruption of the integrity of the skin or mucosa as a result of a trauma. This necrotic condition that occurs in the skin and mucous membranes closes over time with a unique mechanism. This condition is called wound healing and consists of four overlapping physiological stages that usually go smoothly: Homeostasis, inflammation, proliferation and re-maturation. For wounds that do not heal or those that close too late, the removal of tissue debris for various reasons, removal of destructive products such as local infection and/or proteases from the wound bed stops healing [1].

A successful wound healing process depends on effective debridement and infection control [2]. Today, some difficulties are encountered in controlling chronic infected wounds with modern medical (conventional) methods due to the development of resistance to antibiotics and the inability of surgically debriding necrotized scar tissue. In this case, chronic wounds occur.

These kinds of wounds appear as wounds that reduce the quality of human life considerably and also fail to heal by not following the regular healing method, thus increasing the treatment costs considerably [3]. Such situations also require the development of alternative treatment methods or various new treatment methods. "Maggot Debridement Therapy" (MDT), known as larval therapy in some circles, stands out in this sense.

Larvae treatment is the use of sterile larvae of *Lucilia sericata* fly, which is also called green fly among the public, in the treatment of skin wounds to remove dead tissue and inflamed area without damaging the living tissue. Sterile larvae produced in university laboratories or commercial companies are usually applied to the wound by limiting it with cage-style dressings or tea-bag-like structures. With this treatment method, both wound healing takes place and the bad odor caused by necrotic tissue in the cases is significantly reduced [4].

HISTORY OF LARVAE TREATMENT

The history of the therapeutic use of larval therapy (Table 1) to debride necrotic tissue dates back to the beginning of civilization [5]. It was first discovered in the 16th century by the French surgeon Ambroise Pare (1510-1590). Ambroise Pare has tried to treat soldiers' war wounds larvae and achieved successful results. This treatment was first officially documented by John Forney

Zacharias in the American Civil War (1861-1865). It was pushed into the background due to the germ theory developed in the nineteenth century under the leadership of Robert Koch and Louis Pasteur. However, during World War I, when antiseptic devices were not sufficient, it was used by military orthopedist William Baer to treat fractures and wounds and gained popularity again. The first scientific study published on this subject was Prof. Dr. WS Baer who used larvae in the treatment of osteomyelitis in the 1930s. It was used as a standard wound treatment method in chronic infected wounds of soft tissues in hospitals in the United States until the 1940s [4]. After the discovery of antibiotics in the 1940s and the mass production of sulfonamides and penicillin, the use of larvae became limited to stubborn wounds and was out of use for a while Sherman, who conducted one of the oldest (1988) rewiew studies on this subject, used larvae for the treatment of chronic osteomyelitis and reported that antibiotics were ineffective in this treatment and that it was suitable for situations where surgery was not possible [7]. Larva Treatment has come to the fore again with the development of the sterilization methods required for larva treatment with the advancing technology towards the 2000s and the antibiotic resistance problem in bacteria [8].

NEXT TERM STUDIES

Larval therapy was first implemented in 2002, and the treatment of difficult healing wounds with the larvae of Lucilia Sericata in 2007 in Turkey "project is supported by TUBITAK [9]. In the same year, a study was published stating that larval treatment is the first choice in leg ulcers, carbuncles, pressure ulcers and infected traumatic wounds, and its beneficial effects are also seen in eliminating the diabetic foot and malignant tissue [10]. Whitaker et al. (2007) stated that the clinical indications for larvae treatment are diverse, but the main indication is the presence of major comorbidities that prevent surgery and wounds infected with multidrug-resistant bacteria [5].

Falch et al.'s study in 2009 showed that larvae debride wounds by dissolving necrotic tissue, show an antimicrobial effect and support wound healing [11]. In different studies, it has been reported that the larvae provide faster wound granulation [2, 12-14]. Bolaban (2009) studied samples from 23 identified diabetic ulcers and 2 chronic wounds and concluded that larval secretions reduced colonization of MRSA (methicillin resistant *Staphylococcus*), MSSA (methicillin susceptible *Staphylococcus*) and *P. aeruginosa* by 50% [15] Nenoff et al. (2010) used MRSA strains in their study and showed that the larvae have antimicrobial activity against gram-positive bacteria as well as necrectomy and debridement properties [12]. It has been shown in studies that the secretions of the larvae contain allantoin, cysteine, sulfhydryl radicals, glutathione, ammonia, calcium carbonate and growth stimulating factors. It has also been reported that the larvae have many digestive enzymes, including carboxypeptidase A and B, leukine aminopeptidase, collagenase, and serine proteases (trypsin-like and chymotrypsin-like enzymes) while feeding on the wound, thus showing a successful debridement [15-18].

Zarchi et al. (2012) showed three randomized clinical studies and five non-randomized studies evaluating the efficacy of sterile *Lucilia Sericata* applied to ulcers of various etiologies in their systematic study. As a result, it has been reported that there is a significant difference in debridement and recovery in larval treatment compared to conventional treatment such as saline moistened gauze [18]. The treatment of larvae was not only evaluated in terms of its effectiveness and mechanism, but was also examined in terms of its side effects and it was observed that the larvae could sometimes cause an itching sensation, and some patients needed analgesic due to the pain they cause during larva treatment [14].

LARVA TREATMENT APPLICATION METHODS

The method of administration is as important as the effectiveness of larval treatment. A lot of research has been conducted on the application of the treatment, the type of larva used and its production. Historically, *L. sericata* and *L. cuprina* larvae are considered to be beneficial in the treatment of ulcers. Recently, larvae of various other fly species

(*Calliphora vicina, Calliphora vomitoria, Phormia regina, Chrysomya albiceps, Sarcophaga carnaria and Hermetia illucens*) have been shown in vitro to have characteristics that make them favorable (i.e. debridement efficiency and putative antimicrobial potentials). The larvae generally do not damage the healthy dermis and subcutaneous tissue; However, the risk of damaging the healthy epithelium should not be forgotten [3,5,13].

There are two different application methods of Larva Treatment, mainly the Cage method and the Biobag. In the cage method, adhesive hydrocolloid materials are cut according to the wound and the edges are framed with this material so that the wound is exposed. A sterile piece of fine gauze is cut wider than the wound and smaller than the hydrocolloid frame. This tulle is attached to the hydrocolloid frame with adhesive tapes with one end exposed. After the larvae are left to the wound from the exposed end of the tulle, this part is also closed. Sterile tampons are placed on the tulle to ensure drainage. Tulle allows larvae to breathe as well as facilitates the drainage of necrosis tissue [19].

In the biobag method, the larvae are placed between two pieces of tulle made of 0.5 mm thick special material (polyvinylalcohol-hydro-sponge) as in a tea bag and the mouth of the bag is glued. Because the bags are permeable, the larvae can be fed and their secretions penetrate the wound so that infection control and recovery can be achieved. In this method, a cage-style dressing is not needed. After the bags are placed directly on the wound site, they are wrapped with gauze or a bandage in order to remain fixed [19].

There are studies to find out which of these two methods provide more effective wound debridement and how well the larvae can affect the wound with these methods-comparing the advantages and disadvantages. Accordingly, it has been reported that the development of the most appropriate and effective original package designs containing sterile larvae is important for practicality in practice and successful treatment [20]. This method called "biobag" is thought to have advantages such as preventing mechanical irritation due to the larvae walking directly on the wound and reducing pain due to this [19,21].

Disinfection of larvae to be used in treatment is also one of the important points during the application. If it is applied improperly and necessary precautions are not taken, septicemia may develop[22]. For this purpose, 20% sugar solution is given to the larvae to be used in the treatment. Pieces of meat or liver are used to stimulate ovulation. After that the collected eggs are separated from each other and their surfaces are disinfected with 1% sodium sulfite solution added to physiological saline with 2.5% formaldehyde, using a mixture of 3% Bakto Agar and crushed liver (1:1 weight/volume) is transferred to the prepared sterile medium. Approximately 2-36 hours later, the larvae hatched from the eggs are removed from the fattening place and placed in sterile containers for use in treatment. Sterile larvae can live for up to five days without losing their vitality at 5-8 ºC [4]. Studies on the production of the larvae to be used and the supply chain management have lagged behind the investigation of the effectiveness of larval treatment [23].

CURRENT STUDIES AND LATEST STATUS

Larva treatment, which started as a last-resort trial in aggressive chronic wounds and has historically become one of the traditional methods, is now an FDA-approved therapy against MDT, non-healing skin and soft tissue wounds [1]. Given its ability to reach necrotic areas, prevent unnecessary living tissue debridement and only debride dead tissue, accelerate wound healing, antibacterial effects and no side effects, it is an appropriate method in the treatment of pressure sores in patients with MDT resistant, with both cheap and fast and effective recovery [23]. It is widely used in treatment all over the world. Zurairie et al. (2020), with a more comprehensive study involving 580 patients, concluded that larval treatment provides a faster and more effective debridement than conventional treatment, and leads to a faster development of granulation tissue [24] be carried out in Turkey in 2014 by the Traditional and Complementary Medicine Practices regulations and other doctors treating larvae with the traditional treatment has been made official. Özkan et al. (2019) states that

in addition to the treatment of bacterial-induced ulcers, it can also be used for symptoms associated with mycotic infection and leishmaniasis [1].

Many studies have been conducted on which types of wounds the Larva Treatment, which is such an effective method of debridement, can be used in which ones, and many cases have been presented. Four cases concerning the use of maggot therapy in Turkey are listed below;

In case one, on the 45th day of hospitalization, stage 3 pressure sores of the patient developed 7 cm in the sacrum area, 8 cm in the right trochanter and 3 cm in the left trochanter, and the wounds of the patient were resistant to conventional treatment and did not heal. Because of the presence of dense necrotic tissue and infected areas in the pressure wound, the patient was not able to undergo surgical debridement. The larvae were placed with the cage method for 3 days and replaced with new larvae at the end of 3 days and the dressing was changed 4 times a day as they got dirty during this period. Maggot debridement treatment was continued for a total of eight days and MDT was discontinued at the end of the eighth day. As a result of MDT, it was observed that necrotic tissues disappear completely [25]. In case two, a seventy-five-year-old female patient with chronic venous insufficiency for 10 years was treated with different methods for 11 months for 11 months of the infection in the second toe of the right toe, which was ulcerated one year ago and was amputated due to no response. Considering the risk of amputation of other fingers, it was decided to start maggot therapy. Maggot was applied once a day for 3 days, a response was obtained in 3 days, and then autologous skin transplantation was performed on it and complete treatment was achieved. During the application of maggots, the patient did not have any major complaints, except for an increase in pain, which was eliminated by giving analgesics. In conclusion, in patients with chronic cutaneous ulcer who do not respond to conventional treatment methods, maggot debridement treatment is seen as an alternative treatment option that is economical, easy to apply, gives quick results and has few side effects [26]. In the third case report, a 33-year-old male patient with spinal cord injury (SCI) developed a 3*2cm stage 3 pressure sore on the postoperative 2nd day. Although the patient received convectional wound therapy for 3 months, she did not recover and resistance to treatment developed. Mechanical and surgical debridement could not be achieved because of the presence of necrotic and infected areas and preservation of pain sensation; Larvae treatment is planned. Larvae treatment was applied by changing the larvae once every 3 days in cycles of 48-72 hours with the cage method in a way that the 1-2 day-old larvae were 5-10 larvae per cm^2. The tulle on the cage was also changed every 6-8 hours. In this way, it was seen that the wound was completely closed on the 3rd week [27]. In the fourth case report, a 60-year-old 14-year-old DM patient was administered conventional wound care and antibiotic prophylaxis for 6 years due to grade 2 diabetic foot wound in the right foot Achilles tendon area. During the process, the wound got bigger and infected due to circulatory disorders. Two years ago, a left leg below the knee amputation was performed due to a diabetic foot wound. MDT was recommended for the treatment of necrotic tissues before any surgical intervention was planned for the patient who had two strokes and 1 heart attack. MDT was applied to our patient with 2 sessions of cage dressing for 5 days. Since the pain of the patient reached an unbearable level in the first session, the treatment had to be stopped at the 48th hour. In the second session, conventional TENS was applied to reduce the pain that would occur in the patient and the treatment was continued for 72 hours. At the end of 5 days, it was observed that sufficient wound debridement was achieved and the treatment was terminated [28].

In addition to all these, larval treatment, which has a wide area of use in chronic wound treatment, has found application in veterinary medicine. Although its use and studies in animals are very few, successful results have been obtained in a limited number of studies [29]. Studies have also reported that larval treatment on animals has been largely successful in wound healing [19].

Table 1. Chronological order of studies on larval treatment

No	History of The Study	Name of The Study	Author of The Study	Wound-Regıon Worked On	Result
1	1931	The treatment of chronic osteomyelitis with the maggot	Baer[6]	chronic osteomyelitis	Recovery was achieved in almost all patients.
2	1988	Maggot therapy: a review of the therapeutic applications of fly larvae in human medicine, especially for treating osteomyelitis	Sherman et al.[7]	chronic osteomyelitis	It continues to be suitable for situations where antibiotics are ineffective and surgery is not feasible.
3	2001	Clinical applications for maggots in wound care	Mumcuoğlu[14]	Necrosis wound	It is a simple, efficient, well tolerated and cost effective tool in the treatment of wounds and ulcers that do not respond to conventional therapy and surgical intervention.
4	2002	The effect of containment on the properties of sterile maggots	Thomas et al.[21]	Research has been done on the properties of sterile maggots.	showed that free-range maggots survive and grow significantly faster than maggots in the bag
5	2002	Clustering of bloodstream infections during maggot debridement therapy using contaminated larvae of Protophormia terraenovae	Nuesch et al.[22]	chronic ulcer	Application on chronic ulcers seems safe, provided the maggots have been effectively disinfected.
6	2006	Maggot Therapy: The Science and Implication for CAM Part I – History and Bacterial Resistance	Nigam et al.[8]	Necrotic tissue	Maggot therapy can be used successfully in the treatment of chronic, long-term, infected wounds that previously failed to respond to conventional therapy.
7	2007	Treatment of a chronic venous ulcer patient with maggot debridement treatment	Özkan et al.[26]	chronic ulcer	With maggot therapy, it was determined that the ulcer was completely cleared from necrotic and suppurative tissue.
8	2007	Larval therapy from antiquity to the present day: mechanisms of action, clinical applications and future potential	Whitaker et al.[5]	The history of larval therapy has been studied.	The use of larval treatment application is increasing all over the world due to its effectiveness, safety and simplicity.
9	2007	Maggots of *Lucilia Sericata* in treatment of intractable wounds	Orkiszewski[10]	Today, the treatment of maggots has turned into less treatment as a last resort, but it is the first choice for leg ulcers, carbuncules, pressure ulcers and infected traumatic wounds.	Clinical experience has shown that maggot treatment can significantly reduce treatment costs by shortening hospital stay and reducing the use of antibiotics.
10	2008	A review of the use of maggots in wound therapy	Gupta[3]	Chronic wound	The use of larval therapy on chronic wound has been studied.
11	2009	Maggot Debridement Treatment of Suppurative Chronic Wounds	Mumcuoğlu[4]	Suppurative Chronic Wound	Cleaning and granulation of MDT in chronic wounds proven to be an effective method of startup.
12	2009	Maggot therapy in wound management	Falch et al.[11]	Chronic wound	-
13	2009	*Investigation of Antibacterial Effects of Lucilia Sericata Larvae and Secretions on Methicillin Resistant Staphylococcus Aureus (MRSA) and Methicillin Sensitive Staphylococcus Aureus (MSSA) Under In-Vivo and In-Vitro Conditions*	Bolaban[15]	In vitro experiment	In in-vitro experiments conducted in our study, it was observed that larval secretions decreased the number of MRSA, MSSA and P. aeruginosa colonies by 50%.. In our study Lucilia sericata on various lethal microorganisms and enzyme secretions of the larvae and reproductive effects stopper has been demonstrated for the first time in Turkey.
14	2009	Maggot Therapy Application in Conventional Therapy Resistant Sacral Compression Wound: A Case Report	Tuğcu et al.[27]	Pressure sores	There was a dramatic improvement in the patient.

15	2010	Biosurgical débridement using *Lucilia Sericata*-maggots-an update	Nenoff et al.[12]	chronic ulcers that don't heal	Chronic wound debridement, reduced bacterial load, and improved wound granulation.
16	2010	Larval Debridement Treatment	Polat et al.[9]	Foot wound	The wound is completely healed.
17	2012	The efficacy of maggot debridement therapy--a review of comparative clinical trials	Zarchi et al.[18]	3 randomized clinical trials and 5 nonrandomized trials applied to ulcers of various etiology were shown	7 studies had debridement and / or recovery as outcome variables
18	2013	Multiple actions of *Lucilia Sericata* larvae in hard-to-heal wounds: larval secretions contain molecules that accelerate wound healing, reduce chronic inflammation and inhibit bacterial infection	Cazander et al.[16]	Up-to-date information on wound healing mechanism has been compiled.	Molecules involved in wound healing may be candidates for the development of new agents.
19	2014	Sterile *Lucilia Sericata l*arvae in the treatment of chronic wounds uygulamaları.	Tanyüksel et al.[20]	23 diabetic ulcers, 2 chronic wounds	19 full cure, 6 partial debride
20	2015	Maggot Debridement Treatment in Pressure Sore: A Case Report	Yağız et al.[25]	Stage 3 pressure sores	necrotic tissues disappeared completely
21	2016	Maggot debridement therapy as primary tool to treat chronic wound of animals	Choudhary et al.[29]	Limb wounds in animals	A very safe, efficient and easy healing method for chronic wounds.
22	2016	TIME management by medicinal larvae	Pritchard et al.[17]	Venous leg ulcer	Debridement is provided.
23	2017	The Use of Myase Fly Larvae in Wound Treatment	Yaman et al.[19]	Information was given about the history of larvae, method of use, number, indication contraindication, wound healing mechanism..	In recent years, successful results have been obtained by applying maggot therapy to a large number of patients. In the future, maggot therapy may be replaced by larval-derived drugs.
24	2017	Larval Therapy for Chronic Cutaneous Ulcers: Historical Review and Future Perspectives	Raposio et al.[13]	Chronic cutaneous ulcer	It accelerates the wound healing process.
25	2018	Painless approach to maggot debridement treatment in a diabetic foot ulcer patient: a case report	Uçar et al[28]	Stage 2 diabetic foot	Adequate debridement provided
26	2019	Larval Therapy and Chronic Wounds	Gazi et al.[1]	Pressure sores	Debridement is provided.
27	2019	Effectiveness of Chronic Wound Debridement with the Use of Larvae of *Lucilia Sericata*	Bazalinski et al.[2]	Venous ulcer	The wound was treated more cost-effectively.
28	2020	The maggot therapy supply chain: a review of the literature and practice	Stadler[23]	Researching supply chain management through the conceptual and disciplinary framework	There is not enough work.
29	2020	Maggot Therapy in Wound Healing: A Systematic Review	Zubir et al.[24]	The full text of five studies involving 580 patients with chronic wounds was included.	It provided faster and more effective debridement of necrosis tissue.

CONCLUSION

Larvae treatment is an easily applicable and successful method in traditional and complementary medicine. Although it has been discredited from time to time in the historical process, it has become a widely used FDA-approved form of treatment today. The fact that it has almost no side effects, reduced the need for antibiotics and the successful results of the studies

made larval treatment an important reason for preference in chronic wound treatment. It is interesting to see success with MDT in chronic wound treatment, especially in cases where conventional treatment has not been successful. The microdebridement ability and proteolytic enzymes of the larvae appear to be key to this success. Larva treatment in the coming period; It seems that it will continue to be a hope for many desperate patients with its current scientific evidence and application practice and will find a place in conventional medicine applications beyond the field of traditional medicine.

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REVIEW

Medical and Cosmetic Applications of Persimmon (*Diospyros kaki* L.) and Their Toxicity Assessment-A review

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Abstract

In this review study, it is aimed to summarize the information cited about medical and cosmetic applications of the date persimmon (*Diospyros kaki* L.f.) and accordingly the toxicity assessment. For this purpose, the information cited about medical and cosmetic applications of the date persimmon (*Diospyros kaki*) and accordingly the toxicity assessment were summarized. Persimmon (*Diospyros kaki*), which is cultivated in tropical/subtropic regions such as China, Korea, Japan and Brazil, especially in the Far Eastern countries with more hot climate conditions, has been named as Trabzon Persimmon because it entered Turkey through the Black Sea region. This fruit type has a very important role on the immune system thanks to vitamins and some active ingredients. Although it is mostly consumed as fresh fruit and dried in our country, there are also formulations developed as medical support products (dematological and cosmetic applications etc.) in different countries worldwide. However, toxicity assessment studies on natural plants/herbal products are also very few. Since there is scientific evidence on the phytotherapeutic effects of *Diospyros kaki*, and the product scale on the market is very narrow, this is highly promising for future healthcare products.

Keywords: *Diospyros kaki*, Persimmon, Medical Applications, Cosmetic Applications, Toxicity

INTRODUCTION

Fruits and vegetables play a very important role in human nutrition and diet. Consumption of these nutrients plays a role in the development of health with the presence of potentially bioactive components, and also the phytochemicals they contain are various bioactive compounds that are widely accepted for their useful roles in human physiology [1]. The number of plants has gained popularity as healthy food ingredients, but researchers' attention is still required in many respects.

Persimmon (*Diospyros kaki* L.f.) is one of these nutritious fruits given with strong antioxidant activity [2,3]. Persimmon is a pulpy/fibrous tropical and deciduous fruit belonging to the Ebenaceae family. The world's regions with hot climates, such as China, Korea, Japan, Brazil, Turkey and Italy are known to grow it widely [4,5]. The Mediterranean region has a manufacturing potential of up to 110,000 tons per year. The worldwide persimmon (*Diospyros kaki*) manufacturing was found as about 5.75 million tons in 2017, and China is the leading country (2.36 million tons) followed by Korea (0.32 million tons) and Japan (0.25 million tons) [6]. Persimmon is not very popular in European countries, but due to its awareness of its potential for consumer health development, the demand needed increases [2,7,8]. There are more than 400 species of persimmon grown globally. *D. kaki, D. virginiana, D. oleifera and D. lotus* are some of the most important species of them [9]. However, it is interesting that Diospyros kaki is the most promising species. Popular types are widely cultivated in Japan in general [10,11].

Today, nutrition and health-related issues are intertwined, and researchers have focused on a diet-based regimen strategy that has emerged to combat various physiological threats, including

cardiovascular disorders, oxidative stress, diabetes mellitus, etc. At this point of view, consumption of fruits and vegetables is of great importance in protecting human health. However, phytochemicals and bioactive molecules have also become popular as promising therapeutic agents for a variety of ailments. According to the some studies in the literature, persimmons and components of them are thought to be effective because of their rich phytochemistry in reducing oxidative damage caused by reactive oxygen species (ROT). Anti-malignant and anti-melanogenic compounds, which are among the functional components of persimmon, show antioxidant potential. There is, however, evidence that pharmacological administration of persimmon and functional compounds such as proanthocyanidin may help against hyperlipidemia and hyperglycemia. However, the astringent effect and diospirobezoar formation create a gap to increase vitality. Persimmons and their ingredients have the potential to be one of the fully effective modules effective in diet-based therapy; however, meticulously integrated research and meta-analysis are still required [12]. Therefore, it is of great importance to investigate and carry out toxicity studies related to the use of persimmon fruit and related products. For this reason, in this review study, it is aimed to summarize the researches related to the toxicity studies with medical/pharmacological and cosmetic applications of the persimmon (*Diospyros kaki*).

General features of the persimmon

Diospyros is a member of the genus Ebenaceae family and consists of 400 species that have spread in tropical and subtropical regions of the world. However, only 4 of these species have commercial importance (*Diospyros kaki, Diospyros lotus, Diospyros virginia, Diospyros oleifera chen*). Among these 4 species, the most cultivated species in the world is *Diospyros kaki* [13].

The word Diospyros is derived from the words Mythological Jupiter (Dios) and dane (Pyros) in the mythological period, meaning the food of the gods due to the beautiful appearance and taste of the fruit[14].

Place in Systematic:

Kingdom: Plantea
Division: Magnoliophyta
Class: Magnoliopsida
Order: Ebenales
Family: Ebenaceae
Genus: *Diospyros*
Species: *Diospyros kaki*

Persimmon, whose homeland is China, was brought from Japan before. It has been started to be called "Japanese Apple" among the people [15].

In Figure 1, ripe and unripe versions of the fruit of Persimmonare given. Commercially recognized varieties and properties of *Diospyros kaki* are given in Table 1.

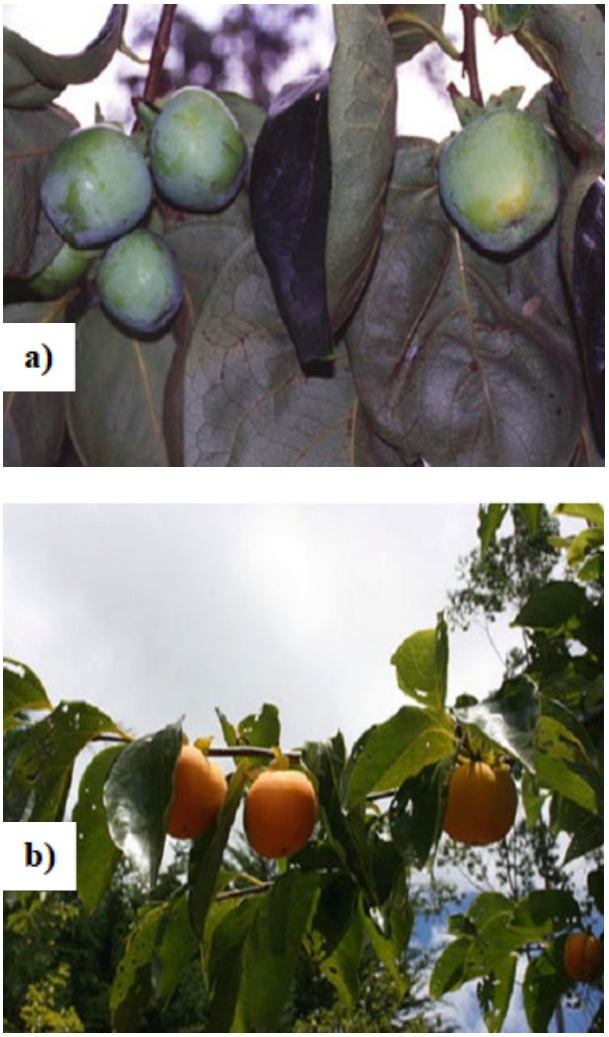

Figure 1. Ripened and unripe versions of the fruit of Persimmon **a)** immature form (stringent form) **b)** ripe form (non-stringent form)

Table 1. Commercially recognized varieties and properties of *Diospyros kaki*

Types	Characteristic
Hiratanenashi	stringent
Ishibashi-ves	bitter pulp and high-soluble tannins
Ton-ves	
Maekawa-jiro (MJ)	non stringent
Matsumoto-wasefuyu (MF)	sweet pulp and low-soluble tannins

Persimmon, which is grown also in economic field in the countries with tropical and subtropic climates such as China, Korea, Japan, and Brazil, is grown economically in the Mediterranean, Aegean, East Black Sea, Southeastern Anatolia and Marmara in our country, Turkey [16]. Persimmon plays very important roles in the immune system in humans thanks to vitamins and some special nutrients. In addition, since it has a rich content in vitamin c, dietary fiber, carotenoids and polyphenols, it has been consumed both fresh and dried since ancient times [7]. In the food industry, it has different uses such as marmalade, cake, puree, various sauces, ice creams, cream and custard making. In addition, fresh or dried leaves are considered as tea in some countries [15]. *Diospyros kaki* species, which enters our country from the Black Sea region, is therefore called the Persimmon, and is also known as paradise fruit, Japanese persimmon and public in some places. Today, due to the changes in consumption habits of the societies and demands for alternative products, the interest in subtropic climate fruits is increasing. Persimmon cultivation, which is mostly cultivated in tropical and subtropic climates, has also started to become widespread in our country (Turkey). Although there is no information about when it was brought to our country, it is known among people as the Trabzon fruit because it first entered our country through Trabzon. Due to the suitability of the climatic conditions in our country, it is a fruit that is cultivated intensively in the Mediterranean region, especially in Hatay, Mersin and Adana, and that the people of the region gain economically. Figures 2, Figure 3 and Figure 4 persimmon production volume in the world, producing country in the world and the number of Persimmon trees in Turkey, area, yield and production quantities are given. When the date of persimmon production in our country is analyzed according to the data in 2017, Adana (9100 tons), 2nd place İzmir (4179 tons), 3rd place Mersin (3403 tons), 4th place Hatay (3172 tons) and 5th place Adıyaman (2991 tons) is located (http://www.tuik.gov.tr/)[17].

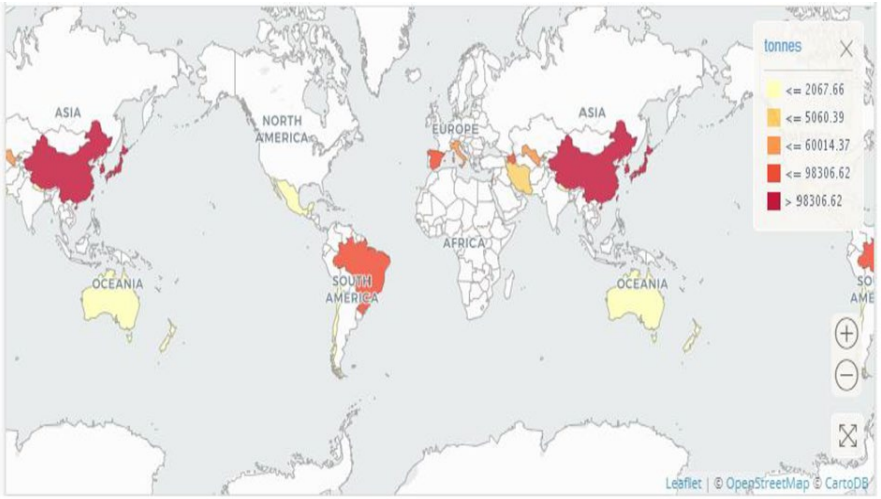

Figure 2. Persimmon production in the world (https://www.mapsofworld.com/world-top-ten/persimmon-producing-countries.html)[18]

The main phytochemicals found in persimmon

Compounds such as proanthocyanidin, flavonoid oligomer, tannins, phenolic acid, carotenoid and catechin are commonly found in persimmon and leaves [2,14,19,20]. Dried fruit is known to consist of 0,16-0,25g/100g polyphenol, 0,002g/100g

carotenoid and 0,64-1,3g/100g proteins. Dried fruit leaves are known to contain 1,15g/100g of phenolic compounds and 63,48g/100g of fiber, and it is thought to have a beneficial effect [19].

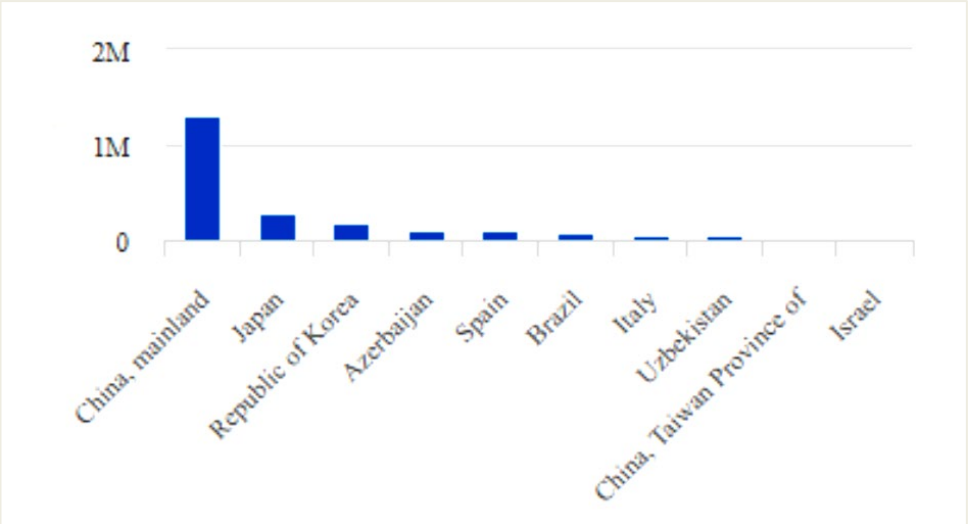

Figure 3. Persimmon manufaturing countries in the world (https://www.researchgate.net/figure/trnL-F-region-and-primers-This-figure-shows-the-coding-and-non-coding-portions-of-the_fig1_8346824)[21]

Years	Number of trees fruiting	Number of trees does not bear fruit	The area of fruit bulk (decares)	Efficiency (kg/trees fruiting)	Production amount (tons)
2010	733.563	194.329	19.741	36	26.277
2011	800.898	197.141	20.900	35	28.295
2012	857.840	188.454	21.317	38	32.392
2013	883.235	172.778	226.42	38	332.32
2014	873.755	184.245	206.19	38	334.70
2015	860.177	197.067	207.89	39	337.25
2016	865.242	275.655	230.24	40	346.50
2017	883.449	303.467	239.32	43	380.43

Figure 4. Number of *Persimmon* tree in Turkey area, yield and production quantity (https://www.mapsofworld.com/world-top-ten/persimmon-producing-countries.html)[18]

Evaluation of the date persimmon in terms of health benefits

It has been found that persimmon leaves have beneficial effects against oxidative stress, hypertension, diabetes mellitus and its complications and atherosclerosis [22,23]. Its bioactive components, especially carotenoids and tannins, are effective in damping free radicals, reducing

cardiovascular risk factors (blood pressure and cholesterol), and reducing the risk of diabetes mellitus, but also against cancer formation [19,24]. The tannins found in persimmon are ultimately responsible for the improvement of physiological threats. It is responsible for antibacterial, anti-allergic, free radical hunt, lowering blood pressure, anticancer and antioxidant activities [20,22,25-27]. The antioxidant activities of the diagnosis depend on the presence of nucleophilic groups with some antimutagenic properties by inhibition of nitrogen reactive compounds [28]. They are also effective in reducing the incidence of stroke and in hypertensive disorders [26]. Similarly, flavonoids inhibit the activity of the angiotensin converting enzyme, which raises blood pressure, and inhibits cyclooxygenase, which forms prostaglandins. Some in vitro studies have illustrated the inhibitory effects of flavonoids in preventing platelet aggregation and thrombosis formation. According to research conducted in the USA, there is an inverse proportion between persimmon consumption and coronary heart disease [19]. Carotenoids and catechins also carry anticancer perspectives against various cancer cell lines [28,29]. Anthocyanidins are similar to other flavonoids for carrying out in vivo and in vitro antioxidative activities and in vivo anti-mutagenic properties. In addition, persimmon fruit has a hypocholesterolemic and antioxidant potential [30-32].

Pharmacological and dermatological applications of persimmon (*Diospyros kaki*)

Persimmon (*Diospyros kaki*) is applied and formulated in the market in dermatological and cosmetic applications, in the form of extraction variations and creams. In Table 2. the dermatological and cosmetic formulations and effects of various active ingredients of *Diospyros kaki* were given.

Jung et al. (2015) [33] applied to melanoma cells B16F10 of mouse stimulated with MSH in the form of acetone-water (70%) extract (10-100 µg/ml) by purifying quercetin-3-O-β-dglucopyranosyl- (1 → 6) -β-dglucopyranoside active substance from *Diospyros kaki* fruit, they encountered results such as hypopigmentation effects, inhibition of melanin synthesis, inhibition of tyrosinase activity and decreased expression of melanogenic proteins. Xue et al. (2011) [34] observed antithrocinase activity (moderate) by in-vitro L-DOPA oxidation, which obtained the active ingredient of chrysontem from the leaves of persimmon as a methanolic extract. Ohguchi et al. (2010) [35] obtained the active substances of isoquercitrin (quercetin-3-oglucoside) and hyperin (quercetin-3-ogalactoside) as acetone extract from the peel of the fruit, and found that the on the mouse it inhibits melanin biosynthesis on b16 melanoma cells (kojic acid and arbutin). Fukai et al. (2009) [36] extracted 2-methoxy-4-vinylphenol from the shell, methanolic and aqueous, and observed antithrosinase activity (higher than arbutin). Thuong et al. (2008) [37] provided rotungenic acid, 24-hydroxyursolic acid, ursolic acid, oleanolic acid and spathodic acid substances from the leaves as methanolic extract and obtained inhibitory effects on protein tyrosine phosphatase 1B (PTP1B). Tiechi et al. (1999) [38] tested the in vitro antithyrosinase activity in ethanolic form of the crude extract of the fruit and observed antithelrosinase activity comparable to the arbutin. An et al. (2005) [39] tested the inhibitory activity against ethanolic extract and their purification from fractions I, II and III xanthine oxidase, collagenase and elastase enzymes, found antithrocinase activity, collagenase inhibition, collagen synthesis in culture-promoted fibroblasts, xanthine oxidase activity and elastase inhibitory effects. Tsang et al. (2016) [40] obtained gallic acid in form of gallic acid dilutions and topical preparation from leaves and fruits, and observed anti-inflammatory, anti-microbial, histamine release inhibition effects by application of eiosinophil-dermal fibroblast. The method used by Kumar et al. (2013) [41] and Tsang et al. (2016) [40] was experienced on Swiss albino rats, zebrafish and UV-B mice tested in skin model, and it was observed suppressing the release of eosinophils-dermal fibroblasts of pro-inflammatory cytokines (IL-6) and chemokines (CCL7 and CXCL8) - oxidants and with modulating of MMP-2/MMP-9 two-stage skin carcinogenesis, depigmentation and skin lightening effect and anti-aging effects were

Table 2. Dermatological and cosmetic formulations and effects of various active ingredients of *Diospyros kaki*

		Effects of dermatologically/cosmetically;
Dermatological and cosmetic formulations of various active ingredients of *Diospyros kaki*:	• Fraction purified from acetone-water (70%) extract • Acetone extract • Methanolic extract and purified fractions • Ethanolic extract • Purified fractions of ethanolic extract • Gallic acid dilutions and topical preparation • Diluted samples • Topical cream • Aqueous methanolic extract [42]	• Hypopigmentation effects • inhibits melanin synthesis • Prevents tyrosinase activity • Decreased expression of melanogenic proteins • Antityrosinase activity (medium) • Inhibitory effects on protein tyrosine phosphatase 1B • Collagenase inhibition • Collagen synthesis promoted in fibroblasts • Xanthine oxidase activity • Elastase inhibitory effects • Anti-inflammatory, • Anti-microbial • Prevents histamine release • 7,12-DMBA/Croton oil from two-stage skin is suppressed by modulatiın of anti-oxidants and MMP- 2/MMP-9 in Swiss albino mice • Depigmentation and skin lightening effect • Anti-aging effects (in-vivo and in-vitro) • Attenuation of oxidative damage from UVA to human skin fibroblasts • Photo protector (UV-B) • Anti-inflammatory • Reduces melanin synthesis • Inhibits tyrosinase activity and suppresses melanogenesis in B16 melanoma cells • Protects skin against UV-ind. oxidative damage • Anti-inflammatory effects • To give the skin “Golden Yellow” color • Protection against UV-skin damage • Suppress release of pro-inflam. cytokine and chemokine from eosinophil-dermal fibroblast[42]

observed induced by 7,12-DMBA/croton oil (in-vivo and in-vitro), modulated by 7,12-DMBA / croton oil. Domingo et al. (2010) [43] and Jeon et al. (2010) [44] extracted the active ingredients of epicatechin and epigallocatechin from fruits and leaves, diluted samples and cultured human skin fibroblasts in topical cream, and healthy human volunteers used split face study design applications, as a result, reducing UVA-induced oxidative damage in human skin fibroblasts, photo protector (UV-B) have achieved anti-inflammatory, melanin synthesis-reducing effects. Li et al. (2014) [45], Kitagawa et al. (2011) [46] and Tsang et al. (2016) [40] obtained chlorogenic acid from fruits and leaves and applied it to B16 melanoma cells with 0-500 µM dilution, as a result, they found tyrosinase activity inhibition and suppression of melanogenesis in B16 melanoma cells. Zaghdoudi et al. (2015, 2016) [47,48] obtained the β-carotene by purifying it from fruit pulp and peel, and determined the effects of giving the skin a "golden yellow" color and protection against UV-skin damage. Anunciato and da Rocha Filho (2012) [49] purified lycopene from the fruit part and determined the effects of reducing the level of skin erythema and regulating cholesterol. Kaulmann et al. (2014) [50] obtained leutin and xeaxanthin fruit, providing protection against UV damage and ROS. Gu et al. (2008) [30] and Zhou et al. (2016) [51] provided tannins and procyanidinoellagitanin,

including flavanoellagitan, as aqueous methanolic extract from fruit pulp, and obtained findings such as lowering ROS levels of exposure to gamma radiation in HEK 293T cells and lowering ROS levels in HEK 293T cells. Kim et al. (2016) [52] purified coussaric and betulinic acid from leaves, applied lipopolysaccharide-induced RAW 264 macrophages and observed anti-inflammatory effects. Xue et al. (2011) [34] obtained reports that chrysontem, isolated from persimmon leaves, shows moderate inhibitory activity for tyrosinase and that chrysontem inhibits carcinogenesis and tumor metastasis caused by the tumor promoter in vivo. Also in the same study, antifungal activity of hyperoside and trifolin; anti-inflammatory activity of isoquercitrin; antiallergic activity of astragalin; and angiotensin converting enzyme inhibitory activity of astragalin, isoquerycrine has also been reported. According to Wang et al. (2011) [53], the vomifoliol 9-O-a-arabinofuranosyl-(1-6)-β-D-glycoranorano side from persimmon leaves may increase peripheral glucose as an insulin sensitizing agent against type 2 diabetes mellitus. According to Chen et al. (2002) [54], ursolic acid (UA), 19-hydroxy ursolic acid and 19, 24-dihydroxy ursolic acid (DHU), stimulated superoxide production and tyrosyl phosphorylation release could help to pharmaceutical applications. Some reports have proved that persimmon leaves increase coronary artery blood flow in the hearts of rabbits and frogs in vitro and coronary blood circulation in anesthetic dogs [55]. Huang et al. (1983) [55] evaluated the effects of alcohol extract from palm leaves on various cardiovascular indices in anesthetic dogs. In addition, it has been reported that persimmon leaves show protective activities against injuries caused by myocardial ischemia. In the experiment of Deng et al. (2004) [56], the acute myocardial ischemia model was stimulated by ligation of the left anterior descending coronary artery in the distal third segment in rat open breast rats. Also, persimmon with its leaves flavonoids can reduce AST, CK and LDH release and MDA production, as well as increased activities of SOD, Naş-ATŞ enzyme and ATP. There are studies showing that Ca2-enzyme PLF has myocardial protective effects against I/R damage in rats [57]. Persimmon leaves showed potential antioxidant activity in in vitro studies. Ethanolic extracts from leaves (600 mg/kg) were effective in delaying lard deterioration, which showed slightly higher antioxidative activity than hesperidin (100 mg/kg) and tea polyphenol (100 mg/kg) [55]. Total flavonoids from persimmon leaves significantly reduced the level of reactive oxygen species and malondialdehyde with increased catalase, SOD and glutathione peroxidase (GSH-Px) activity in E1 cells [31]. This indicates that persimmon extract and palm leaf tea leaves have an oxygen-free radical and antioxidant cleaning effect. To investigate the mechanisms in osteoblast cells injured by oxidative stress, Sun et al. (2014) [58] discussed potential therapeutic or toxic effects on MC3T3-E1 cells stimulated with H_2O_2 and found that flavonoids from persimmon leaves (FPL) reduced H_2O_2-induced apoptosis in MC3T3-E1 cells via the NF-kB pathway. The results suggest that the molecular mechanism of FPL in antiapoptosis is associated with the suppression of the translocation of NF-kB/p65 into the nucleus. The protective effect of FPL can provide a promising approach for the treatment of osteoporosis. Some studies have proven that palm leaves have free radical scavenging activity [26,59]. Han et al. (2002)'s [59] experiment showed that the IC 50 value of the methanol extract of persimmon leaves was 0,11 mg/mL against the DPPH radical. The effect of PLF on DPPH's free radical clearance is not routine as the EC50 is 96,367, 2,63 and 41,567 1,96 µg/mL, respectively [31].

In the study of Ercisli et al. (2008) [60], the highest antioxidant activity was observed in the 08 TH 10 genotype with 91,6%, while the lowest antioxidant activity was found as 14 TH 01 (51,7%), respectively. The antioxidant activities of butylated hydroxyanisole and butylated hydroxytoluene were 93,4% and 91,8%, respectively. A low correlation (R = 0,711) was obtained between total phenolic content and antioxidant activity between genotypes. The results showed that the antioxidant activity in palm fruits is strongly managed by the genotype. Jang et al. (2010) [61] found that palm seed and calyx extracts showed higher antioxidant

activities and phenolic contents than shell and pulp extracts when evaluated with DPPH (1,1-diphenyl-pyridylhydrazyl) radical scavenging activity and reducing power (RP). Ethanol has been found to be more effective on extraction of antioxidant compounds than other solvents (acetone, methanol and water). Antigenotoxic effects of persimmon extracts on DNA damage induced by H_2O_2 in human leukocytes were evaluated by Comet test. All persimmon extracts inhibited DNA damage caused by 200 µM H_2O_2. Calyx and seed extracts showed stronger inhibition activity than shell and meat extracts. The results suggested that persimmon extracts may have protective toxins against beneficial antioxidant and protective effects. Akter et al. (2010) [62] has shown that palm kernel extracts can potentially be used as a cheap source of natural antioxidants in the food and pharmaceutical industries. EC50 values of persimmon seeds were found in the radical hunter assay, while extracts from absolute ethanol and methanol were 49,71 and 51,15 µg/ml, respectively, while the EC50 of butylated hydroxyanisole extract was found as 70,82 mg/ml. The EC50 value of reducing power for absolute acetone extract was higher (210.06 µg/ml) than butylated hydroxyanisole extract (212,67 µg/ml). Absolute methanol extract has the highest antioxidant activity, but it has the lowest total phenolics and flavonoids. In contrast, the antioxidant activities of aqueous solvent extracts showed good correlation with total phenolics and flavonoids compared to absolute solvent extracts.

Kim et al. (2010) [63] reported that treatment of human leukemia HL-60 cells with zero to 100 mg/ml D. kaki leaves (KV-1) for 72 hours caused a small increase in cell differentiation. When HL-60 cells were treated with all-trans retinoic acid (ATRA) and persimmon leaf extract, a synergistic differentiation induction was observed. Protein kinase C (PKC) (a and b I) and extracellular signal regulated kinase (ERK) inhibitors, but phosphoinositide 3-kinase (PI3-K) and c-Jun N-terminal kinase (JNK) inhibitors ATRA or 1.25 HL-60 differentiation induced by the extract with the combination of (OH) 2D3 indicates that PKC and ERK are involved in the development of cell differentiation by the extract. The results showed that acetone extract of *D. kaki* leaves has the ability to increase HL-60 cell differentiation and may be useful in the treatment of acute promyelocytic leukemia.

Diets supplemented with dried and powdered young and ripe fruits of persimmon Fuyu-kaki and Hachiya-kaki varieties significantly reduced plasma lipids, including total cholesterol, triglyceride and LDL cholesterol [64]. The fruit-supported young diets of both varieties evenly regulated the three-fold expression of the cholesterol 7 α-hydroxylase (CYP7A1) gene in the liver. CYP7A1 plays an important role in maintaining cholesterol homeostasis by regulating bile acid synthesis, suggesting that increased conversion of cholesterol to bile acids may cause cholesterol-lowering effects of young fruits. The results showed that young palm fruits are useful in the development of protective and therapeutic agents against dyslipidemia. In a follow-up study, young persimmon fruit treatment was found to significantly reduce plasma chylomicron, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol and triglyceride, with increased fecal bile acid excretion [64].

Matsumoto et al. (2006)[64], diets supplemented with dried and powdered young and mature fruits of persimmon Fuyu-kaki and Hachiya-kaki varieties significantly reduced the increase in plasma lipids, including total cholesterol, triglyceride and LDL cholesterol. The fruit-supported young diets of both varieties evenly regulated the three-fold expression of the cholesterol 7 α-hydroxylase (CYP7A1) gene in the liver. CYP7A1 plays an important role in maintaining cholesterol homeostasis by regulating bile acid synthesis, suggesting that increased conversion of cholesterol to bile acids may cause cholesterol-lowering effects of young fruits. The results showed that young palm fruits are useful in the development of protective and therapeutic agents against dyslipidemia. Matsumoto et al. (2008)[65] found in a follow-up study that young persimmon fruit therapy reduced plasma chylomicron, low-density lipoprotein and low-

density lipoprotein cholesterol and triglyceride with increased fecal bile acid excretion.

According to the study of Fukai et al. (2009) [36], it was found that triterpenoids with 3b-hydroxy group inhibit protein tyrosine phosphatase 1B (PTP1B) activity while IC50 values ranged from 3,1 to 18,8 mM, while those with 3a-hydroxy moiety were inactive. 2-methoxy-4-vinylphenol, which is a component of persimmon bark, has been found to have high antioxidant activity on DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenger and SOD (superoxide dismutase) assays. The compound exhibited higher tyrosinase-inhibiting activity than that of arbutinin using both L-tyrosine and L-DOPA as substrates. In addition, the synthesized 2-methoxy-4-vinylphenol glycoside exhibited tyrosinase-inhibiting activity, suggesting its potential as a cosmetic component with a whitening effect. The acetone extract (*Diospyros kaki* 'Fuyu') of the Japanese persimmon bark has been found to inhibit melanin biosynthesis in mouse B16 melanoma cells [35]. Two active compounds were isolated and flavonoid glycosides were defined as isocercitrin (quercetin-3-O-glucoside) and hyperin (quercetin-3-O-galactoside). It has strongly inhibited the production of isoercitrin and hyperin, melanin with IC50 values of 21,7 and 18,2 mM, respectively. Inhibitory effects were mediated by the suppression of tyrosinase expression.

***Diospyros kaki*'s current formulations on the market**

The formulations of *Diospyros kaki* available worldwide are very limited and some of the products are shown in Figure 5. In Figure 5a, there is a patented sedative with content (camellia leaf, persimmon and carob bean) that plays a role in hair growth produced in South Korea and soothes skin irritation. In addition, in Figure 5b, acol-based/non-alcoholic extracts, teas, food supplement products and skin care products produced from plants made in Japan consisting of persimmon (*Diospyros kaki*) and dry sepals as liquid extracts. Persimmon in Turkey found in the form of that more fresh fruits and dried fruits in the market and is known to be consumed.

Figure 5. Some of *Diospyros kaki*'s existing formulations on the market **a)** a patented skin soothing developed in South Korea **b)** supplement food product.

Toxic effects of medicinal plants and herbal products

The interest in herbal products has started to increase especially in recent years for preventive/therapeutic purposes. Incomplete information in the market creates a perception that herbal products are completely harmless, and this situation may have harmful consequences. Plants/herbal products can have unexpected adverse effects due to their different content. In addition, some of them may have toxic effects or interact with medicines taken together. Therefore, it can cause other conditions or increase the size of the condition. Scientific studies regarding the side effects that can be seen in treatment with plants

should be increased and necessary legal arrangements should be completed in this regard. Plants/herbal products on the market as supplementary products could be sold unsupervised. However, it is legally mandatory to have the necessary information on the drugs. In plant treatment/phytotherapy, the systematic identification and naming of the plant inaccurately leads to undesired results. For example, only the diagnosis based on the appearance may cause serious results due to morphological similarity (the very poisonous hemlock leaf is compared to parsley). However, the subspecies of the plant can be of very different structures, so scientific diagnosis on the subspecies basis is important besides the species base [66].

Plants and herbal products used for therapeutic purposes can be found contaminated with pesticides, heavy metals, toxic substances, synthetic drug residues and microorganisms (plants irrigated with well-treated recycled wastewater, etc.) [67]. There are very few herbal products, such as vinblastin, vincristine and paclitaxel, among the cancer drugs that are being used. However, the tobacco plant causing cancer creates a contradiction with the aforementioned situations. Some slimming teas in the market have been found with high amounts of diuretics and laxatives. In addition, some of the ingredients in these teas may have blood pressure-increasing or sodium, potassium, plasma aldosterone and lowering effects, and may even lead to death.

In the formulation of products sold in the market as vitamin supplements, stimulant foreign active ingredients not mentioned on the label were found. It is also known that some athletes who consume herbal products containing ephedrine active ingredient in the sports sector are also disqualified as a result of doping controls [66].

Toxicity studies on persimmon (*Diospyros kaki*)

Xie et al. (2015), according to their research, the toxicity studies conducted in the literature related to the fruit of persimmon are mostly related to leaves, and there have been no cases of toxicity in various uses of the leaves during the last hundred years [55]. Modern toxicity studies on animals have not been toxic in leaves. Studies on the fruit portion are very limited and no toxicity has been found. While this appears to be reliable in widespread use, further studies of toxicity are essential and necessary to fill the literature gap today, especially in different effective extracts (stem, fruit).

According to Wu et al. (2012) [68], in the acute toxicity test, after pretreatment of leaves with water extract, both LD50 in male and female mice were higher than 21,5 g/kg (equal to 597,2 g/kg in raw medicinal material), which suggested that the extract was non-toxic. In the mouse bone marrow micronucleus test (MNT), the ratio of polychromatic erythrocytes/normrochromatic erythrocytes (PCE/NCE) drops to 10 g/kg in the normal range compared to 20 mg/kg cyclophosphamide. It is implied that palm leaf extracts do not have a mutagenic effect on somatic cells. Therefore, 10,0 g/kg palm leaf extracts did not show the effect of sperm malformation. In the study of Chen et al. (2005) [69], subchronic toxicity test was performed on 100 SD rats by oral administration of 0,5, 1,0, 3,0, 6,4 g/kg preparations (ethanol extract of leaves) for 90 days. The blood and physiological indices of the rats were not significantly different from the normal diet control group. Teratogenicity was evaluated using 100 pregnant rats at preparation doses of 1,0, 3,0, 6,4 g kg and compared to negative and positive (control group). No significant changes were observed in weight gain at each dose. The mean live fetus, absorbent fetus, and dead fetus amount were not significantly different in the negative control group. Anomaly of physiological features was also not observed at all doses. Therefore, NOAEL ethanol extract from leaves was 6,4 g/kg, no maternal toxicity, embryo toxicity and teratogenesis were observed at this dose.

Xie et al. (2015) [55], the long-term toxicity of Naoxinqing Tablets (persimmon leaf extract) was investigated in an experimental study on rats. Rats sat intragastric administration Naoxinqing tablet at doses of 35, 70, 140 g/kg once daily for 180 days. There was a slight decrease in food intake of the high dose group (140 g/kg), compared to the control group for serum total bilirubin, medium

dose group (140 g/kg) and high dose group (140 g / kg), no abnormal changes were observed for other indicators. The safe dose for persimmon leaf extract was 35 g/kg.

El-Hawary et al. (2019) [70] and the results of the study and results for the leaf and fruit part of the fruit are detailed below; fifteen male Wistar rats (200-220 g) were used. Conventional laboratory conditions: temperature with free access to water and food (20-25 °C). Kits for plasma albumin, total protein, alkaline phosphatase, GOT and GPT, creatinine, urea, total cholesterol, HDL cholesterol, LDL-cholesterol, total lipids, triglycerides, malondialdehyde and catalase were obtained from Biodiagnostic Company, BIODIAGNOSTIC. Blood glucose was recorded by Accu-Chek Go and strips purchased from Roche. Drabkin reagent was taken from Vitro Scientific for hemoglobin testing. Rats were randomly divided into three experimental groups. All groups were fed a balanced diet, 15% casein (80% protein), 5% cellulose, 8% corn oil, 10% sugar, enough vitamins and mineral mixtures for a week. Then, the control group: only a balanced diet was applied to the rats. Fruit group: rats received a balanced diet, but were fortified with fruit powder (10%). Fresh fruits were collected, dried in an oven, then ground into powder (as specified in plant material), then added to a balanced diet at 10:90. Leaves group: rats received a balanced diet, but supplemented with leaf powder (10%) Fresh leaves were collected, dried in an oven, then ground into powder (as specified in plant material) and then added to a balanced diet (10:90). All diets were completed to 100 g using starch. Diets were prepared and stored frozen for the duration of the experiment (4 weeks). At the end of the experiment, the mice were fasted overnight, total food intake, final rats body weight gain recorded. Food efficiency rate was calculated according to the equation: food efficiency rate=body weight gain/food intake. Blood samples were drawn from the retro-orbital venous plexus in heparinized tubes under light ether anesthesia and serum was separated by centrifugation at 3000 rpm for 15 min. Plasma was liquefied and stored at -20°C until used for biological analysis. Blood glucose levels (mg/dl) were measured in a glucometer using the Accu-check Go strip (Roche) test using the albumin function (g/dl). Total proteins (g/dl) were measured and Globulin (g/dl) was calculated by the difference between the total protein level and the albumin level. Liver functions such as alkaline phosphatase (ALP), GOT and GPT activities were determined by Reitman and Frankel (1957) method [71]. For kidney function; creatinine and urea levels were measured. Serum lipid profile containing total cholesterol, total lipids, triglycerides, HDL and LDL-cholesterol was determined. Antioxidant enzymes; Catalase activity and malondialdehyde (MDA) were measured. The results showed that there was no significant difference in overall food intake or food efficiency rates across all experimental groups. In addition, at the end of the experiment period, the group fed the fruit diet showed a significant decrease in blood sugar level and also a significant increase in blood hemoglobin level. The results obtained for liver and kidney functions did not show a significant difference in all parameters evaluated, in all values determined by the control group fed with a balanced diet. The healthy effect of *Diospyros kaki L.* fruits and leaves on the plasma lipid profile showed a significant reduction ($p<0,05$) in plasma total cholesterol and triglyceride values, but not a significant reduction in total lipids. Therefore, there was an insignificant increase in HDL/LDL ratio. For antioxidant enzymes; catalase and MDA did not show a significant reduction in activities when comparing the results of the fruit and leaf groups with the control group.

CONCLUSIONS

In this study, the pharmacological/cosmetic applications and toxicity assesment of the persimmon (*Diospyros kaki*) fruit and leaves were evaluated with the existing phytochemical content. Various active ingredients obtained with crude extracts, purified fractions have been found to have great potential for dermatological and cosmetic applications. In addition, it has been proved that the leaves of the persimmon are rich in bioactive substances with rich nutritional values as well as of

the fruit. Some significant evidence-based scientific studies for microbial inhibition have been conducted in Asian countries such as China, Korea and Japan, and most importantly, despite many years of research, no side effects or toxicity reports have been found on these plants. As a result of the study, persimmon fruits and leaves are traditionally consumed as a support product, but health-related products are not common in the market worldwide. Since there is scientific evidence on the phytotherapeutic effects of *Diospyros kaki*, and the product scale on the market is very narrow, this research area is highly promising for future healthcare products.

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