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ORIGINAL RESEARCH

The Effects of Sacral Massage and Music Therapy Applied to Primaparous Women in Labor on Labor Pain, Postpartum Comfort, and Posttraumatic Growth: A Randomized Controlled Trial

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Abstract
Objective: In this study, the researchers investigated the effects of sacral massage and music therapy applied to primiparous women in labor on labor pain, postpartum comfort, and posttraumatic growth.
Materials-Methods: This randomized controlled study was conducted with 105 primiparous women admitted to the delivery room of a public hospital in a province south of Türkiye. Data from the study was collected with the "Visual Analog Scale (VAS)", "Postpartum Comfort Questionnaire (PPCQ)", and "Posttraumatic Growth Inventory (PTGI)".
Results: After the intervention, the latent, active, and transitional phases' VAS scores of the pregnant women in the massage, music, and control groups were compared, and the difference between the groups was statistically significant ($p<0.001$). It was determined that the PPCQ total mean scores were 127.0 (119.0-136.0) in the massage group, 121.0 (113.0-127.0) in the music group, 112.0 (106.0-115.0) in the control group, and the difference between the groups was statistically significant ($p<0.001$). It was determined that the PTGI total mean scores were 69.0 (62.0-75.0) in the massage group, 57.0 (49.0-72.0) in the music group, 55.0 (40.0-65.0) in the control group, and the difference between the groups was statistically significant ($p<0.001$).
Conclusion: Sacral massage and music therapy applied to primiparous women in labor were found to be effective in reducing the pain levels of pregnant women and increasing postpartum comfort and posttraumatic growth levels.
Keywords: Comfort, Music Therapy, Posttraumatic Growth, Primiparous Women, Sacral Massage.

INTRODUCTION

Although labor pain is a stressful and frightening experience for some women, it is an important part of the physiological and natural birth processes.[1] Failure to manage labor pain may cause pregnant women to perceive birth negatively and decrease their satisfaction with birth.[2] There are pharmacological and non-pharmacological interventions to cope with labor pain.[3-5] As an alternative to the possible side effects of pharmacological interventions (fever, headache, hypotension, urinary retention, bladder distention, fetal respiratory depression, etc.) and some negative consequences (delay in the second stage of labor, delivery with intervention, cesarean section, etc.), the use of non-pharmacological methods is both cost-effective and can help women cope with the labor pain.[4-9] Non-pharmacological methods used to cope with labor pains include breathing techniques, yoga, hypnosis, moving and positioning, hydrotherapy, acupuncture, acupressure, aromatherapy, massage, and music therapy.[2,5,8]

Insufficient support provided for the woman during labor can lead to a negative birth experience.[10] The application of massage and music practices in labor are among the most effective strategies to prevent negative birth experiences.[11] Massage is one of the complementary treatment methods used in labor that helps relieve pain, increase relaxation, and reduce emotional stress.[4,12] Massage can be applied to the woman's back, hips, thighs, or sacral region to cope with labor pain.[1,4,13-15] Türkmen & Tuna-Oran (2020) stated that sacral massage is an effective intervention that reduces the pain perception of women in labor and increases their comfort.[15] Akköz-Çevik & Karaduman (2020) similarly reported in their study that sacral massage applied in labor reduces the labor pain, agitation, and anxiety levels of women,

increases the satisfaction levels associated with labor, and positively affects the perception of birth.[14]

Music therapy can reduce the feeling of anxiety and pain by distracting the senses and reducing the individual's stimulus concentration.[9] In addition, music supports the mother-baby relationship and improves mothers' courage and self-confidence.[2] It has been stated in the literature that music reduces pain and anxiety in labor.[16-19] Music therapy applied in labor is an application that increases maternal satisfaction while reducing anxiety, pain, and early depression rates in the postpartum period. Therefore, music therapy is recommended as an alternative and safe method for postpartum well-being.[20]

Labor pain may cause discomfort in the mother or may be perceived as traumatic by some mothers.[21] In addition, labor pain can prepare the ground for many disorders that affect the mother and baby during the postpartum period.[22-23] Some non-pharmacological methods used to cope with labor pain increase postpartum comfort levels.[24,25] Postpartum comfort and post-traumatic growth are closely related[26], and the mental and physical health of the mother plays an important role in post-traumatic growth.[27] Labor is a challenging life event with the potential to experience positive and negative experiences. Growth is not limited to traumatic experiences, but life events such as birth also have the potential to encourage growth.[28] The most important aspect of midwifery is to relieve and care for women during labor pain.[12,14] Therefore, midwives should support and encourage women to use non-pharmacological methods.[2,7] To the best of our knowledge, no research has been found on the effects and comparison of sacral massage and music therapy, which are supportive and alternative methods in labor, on both labor pain, postpartum comfort, and post-traumatic growth. It is aimed that the results of this research, which was conducted to determine the effects of sacral massage and music therapy applied to primiparas in labor on labor pain, postpartum comfort, and post-traumatic growth, will provide important contributions to midwives who provide care services in the labor, delivery, and postpartum services regarding the effectiveness of non-pharmacological methods.

MATERIALS AND METHODS

Research design

This study was conducted in a randomized controlled manner to determine the effects of sacral massage and music therapy applied to primiparas in labor on labor pain, postpartum comfort, and posttraumatic growth. The research was conducted with primiparous women who were admitted to the delivery rooms of a public hospital in a province in the south of Türkiye between April and July 2022. There are 11 beds in the delivery room of the relevant hospital, and 19 midwives work here. In hospital routine care, non-pharmacological coping methods are not used to cope with pain.

Population and sample of the research

The population of the research consisted of pregnant women who were admitted to the delivery room of a public hospital in a province in the south of Türkiye. When the power analysis was performed, the sample size was calculated with a 5% error level, bidirectional significance level, 95% confidence interval, and 80% ability to represent the universe. It was found that at least 32 participants for each group and 96 primiparous women in total were needed (32 participants for the massage group, 32 participants for the music group, and 32 participants for the control group), assuming that the intervention would create 11 points increase in the PPCQ total mean score (115.43±15.51).[29] A total of 114 primiparous pregnant women (38 participants for the massage group, 38 participants for the music group, and 38 participants for the control group) were planned to be included in the study, considering possible losses (approximately 20%). Pregnant women who were pregnant at the term, primiparous, expecting a single baby, and voluntarily accepted to participate in the study were included in the study. Pregnant women who had hearing problems, impaired skin integrity, using analgesia/anesthesia during labor, and delivery by caesarean section were excluded from the study.

Columns between 1 and 114 were created using the Random Integer Generator method in the Numbers subheading of the random.org site for women who met the sampling criteria. Numbers 1, 2, and 3 for massage, music, and control groups were observed in the column. Which number would represent the massage group, music group or control group was determined by drawing a lot at the beginning of the study. As a result of the drawing lots, women who were matched with the number 1 were assigned to the massage group, those who were matched with the number 2 were assigned to the control group, and those who were matched with the number 3 were assigned to the music group.[30]

Data collection tools

Data of the study was collected using the "Personal Information Form", "Visual Analog Scale", "Postpartum Comfort Questionnaire" and "Posttraumatic Growth Inventory".

Personal information form

It is a form consisting of 9 questions developed by

researchers in line with the literature in order to determine some sociodemographic and obstetric (age, educational level, employment, location of residency, income level, family structure, the status of having regular health check-ups during pregnancy, etc.) characteristics of pregnant women.[1,4,16,19,20]

Visual analog scale (VAS)

The visual analog scale was developed by Price et al. (1983). VAS is used to assess pain severity. It is used to self-evaluate perceived pain on a 10-cm ruler scale, where 0 indicates no pain, and 10 indicates the most severe pain. High scores indicate more severe pain.[31]

Postpartum comfort questionnaire (PPCQ)

The "General Comfort Questionnaire" developed by Kolcaba (1992) was adapted to Turkish society by Kuğuoğlu & Karabacak (2004). The "Postpartum Comfort Questionnaire" was developed by Karakaplan in 2007 from the Turkish form of the General Comfort Questionnaire. The PPCQ is a five-point Likert-type questionnaire consisting of 34 items and 3 subscales: physical comfort, psychospiritual comfort, and sociocultural comfort. Each item is scored between 1 and 5, and a score of 34-170 can be obtained from the questionnaire. If the scores obtained from the questionnaire are close to 170, it means that the comfort is high. The Cronbach's Alpha coefficient of the questionnaire was 0.78.[32-34] In this study, the Cronbach's Alpha coefficient was found to be 0.77.

Posttraumatic growth inventory (PTGI)

It was developed by Tedeschi & Calhoun (1996) to measure perceived positive changes after traumatic life events. In addition, the inventory was adapted into Turkish by Dirik & Karanci (2008). The Posttraumatic Growth Inventory is a six-point Likert-type inventory consisting of 21 items and 3 subscales: A Changed Sense of Relationships with Others, A Changed Philosophy of Life, and Perceived Changes in Self. The inventory's items are scored between 0-5, and a score between 0-105 can be obtained. As the score obtained from the inventory increases, it is thought that the level of posttraumatic growth is higher. The Cronbach's Alpha coefficient of the inventory was 0.94.[35-36] In this study, the Cronbach's Alpha coefficient was found to be 0.95.

Procedures

The data was collected by the researchers through face-to-face interviews with pregnant women admitted to the delivery rooms of a public hospital in a province in the south of Türkiye.

Massage Group; The sacral massage was applied to pregnant women in the massage group by the researcher (H.G.Ö.), who received training in the field of pregnant massage (10 hours). Before the application, the pregnant women were informed, their consent was taken, and gloves were worn. A lubricant that does not contain any harmful substances was used to provide lubricity during the massage. The sacral massage was applied to the pregnant women in this group three times, in the latent (1-4 cm), active (5-7 cm), and transitional phases (8-10 cm). Pregnant women cling to the bed, sitting or lying on their left side, to the area where the sacral (S1-S4) vertebrae are. The massage was applied to the right and left 4-5 cm lateral parts of the midline with effleurage and friction techniques. The massage application was continued for 10 minutes without interruption. Each application took an average of 10 minutes.

Music Group; The pregnant women in the music group were informed about the music application, and their consent was obtained. Headphones were used during the music application. In addition, suitable disposable caps were attached to the earplugs before the application. The pregnant women in this group listened to music in Rehavi mode for 20 minutes, 3 times in the latent (1-4 cm), active (5-7 cm), and transitional phases (8-10 cm). The Rehavi mode was preferred for the study as it is a mode that gives people the idea of eternity and is thought to be effective on labor pain.[37]

Data analysis

The obtained data was analyzed in the statistical package program "Statistical Package for Social Sciences" (SPSS) for Windows 25.0. Kolmogorov Smirnov test was used for the normality analysis of the data, and it was seen that the data were not normally distributed. Descriptive statistics (number, percentage distribution, median, and Q1-Q3), chi-square test, Kruskal Wallis test, and Wilcoxon test were used in the analysis of the data. The Post-Hoc Tamhane test was used for further analysis. The error level of $p<0.05$ was considered statistically significant when interpreting the results.

Ethics

Ethical approval was obtained from the Scientific Research and Publication Ethics Committee of Inonu University (Decision Number: 2022/2889), and the study was registered at the Clinical Trials (registration number: NCT05317052). In addition, necessary permissions were obtained from the relevant institution in order to conduct the research. Informed consent was obtained from the pregnant women who participated in the study, and they were informed that they were free to participate in the study and could withdraw from the study at any time.

RESULTS

Nine pregnant women were not included in the study because three pregnant women in the massage group did not want to continue the research, three pregnant women in the music group received an emergency cesarean section, and one pregnant woman in the control group received an emergency cesarean section, and two pregnant women in the control group did not want to continue the research. The study was completed with 105 primiparous women (Figure 1. Research Consort Diagram).

The comparison of pregnant women in massage, music, and control groups in terms of descriptive characteristics is given in Table 1. In the analysis made, it was determined that there was no statistical difference in terms of descriptive characteristics such as age, educational level, employment, age of spouse, educational level of spouse, income level, location of residency, family structure, the status of having regular health check-ups during pregnancy, and all three groups had similar characteristics ($p>0.05$).

Intra-group comparison of the pre-test and post-test VAS scores of pregnant women in labor in the massage, music, and control groups is presented in Table 2. When the pre-application VAS scores of the pregnant women in the massage, music, and control groups were compared in the latent, active, and transitional phases, it was determined that the difference between the groups was not statistically significant. The pregnant women in all groups had similar pain levels before the application ($p>0.05$).

After the intervention, when the latent, active, and transitional phases' VAS scores of the pregnant women in the massage, music, and control groups were compared, the VAS scores in the latent phase were 2.0 (1.0-2.0) in the massage group, 2.0 (2.0-3.0) in the music group, and 3.0 (3.0-4.0) in the control group. The difference between the groups was statistically significant ($X^2=40.021$, $p<0.001$). As a result of the further analysis performed to determine which group caused the difference, it was determined that the VAS score of the pregnant women in the massage group was lower than those in the music and control groups. The VAS score of the pregnant women in the music group was lower than those in the control group ($1<2<3$). It was determined that the VAS scores after the intervention in the active phase were 5.0 (3.0-5.0) in the massage group, 6.0 (5.0-7.0) in the music group, and 6.0 (6.0-7.0) in the control group. The difference between the groups was statistically significant ($X^2=32.124$, $p<0.001$). As a result of further analysis performed to determine from which group the difference originated, it was determined that the VAS score of the pregnant women in the massage group was lower than those in the music and control groups ($1<2$, $1<3$). In the transitional phase, the post-intervention VAS scores were 8.0 (6.0-9.0) in the massage group, 9.0 (8.0-9.0) in the music group, and 9.0 (8.0-10.0) in the control group, and the difference between the groups was statistically significant ($X^2=28.228$, $p<0.001$). As a result of further analysis performed to determine from which group the difference originated, it was determined that the VAS score of the pregnant women in the massage group was lower than those in the music and control groups ($1<2$, $1<3$).

When the pre- and post-application VAS scores of the pregnant women in the massage group were compared within the group in the latent, active, and transitional phases, it was determined that the post-test measurements were statistically significantly reduced compared to the pre-test ($p<0.001$). When the pre- and post-application VAS scores of the pregnant women in the music group were compared within the group, it was seen that the post-test measurements decreased statistically significantly compared to the pre-test ($p<0.05$). However, the VAS scores in the transitional phase did not create a statistically significant difference between the pre-test and post-test measurements ($p>0.05$).

When the pre- and post-application VAS scores of the pregnant women in the control group were compared within the group in the latent, active, and transitional phases, it was seen that there were no statistically significant differences between the pre-test and post-test measurements ($p>0.05$).

The inter-group comparison of the postpartum PPCQ and PTGI scores of the women in the massage, music, and control groups is presented in Table 3. It was determined that the scores of PPCQ's subscale of *physical comfort* were 50.0 (48.0-55.0) in the massage group, 47.0 (43.0-48.0) in the music group, and 42.0 (40.0-45.0) in the control group. The difference between the groups was statistically significant ($X^2=39.609$, $p<0.001$). As a result of the further analysis carried out to determine which group caused the difference, it was determined that the score of PPCQ's subscale of the *physical comfort* of the women in the massage group was higher than those in the music and control groups. The score of PPCQ's subscale of the *physical comfort* of the women in the music group was higher than those in the control group ($1>2>3$). It was determined that the scores of PPCQ's subscale of the *psychospiritual comfort* were 46.0 (43.0-48.0) in the massage group, 42.0 (39.0-46.0) in the music group, 40.0 (37.0-43.0) in the control group.

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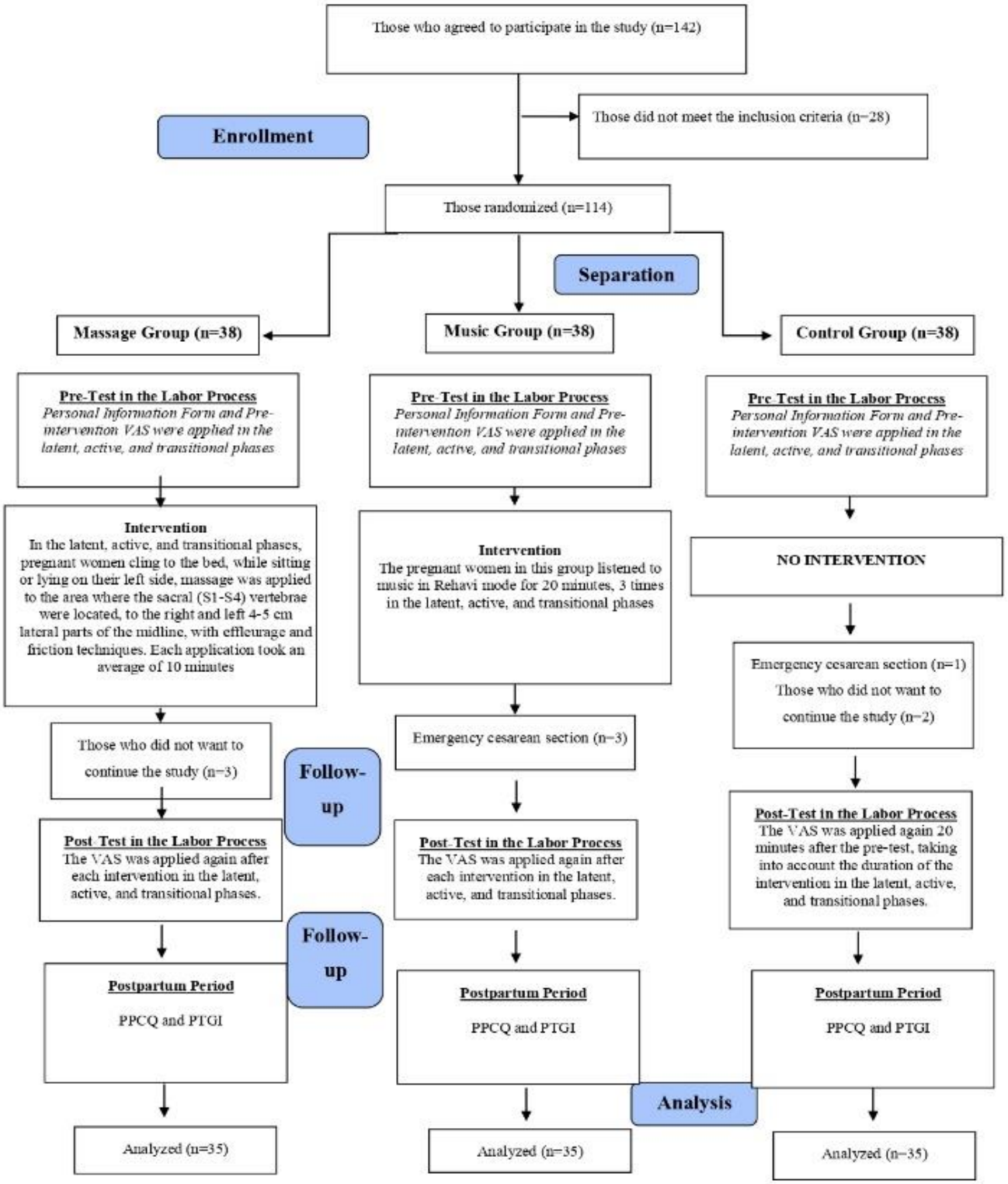

Figure 1. Research Consort Diagram

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Table 1. The Comparison of Pregnant Women in Massage, Music, and Control Groups in terms of Descriptive Characteristics (n=105)

Descriptive Characteristics	**Massage Group (n=35)**		**Music Group (n=35)**		**Control Group (n=35)**		**Test and p Values**
	N	**%**	**N**	**%**	**N**	**%**	
Educational Level							
Literate	-	-	-	-	1	2.9	
Primary School	6	17.1	5	14.3	7	20.0	X^2=6.291
Middle School	6	17.1	10	28.6	10	28.6	p=0.615
High School	19	54.3	14	40.0	11	31.4	
University and Higher	4	11.4	6	17.1	6	17.1	
Employment							
Unemployed	4	11.4	6	17.1	8	22.9	X^2=1.609
Employed	31	88.6	29	82.9	27	77.1	p=0.447
Educational Level of the Spouse							
Illiterate	-	-	2	5.7	-	-	
Literate	-	-	-	-	2	5.7	
Primary School	4	11.4	3	8.6	8	22.9	
Middle School	7	20.0	7	20.0	7	20.0	X^2=14.410
High School	20	57.2	18	51.4	11	31.4	p=0.155
University and Higher	4	11.4	5	14.3	7	20.0	
Income Level							
Income is less than expenses	7	20.0	7	20.0	11	31.4	
Income is equal to expenses	26	74.3	28	80.0	20	57.2	X^2=6.685
Income is more than expenses	2	5.7	-	-	4	11.4	p=0.153
Location of Residency							
Province	23	65.8	23	65.8	19	54.3	X^2=1.455
District	6	17.1	6	17.1	9	25.7	p=0.835
Village	6	17.1	6	17.1	7	20.0	
Family Structure							
Core	29	82.9	31	88.6	32	91.4	X^2=1.229
Traditional	6	17.1	4	11.4	3	8.6	p=0.541
The status of having regular health check-ups during pregnancy							
Regular	35	100.0	33	94.3	33	94.3	X^2=2.079
Irregular	-	-	2	5.7	2	5.7	p=0.354
	Med (Q1-Q3)		**Med (Q1-Q3)**		**Med (Q1-Q3)**		
Age (years)	22.0 (20.0-25.0)		22.0 (20.0-24.0)		22.0 (20.0-25.0)		*X^2=0.210 p=0.900
Age of the spouse (years)	26.0 (24.0-30.0)		26.0 (24.0-29.0)		27.0 (25.0-29.0)		*X^2=0.678 p=0.713

X^2: Chi-square test, *X^2:Kruskal Wallis test, Med: Median, N:Number

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The difference between the groups was statistically significant (X^2=22.757, p<0.001). As a result of further analysis carried out to determine which group caused the difference, it was determined that the score of PPCQ's subscale of the *psychospiritual comfort* of the women in the massage group was higher than those in the music and control groups (1>2, 1>3). It was determined that the scores of PPCQ's subscale of the *sociocultural comfort* were 32.0 (28.0-35.0) in the massage group, 32.0 (30.0-34.0) in the music group, and 29.0 (27.0-32.0) in the control group. The difference between the groups was statistically significant (X^2=7.811, p<0.05). As a result of further analysis carried out to determine from which group the difference originated, it was determined that the score of PPCQ's subscale of *sociocultural comfort* of the women in the music group was higher than those in the control group (2>3). It was determined that the PPCQ total scores were 127.0 (119.0-136.0) in the massage group, 121.0 (113.0-127.0) in the music group, 112.0 (106.0-115.0) in the control group, and the difference between the groups was statistically significant (X^2=34.159, p<0.001). As a result of the further analysis performed to determine which group caused the difference, it was determined that the PPCQ total score of the women in the massage group was higher than those in the music and control groups. The PPCQ total score of the women in the music group was higher than the women in the control group (1>2> 3).

Table 2. Intra-Group and Inter-Group Comparisons of the Pre-Test and Post-Test VAS Scores of Pregnant Women in Labor in the Massage, Music, and Control Groups (n=105)

Scales		Massage Group (n=35) Med (Q1-Q3)	Music Group (n=35) Med (Q1-Q3)	Control Group (n=35) Med (Q1-Q3)	[a]Test and p Values	Significance[*]
LATENT PHASE (1-4 cm)	**VAS** Pre-Test	4.0 (3.0-4.0)	3.0 (3.0-3.0)	3.0 (3.0-4.0)	X^2=3.954 p=0.138	
	VAS Post-Test	2.0 (1.0-2.0)[1]	2.0 (2.0-3.0)[2]	3.0 (3.0-4.0)[3]	X^2=40.021 **p=0.000**	1<2<3
	[b]Test and p Values	Z=-5.514 **p=0.000**	Z=-4.823 **p=0.000**	Z=-1.000 p=0.317		
ACTIVE PHASE (5-7 cm)	**VAS** Pre-Test	6.0 (6.0-8.0)	6.0 (5.0-7.0)	6.0 (6.0-7.0)	X^2=1.236 p=0.539	
	VAS Post-Test	5.0 (3.0-5.0)[1]	6.0 (5.0-7.0)[2]	6.0 (6.0-7.0)[3]	X^2=32.124 **p=0.000**	1<2,1<3
	[b]Test and p Values	Z=-5.194 **p=0.000**	Z=-2.236 **p=0.025**	Z=-1.000 p=0.317		
TRANSITIONAL PHASE (8-10 cm)	**VAS** Pre-Test	9.0 (8.0-9.0)	9.0 (8.0-9.0)	9.0 (8.0-10.0)	X^2=2.599 p=0.273	
	VAS Post-Test	8.0 (6.0-9.0)[1]	9.0 (8.0-9.0)[2]	9.0 (8.0-10.0)[3]	X^2=28.228 **p=0.000**	1<2,1<3
	[b]Test and p Values	Z=-4.606 **p=0.000**	Z=-1.000 p=0.317	Z=-1.342 p=0.180		

[a]X^2: Kruskal Wallis test, [b]Wilcoxon test, [*]Post-Hoc Tamhane test, Med: Median

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Table 3. The Comparison of Postpartum PPCQ and PTGI Scores of Women in Massage, Music, and Control Groups (n:105)

	Scales	Massage Group (n=35) Med (Q1-Q3)	Music Group (n=35) Med (Q1-Q3)	Control Group (n=35) Med (Q1-Q3)	[a]Test and p Values	Significance[*]
PPCQ's Subscales	Physical Comfort	50.0 (48.0-55.0)[1]	47.0 (43.0-48.0)[2]	42.0 (40.0-45.0)[3]	X^2=39.609 **p=0.000**	1>2>3
	Psychospiritual Comfort	46.0 (43.0-48.0)[1]	42.0 (39.0-46.0)[2]	40.0 (37.0-43.0)[3]	X^2=22.757 **p=0.000**	1>2,1>3
	Sociocultural Comfort	32.0 (28.0-35.0)[1]	32.0 (30.0-34.0)[2]	29.0 (27.0-32.0)[3]	X^2=7.811 **p=0.020**	2>3
	PPCQ TOTAL	127.0 (119.0-136.0)[1]	121.0 (113.0-127.0)[2]	112.0 (106.0-115.0)[3]	X^2=34.159 **p=0.000**	1>2>3
PTGI's Subscales	A Changed Sense of Relationships with Others	23.0 (20.0-26.0)[1]	20.0 (17.0-24.0)[2]	17.0 (12.0-23.0)[3]	X^2=15.528 **p=0.000**	1>2>3
	A Changed Philosophy of Life	16.0 (13.0-18.0)[1]	12.0 (10.0-16.0)[2]	13.0 (8.0-16.0)[3]	X^2=10.110 **p=0.006**	1>2,1>3
	Perceived Changes in Self	29.0 (27.0-31.0)[1]	25.0 (22.0-31.0)[2]	24.0 (17.0-28.0)[3]	X^2=14.774 **p=0.001**	1>3
	PTGI TOTAL	69.0 (62.0-75.0)[1]	57.0 (49.0-72.0)[2]	55.0 (40.0-65.0)[3]	X^2=15.237 **p=0.000**	1>2,1>3

[a]X^2=Kruskal Wallis test, [*]Post-Hoc Tamhane test, Med = Median

It was determined that the scores of PTGI's subscale of *a changed sense of relationships with others* were 23.0 (20.0-26.0) in the massage group, 20.0 (17.0-24.0) in the music group, 17.0 (12.0-23.0) in the control group. The difference between the groups was statistically significant (X^2= 15.528, p<0.001). As a result of the further analysis carried out to determine from which group the difference originated, it was determined that the women in the massage group had a higher score on PTGI's subscale of *a changed sense of relationships with others* than those in the music and control group. It was determined that the score of PTGI's subscale of *a changed sense of relationships with others* of the women in the music group was higher than the women in the control group (1>2>3). It was determined that the scores of PTGI's subscale of *a changed philosophy of life* were 16.0 (13.0-18.0) in the massage group, 12.0 (10.0-16.0) in the music group, 13.0 (8.0-16.0) in the control group. The difference between the groups was statistically significant (X^2=10.110, p<0.05). As a result of further analysis carried out to determine from which group the difference originated, it was determined that the score of PTGI's subscale of *a changed philosophy of life* of the women in the massage group was higher than those in the music and control groups (1>2, 1>3). It was determined that the scores of the PTGI's subscale of *perceived changes in self* were 29.0 (27.0-31.0) in the massage group, 25.0 (22.0-31.0) in the music group, 24.0 (17.0-28.0) in the control group. The difference between the groups was statistically significant (X^2=14.774, p<0.05). As a result of further analysis carried out to determine from which group the difference originated, it was determined that the score of PTGI's subscale of *perceived changes in self* of women in the massage group was higher than those in the control group (1>3). It was determined that the PTGI total scores were 69.0 (62.0-75.0) in the massage group,

57.0 (49.0-72.0) in the music group, 55.0 (40.0-65.0) in the control group, and the difference between the groups was statistically significant (X^2=15.237, p<0.001). As a result of further analysis performed to determine which group caused the difference, it was determined that the PTGI total score of the women in the massage group was higher than those in the music and control groups (1>2, 1>3).

DISCUSSION

Massage is a traditional method that has been used for centuries in many cultures to relieve and alleviate many illnesses.[38] Music therapy, on the other hand, is an application that is simple to apply and has no side effects, and it is used for physical, psychological, emotional, and spiritual healing.[39] Sacral Massage and music therapy are among the non-pharmacological interventions used to relieve labor pain.[14,15,40,41] In this study, which was conducted to determine the effects of sacral massage and music therapy applied to primiparas in labor on labor pain, postpartum comfort, and posttraumatic growth, it was determined that the labor pain scores of the pregnant women in the sacral massage and music therapy groups decreased. In contrast, their postpartum comfort and posttraumatic growth levels increased.

In the current study, it was determined that there were no statistically significant differences between the descriptive characteristics of the pregnant women in the massage, music, and control groups. In line with these results, it can be said that the groups were homogeneously distributed. In the current study, it was determined that the pain levels of the pregnant women in the massage group decreased in the latent, active, and transitional phases, and in the latent and active phases of the music group after the application. It was determined that massage application in the latent phase was an effective application compared to music therapy, and music therapy was an effective application compared to the no intervention in the control group. Additionally, it was determined that the massage application in the active and transitional phases was a more effective application than the music therapy in the music group, and there was no intervention in the control group. Taghinjad et al. (2010), in a study comparing massage and music therapies to relieve the severity of labor pain in primiparous women, stated that massage is an effective method to lower level and relieve labor pain compared to music therapy.[9] Furthermore, Kimber et al. (2008), in their pilot study in which they examined the effectiveness of massage and music in relieving labor pain, stated that although there was no statistically significant difference, the pregnant women in the massage group had lower pain scores than the music and control groups.[42] Studies in which sacral massage and music interventions were examined separately were found in the literature. In the study of Phumdoung & Good (2003), music provided relief during severe pain in primiparous women[41]; in the study of Türkmen & Tuna-Oran (2021), sacral massage was found to be effective in reducing the perception of the pain of pregnant women.[15] Additionally, Akköz-Çevik & Karaduman (2020) also stated in their study that sacral massage applied during labor reduced the labor pain of women.[14] Although our results were similar to the literature, it has been determined that both sacral massage and music therapies are effective as non-pharmacological techniques. For this reason, it is possible to say that these methods can be used as an alternative application, especially in the delivery rooms, starting with the latent phase.

Postpartum comfort is very important in ensuring the adaptation of the mother to the postpartum process, early initiation and continuation of breast milk, a healthy mother-infant relationship, ensuring recovery in a short time, and the care to be given to both mother and baby by preventing risk factors that may occur.[43] In our study, it was determined that the scores of PPCQ's subscales of *physical comfort*, *psychospiritual comfort*, and the total PPCQ of the primiparous women in the massage group were statistically significantly higher than the music and control groups. Moreover, it was determined that the scores of PPCQ's subscales of *physical comfort*, *sociocultural comfort*, and the total PPCQ of the primiparous women in the music group were statistically significantly higher than the control group. Although there is no study in the literature in which both sacral massage and music application were performed, and postpartum comfort levels were evaluated; however, in studies where different massage techniques were applied to different groups, it was stated that deep tissue massage, which was applied to the back after cesarean section, increased the comfort levels of women; furthermore, İçke & Genç (2021) stated that foot massage improved the comfort level of mothers after vaginal delivery.[44,45] According to these results, it is possible to say that the application of sacral massage and music therapy is an effective intervention that increases the postpartum comfort levels of primiparous women.

The positive psychological change that occurs after struggling with difficult life crises is called post-traumatic growth.[46] Post-traumatic growth is an

important concept to address in the postpartum period.[47] No studies on this subject have been found in the literature. In the study of Nishi & Usuda (2017), it was determined that the birth experience could positively affect physical pain and psychological distress, as well as emotions such as joy and success.[47] In our study, while the scores of PTGI's subscales of *a changed sense of relationships with others*, *a changed philosophy of life*, and the total PTGI of the primiparous women in the massage group were found to be statistically significantly higher than the music and control groups, it was determined that the score of PTGI's subscale of *perceived changes in the self* of the primiparous women in the massage group was significantly higher than the control group. In addition, the score of PTGI's subscale of *a changed sense of relationships with others* of the primiparous women in the music group was found to be statistically significantly higher than the control group. In line with the results, it is possible to say that sacral massage and music therapy are effective interventions in increasing the positive effects, such as post-traumatic growth as well as in decreasing the negative effects, such as anxiety, stress, and depression caused by labor.

CONCLUSION

According to the results of the study, sacral massage and music therapy applied to primiparous women both reduced pain levels and increased their postpartum comfort and posttraumatic growth levels. It is recommended to include sacral massage and music therapy practices as non-pharmacological methods in midwifery care practices in order to facilitate women's coping with labor pain and to improve their well-being in the postpartum period.

Limitations

This study has some limitations. First, the findings cannot be generalized to all pregnant women since the study was conducted only with primiparous women admitted to the delivery rooms of a public hospital in a province south of Türkiye. Another is that all pregnant women were primiparous and were admitted to the delivery rooms in the latent phase. Therefore, the results of the research are limited to this group only.

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ORIGINAL RESEARCH

Evaluation of University Students' Attitudes towards Traditional and Complementary Medicine Practices: A Study at a University in Türkiye

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Abstract
Objective: This study aimed to evaluate the attitudes of students continuing their education in the medical and dental faculties, as well as the nursing and physiotherapy and rehabilitation departments of the Faculty of Health Sciences at Afyonkarahisar Health Sciences University, towards traditional and complementary medicine (T&CM) practices.
Materials-Methods: The study population consisted of students enrolled in the medical, dental, nursing, physiotherapy, and rehabilitation departments of the Faculty of Health Sciences. To reach more participants, the questionnaires and scales were transferred to the Google Forms application, and the survey link was sent to the students via platforms such as email, WhatsApp, and Telegram. The statistical data analysis was performed using the SPSS 25.0 package program, and a significance level of $p<0.05$ was determined.
Results: In our study, which included 1085 students, 64.1% (n=695) were female and 35.9% (n=390) male. The best-known T&CM methods among the participants were reflexology (n=374, 34.5%), osteopathy (n=214, 19.7%), mesotherapy (n=204, 18.8%), hypnosis (n=203, 18.7%), cupping therapy (n=196, 18.1%), chiropractic (n=196, 18.1%), music therapy (n=194, 17.9%), apitherapy (n=188, 17.3%), herbal therapy (n=176, 16.2%), prolotherapy (n=175, 16.1%), and ozone therapy (n=160, 14.7%). Leech therapy (n=153, 14.1%), homeopathy (n=149, 13.7%), and maggot therapy (n=110, 10.1%) were found to be less known.
Conclusion: It was observed that students lack sufficient knowledge about T&CM practices but are interested in receiving education on the subject. Adjustments to the current curriculum of medical, dental, nursing, physiotherapy, and rehabilitation departments would positively contribute to students' attitudes and approaches toward T&CM practices.
Keywords: Traditional and Complementary Medicine, Medical Faculty, Dental Faculty, Faculty of Health Sciences.

INTRODUCTION

According to the World Health Organization (WHO), health is not merely the absence of disease or disability but a state of complete physical, mental, and social well-being.[1] The concept of well-being is defined differently based on values and beliefs. The perception of well-being is determined not only by family, community, social status, biological and environmental factors but also by cultural practices.[2]
In recent years, there has been an increase in the use of traditional and complementary medicine (T&CM) in Türkiye and many other countries. In Türkiye, the "Regulation on Traditional and Complementary Medicine Practices" was published in the Official Gazette on October 27, 2014, under Law No. 29.158. This regulation specifies the methods of T&CM, the training, and authority of practitioners, and the working principles of healthcare institutions. Methods such as acupuncture, apitherapy, phytotherapy, hypnosis, leech therapy (hirudotherapy), homeopathy, chiropractic, cupping therapy, larva therapy, mesotherapy, osteopathy, ozone therapy, reflexology, and music therapy are defined as T&CM.[3]
A multinational cross-sectional survey was conducted on the use of traditional, complementary, and alternative medicine in several countries, including Germany, the United States, Japan, China, Malaysia, Vietnam, Russia, Kazakhstan, and the

United Arab Emirates. In the survey, which involved 1071 participants, more than 50% of the respondents reported being aware of traditional and complementary medicine methods (n = 608, 56.8%) and using various herbal products, including functional health foods or dietary supplements (n = 601, 56.1%). Participants who reported no experience with traditional and complementary medicine mentioned a lack of knowledge as the reason for not using them (n = 222, 20.7%). Furthermore, the potential application of traditional and complementary medicine methods for the treatment and management of infectious diseases such as COVID-19 was emphasized.[4]

In a study conducted with university students in Türkiye, the most well-known T&CM methods were reported as acupuncture (77.5%), cupping therapy (75.3%), and phytotherapy (67.3%). The most commonly used methods were stated as phytotherapy (33.3%) and cupping therapy (11.5%). 94.2% of the participants indicated that medical faculties did not provide sufficient education about T&CM practices, and 82.6% emphasized the necessity of integrating T&CM practices into modern medicine and their implementation by physicians. The study showed that students had insufficient knowledge about T&CM practices and acquired information from other sources.[5]

This study aims to evaluate the attitudes of students continuing their education in the faculties of medicine, dentistry, and health sciences, specifically in nursing and physiotherapy and rehabilitation departments, at Afyonkarahisar Health Sciences University towards traditional and complementary medicine (T&CM) practices.

MATERIALS AND METHODS

Study design

This study is cross-sectional, and a survey link prepared through the Google Forms application was distributed to students between January and March 2023.

Population and sample

The study included students from medical faculties (1st, 2nd, 3rd, 4th, 5th, and 6th grades), dentistry faculties (1st, 2nd, 3rd, 4th, and 5th grades), as well as nursing (1st, 2nd, 3rd, and 4th grades) and physiotherapy and rehabilitation (1st, 2nd, 3rd, and 4th grades) departments of health sciences faculties, who were enrolled in the 2022-2023 academic year.

Data collection tools

The data was collected using the following instruments: "Sociodemographic Characteristics Information Form," "Level of Knowledge About Complementary and Alternative Medicine (T&CM) Practices Form," and "Attitude Scale Towards Traditional and Complementary Medicine."

Sociodemographic characteristics information form

This form consists of 15 questions (such as age, gender, class, faculty, etc.) to determine the participants' sociodemographic characteristics.

Level of knowledge about traditional and complementary medicine (t&cm) practices form

This form is designed to assess the participant's level of knowledge regarding complementary and alternative medicine practices. It includes 15 items related to different T&CM methods, and participants are asked to rate their knowledge level as "know well," "know a little," "aware of," "unaware of," or "don't know."

Attitude scale towards traditional and complementary medicine

The Comprehensive Attitude Scale Towards Complementary and Tradinational Medicine, developed by Hyland (4), has been validated in our country Erci et al.[6] The scale is a Likert-type scale consisting of a total of 11 items, and scores can range from a minimum of 11 to a maximum of 66. As the score on the scale decreases, the positive attitude towards complementary and alternative medicine increases.6 The reliability coefficient of the scale, Cronbach's alpha value is 0.72; for this study, Cronbach's alpha value was calculated as 0.93.

Statistical analysis

After the research, a power analysis was performed using the G*Power 3.1.9.2 software package. Number, percentage, and frequency were used for descriptive statistics. The normality of the data was examined using the Shapiro-Wilk test to determine whether the data followed a normal distribution. For data determined to follow a normal distribution, independent t-tests, Mann-Whitney U tests, one-way analysis of variance (ANOVA), and Kruskal-Wallis H tests were applied for two-group and more than two-group comparisons. The statistical analysis of the data was conducted using the Statistical Package for the Social Sciences (SPSS) version 25.0 software package, and a significance level of $p < 0.05$ was set.

Ethical statement

Approval was obtained from the Afyonkarahisar Health Sciences University Non-Interventional Clinical Research Ethics Committee (date: 04.11.2022, decision no: 2022/548) and the relevant departments' dean's offices.

RESULTS

A total of 1085 individuals participated in the study. Among the participants, 44.5% (n=483) were from the medical faculty, 19.4% (n=211) were from the dentistry faculty, 19.1% (n=207) were nursing students, and 17% (n=184) were physiotherapy and rehabilitation students. It was observed that 64.1% (n=695) of the participants were female, and 35.9% (n=390) were male. Regarding their academic year, 26.6% (n=289) were in the first year, 19.3% (n=209) were in the second year, 23% (n=250) were in the third year, 13.5% (n=146) were in the fourth year, 10.7% (n=116) were in the fifth year, and 6.9% (n=75) were in the sixth year (Table 1).

When asked about their aspirations in terms of specialization, 43.5% (n=472) of the students expressed their interest in surgical sciences, 26.1% (n=283) in internal medicine, 12.1% (n=131) in basic sciences, while 18.3% reported being undecided. Among the participants, 91.4% (n=992) stated that they chose their department willingly, while 8.6% (n=93) did not choose their department willingly (Table 1).

In terms of age distribution, 28.9% (n=314) of the students were aged 17-18, 45% (n=488) were aged 19-20, 21.8% (n=237) were aged 21-22, 2.8% (n=30) were aged 23-24, and 1.5% (n=16) were 25 years old or older. Regarding family structure, 74.6% (n=809) of the participants came from nuclear families, while 25.4% (n=276) came from extended families. In terms of income level, 19.7% (n=214) reported an income between 0-5000 TL, 20.9% (n=227) reported an income between 5001-8000 TL, and 59.4% (n=644) reported an income of 8001 TL and above. It was found that 93.5% (n=1015) of the participants did not have any chronic diseases, while 6.5% (n=70) reported having a chronic illness. Among those with chronic diseases, 1.2% (n=13) had allergic rhinitis, 1.6% (n=13) had asthma, 0.5% (n=5) had thyroid dysfunction, 0.6% (n=6) had reflux, 0.8% (n=9) had attention deficit hyperactivity disorder (ADHD), 0.6% (n=7) had rheumatic disease, 0.7% (n=8) had sinusitis, and 0.5% (n=5) had hypertension (Table 1). 31.4% of the students (n=341) stated that they had previously used the T&CM method, while 68.6% (n=744) mentioned that they had not used it. When asked about their beliefs regarding the use of T&CM, 39.4% of the participants (n=428) recommended its use, while 60.6% (n=657) did not recommend it. When asked about the factors influencing students' belief in T&CM, 39.7% (n=431) attributed it to personal experience, 17.5% (n=190) to scientific evidence, 5.8% (n=63) to university education, 19.8% (n=215) to cultural background, 10.7% (n=116) to T&CM training, and 6.5% (n=70) to media.

Regarding the sources of information about GETAT, 17.9% (n=194) mentioned TV, 3.5% mentioned newspapers, 3.2% (n=35) mentioned the internet, 17.3% (n=188) mentioned social media, 7.6% (n=82) mentioned family members or neighbors, 16.1% (n=175) mentioned doctors, 1.4% (n=15) mentioned medical school education, and 33% (n=358) said they had no knowledge and wished for it to be taught as a course (Table 1).

Among the faculties, the most well-known T&CM methods were reflexology (n=374, 34.5%), osteopathy (n=214, 19.7%), mesotherapy (n=204, 18.8%), hypnosis (n=203, 18.7%), cupping therapy (n=196, 18.1%), chiropractic (n=196, 18.1%), music therapy (n=194, 17.9%), apitherapy (n=188, 17.3%), phytotherapy (n=176, 16.2%), acupuncture (n=175, 16.1%), prolotherapy (n=172, 15.9%), ozone therapy (n=160, 14.7%), leech therapy (n=153, 14.1%), homeopathy (n=149, 13.7%), and maggot therapy (n=110, 10.1%) (Table 2).

The three most well-known T&CM methods among medical faculty students are cupping therapy (n=207, 29.8%), music therapy (n=117, 24.2%), and chiropractic (n=107, 22.2%). In the dentistry faculty, the three most commonly known T&CM methods among students are reflexology (n=162, 76.8%), acupuncture (n=47, 22.3%), and homeopathy (n=23, 10.4%). In contrast, 64% of students (n=135) stated that they were not familiar with osteopathy, and 64% (n=135) were not familiar with mesotherapy methods. Among nursing students in the faculty of health sciences, the most well-known T&CM methods are osteopathy (n=103, 49.8%), reflexology (n=93, 44.9%), and mesotherapy (n=89, 43%), while the least known method is phytotherapy (n=74, 35.7%). Among students in the physiotherapy and rehabilitation department of the faculty of health sciences, the most well-known T&CM methods are phytotherapy (n=69, 37.5%), hypnosis (n=59, 32.1%), and chiropractic (n=54, 29.3%). In contrast, the least known method is larval therapy (n=44, 23.9%) (Table 2).

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Table 1. Comparison of students' T&CMS score averages according to some sociodemographic characteristics

Variable	n	%	T&CMS*			Test (p)
Gender			**X**	**±**	**SS**	
Female	695	64.1	31.25	±	6.08	t= 0.01
Male	390	35.9	31.24	±	6.31	p= 0.98
Age						
17-18 age	314	28.9	30.28	±	4.43	
19-20 age	488	45	31.49	±	6.61	
21-22 age	237	21.8	32.52	±	7.19	
23-24 age	30	2.8	29.50	±	2.54	
25 age and above	16	1.5	27.06	±	4.13	
Income level						
0-5000 TL	214	19.7	33.78	±	5.37	F= 35.58
5001-8000 TL	227	20.9	32.25	±	6.87	p= 0.00
8001 TL and above	644	59.4	30.04	±	5.82	
Family type						
Nuclear family	809	74.6	30.67	±	6.62	t= 5.29
Large family	276	25.4	32.92	±	4.16	p= 0.00
Marital status						
Married	3	0.3	28.66	±	15.27	Z= 0.10
Single	1082	99.7	31.25	±	6.13	p= 0.91
Faculty						
Faculty of Medicine	483	44.5	26.78	±	5.39	
Faculty of Dentisty	211	19.4	30.88	±	1.67	F=430.6
Health Sciences-Nursing	207	19.1	36.94	±	2.62	p=0.00
Health Sciences-physiotherapy and rehabilitation	184	17	36.98	±	3.88	
Class						
1st Class	289	26.6	32.63	±	6.37	
2nd Class	209	19.3	31	±	5.85	
3rd Class	250	23	32.55	±	5.95	F= 29,24
4th Class	146	13.5	32.40	±	5.54	p= 0.00
5th Class	116	10.7	26.78	±	4.89	
6th Class	75	6.9	26.89	±	4.39	
Department preference						
Yes	992	91.4	31.53	±	6.04	t=5.10
No	93	8.6	28.16	±	6.62	P=0.00
Chronic Disease						
No	1015	93.5	31.19	±	6.19	F=1.382
Yes	70	6.5	32.08	±	5.74	P=0.24
If You Have Chronic Disease						
No	1015	93.5	31.38	±	6.17	
Allergic rhinitis	13	1.2	33.07	±	7.21	
Asthma	17	1.6	30.05	±	4.26	X^2= 24.83
Thyroid dysfunction	5	0.5	26.8	±	3.63	p= 0.00
Reflux	6	0.6	31.5	±	3.93	
Attention deficit hyperactivity disorder	9	0.8	30.44	±	6.2	
Rheumatic disease	7	0.6	26	±	5.97	
Sinusitis	8	0.7	26.12	±	4.54	
Hypertension	5	0.5	24.6	±	3.28	
T&CM belief						
Personal Experience	431	39.7	33.11	±	5.29	
Scientific Evidence	190	17.5	28.93	±	6.31	
Education at the University	63	5.8	26.98	±	5.49	F=31.19
Cultural background	215	19.8	30.41	±	6.62	P=0.00
T&CM trainings	116	10.7	33.77	±	5.72	
Media	70	6.5	28.21	±	4.43	
T&CM information resource						
TV	194	17.9	35.89	±	5.05	
Newspaper	38	3.5	25.6	±	4.51	
İnternet	35	3.2	29.74	±	6.14	
Social media	188	17.3	35.48	±	4.76	X^2= 395.9
Neighbors, neighbors	82	7.6	30.76	±	7.17	p= 0.00
Doctors	175	16.1	27.56	±	5.46	
Medical faculty education	15	1.4	30.4	±	2.02	
I have no information. I want it to be given as a course.	358	33	29.19	±	4.67	
Using T&CM before						
Yes	341	31.4	30.99	±	7.19	t=0.90
No	744	68.6	31.36	±	5.67	P=0.36
Suggesting the use of getat						
No	428	39.4	28.05	±	5.45	t=15.16
Yes	657	60.6	33.3	±	5.69	P=0.00
The branch you want to progress						
Surgical Sciences	472	43.5	34.5	±	4.79	
Internal Sciences	283	26.1	30.6	±	6.04	F=127.3
Basic Sciences	131	12.1	26.58	±	5.24	P= 0.00
None/I am undecided	199	18.3	27.51	±	5.39	

* T&CMS: Attitude Scale Towards Traditional and Complementary Medicine

In the past 30 years, there has been an increase in the use of Traditional and Complementary Medicine (T&CM) in developed and developing countries. In a study conducted among the adult population in Türkiye, the prevalence of complementary medicine practices was 60.5%.[7] In our study, 31.4% of the students reported using T&CM methods previously, while 68.6% stated that they had not used them. Among the participants, 60.6% recommended the use of T&CM methods, while 39.4% did not recommend their use.

A review of 26 studies evaluating medical undergraduate curricula examined the extent to which complementary and alternative medicine (CAM) was incorporated into the curriculum. It assessed the teaching, presentation, and evaluation approaches used. The review indicated inconsistencies in the curriculum, lack of defined graduate competencies, and the need for different research to determine whether CAM programs' design, content, and assessment affect clinical practice and/or patient outcomes.[8]

In a study conducted with students from Sakarya University Medical School, when asked about their sources of information on complementary medicine practices, 60.7% of the participants indicated their surroundings (family, relatives), 59.1% mentioned the Internet, 42.1% mentioned television, 9% mentioned healthcare professionals, and 5.9% mentioned school as their sources (7). In our study, when students were asked about the factors influencing their belief in T&CM, 39.7% mentioned personal experience, 17.5% mentioned scientific evidence, 5.8% mentioned education at the university, 19.8% mentioned cultural background, 10.7% mentioned T&CM training, and 6.5% mentioned media as influential factors. Our study results differ in this aspect. Furthermore, when asked about their sources of information on T&CM, 33% of the participants stated that they had no knowledge and expressed their desire to be taught as a course. This finding is consistent with the study conducted by Sonmez et al., where it was reported that students lacked sufficient knowledge about T&CM practices, obtained information from other sources, and desired its inclusion in the curriculum and its integration with modern medicine.[9]

A study was conducted to investigate the attitudes of dentistry faculty students towards traditional and complementary medicine (T&CM) methods, using various questionnaires, with a total of 243 volunteer students. The methods that the students were most familiar with were reported to be hirudotherapy-leeches (59.7%), acupuncture (56%), and cupping therapy (51%). The most commonly used methods were cupping therapy (8.2%), hirudotherapy-leeches (4.1%), and music therapy (4.1%). It was also noted that nobody used prolotherapy, apitherapy, and chiropractic methods.[10] In our study, the three most known T&CM methods among dentistry faculty students were reflexology (76.8%), acupuncture (22.3%), and homeopathy (10.4%). In contrast, 64% of the students were unaware of osteopathy and mesotherapy methods. In this aspect, our study differs.

In a study evaluating the attitudes of nursing students toward holistic complementary and alternative medicine, it was stated that 82.5% of the students used the Internet as a source of information. It was reported that they had previously used yoga (31.1%), meditation (25.7%), diet support (23.9%), music therapy (21.4%), and acupuncture (14.6%) methods. In comparison, they had not heard of or used chiropractic (91.8%), shiatsu (90.7%), bioresonance (85%), and homeopathy (77.5%) methods.[11] In our study, the most known TCM methods among nursing students in the health sciences faculty were osteopathy (49.8%), reflexology (44.9%), and mesotherapy (43%), while the least known method was phytotherapy (35.7%).

In a study investigating the usage of Complementary and Alternative Medicine (CAM) practices by patients attending the Physical Medicine and Rehabilitation clinic, it was reported that 63.6% of the patients had heard of CAM practices, and 17.8% had tried at least one of these practices. The most well-known CAM practices were acupuncture (74.7%), herbal therapy (73.3%), and cupping therapy (62.7%). The most commonly utilized CAM practices were cupping therapy (71.4%), herbal therapy (23.8%), and phytotherapy (19%).[12] In our study conducted with physiotherapy and rehabilitation department students from the health sciences faculty, the most well-known T&CM methods were phytotherapy (37.5%), hypnosis (32.1%), and chiropractic (29.3%). In comparison, the least known method was larval therapy (23.9%). It was observed that most of the existing studies have been conducted on patients, and there is a lack of studies focusing on the knowledge level of physiotherapy and rehabilitation department students. Thus, our study can be considered the first comparative study conducted between faculties in this regard.

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Table 2. Comparison of students' knowledge about T&CM methods according to faculties

	Medicine					Dentisty					Nursing					Physiotherapy And Rehabilitation					Total[a]	Total[b]
T&CM implementation	**A** **n** **(%)**	**B** **n** **(%)**	**C** **n** **(%)**	**D** **n** **(%)**	**E** **n** **(%)**	**A** **n** **(%)**	**B** **n** **(%)**	**C** **n** **(%)**	**D** **n** **(%)**	**E** **n** **(%)**	**A** **n** **(%)**	**B** **n** **(%)**	**C** **n** **(%)**	**D** **n** **(%)**	**E** **n** **(%)**	**A** **n** **(%)**	**B** **n** **(%)**	**C** **n** **(%)**	**D** **n** **(%)**	**E** **n** **(%)**	**n** **(%)**	**n** **(%)**
Acupuncture	97 20.1	258 53.4	108 22.4	20 4.1	-	47* 22.3	29 13.7	135 64	-	-	22 10.6	17 8.2	78 37.7	90 43.5	-	6 3.3	44 23.9	21 11.4	113 61.4	-	172 15.9	-
Apitherapy	51 10.6	131 27.1	122 25.3	179 37.1	-	17 8.1	8 3.8	129 61.1	57 27	-	83 40.1	101 48.8	-	21 10.1	2 1	37 20.1	44 23.9	6 3.3	60 32.6	37 20.1	188 17.3	39 3.6
Phytotherapy	65 13.5	190 39.3	147 30.4	81 16.8	-	6 2.8	154 73	45 21.3	6 2.8	-	36 17.4	30 14.5	-	67 32.4	74 35.7	69* 37.5	45 24.5	15 8.2	46 25	9 4.9	176 16.2	83 7.6
Hypnosis	84 17.4	183 37.9	154 31.9	62 12.8	-	17 8.1	28 13.3	29 13.7	137 64.9	-	43 20.8	106 51.2	8 3.9	49 23.7	1 0.5	59* 32.1	53 28.8	-	72 39.1	-	203 18.7	1 0.1
Leech Treatment	66 13.7	192 39.8	159 32.9	66 13.7	-	6 2.8	162 76.8	29 13.7	14 6.6	-	62 30	112 54.1	-	15 7.2	18 8.7	19 10.3	66 35.9	10 5.4	66 35.9	23 12.5	153 14.1	41 3.8
Homeopathy	64 13.3	125 25.9	112 23.2	182 37.7	-	129* 61.1	22 10.4	46 21.8	14 6.6	-	28 13.5	109 52.7	15 7.2	16 7.7	39 18.8	35 19	76 41.3	5 2.7	68 37	-	149 13.7	39 3.6
Kayropraktic	107* 22.2	192 39.8	112 23.2	72 14.9	-	6 2.8	30 14.2	40 19	135 64	-	29 14	50 24.2	2 1	125 60.4	1 0.5	54* 29.3	29 15.8	23 12.5	78 42.4	-	196 18.1	1 0.1
Mug Treatment	144* 29.8	207 42.9	93 19.3	39 8.1	-	11 5.2	29 13.7	171 81	-	-	16 7.7	108 52.2	1 0.5	15 7.2	67 32.4	25 13.6	47 25.5	16 8.7	57 31	39 21.2	196 18.1	106 9.8
Larva Treatment	40 8.3	115 23.8	153 31.7	175 36.2	-	23 10.9	30 14.2	29 13.7	129 61.1	-	23 11.1	44 21.3	1 0.5	119 57.5	20 9.7	24 13	40 21.7	-	76 41.3	44 23.9	110 10.1	64 5.9
Mesotherapy	59 12.2	132 27.3	128 26.5	164 34	-	11 5.2	6 2.8	14 6.6	45 21.3	135 64	89* 43	19 9.2	15 7.2	84 40.6	-	45 24.5	11 6	5 2.7	123 66.8	-	204 18.8	135 12.4
Prolotherapy	24 5	77 15.9	126 26.1	256 53	-	23 10.9	22 10.4	14 6.6	23 10.9	129 61.1	80 38.6	82 39.6	2 1	42 20.3	1 0.5	48 26.1	21 11.4	10 5.4	100 54.3	5 2.7	175 16.1	135 12.4
Osteopathy	40 8.3	98 20.3	160 33.1	185 38.3	-	17 8.1	22 10.4	14 6.6	23 10.9	135 64	103* 49.8	16 7.7	2 1	86 41.5	-	54 29.3	14 7.6	13 7.1	98 53.3	5 2.7	214 19.7	140 12.9
Ozone Treatment	78 16.1	129 26.7	157 32.5	119 24.6	-	11 5.2	30 14.2	29 13.7	141 66.8	-	34 16.4	52 25.1	9 4.3	74 35.7	38 18.4	37 20.1	69 37.5	8 4.3	65 35.3	5 2.7	160 14.7	43 4
Reflexology	71 14.7	133 27.5	155 32.1	124 25.7	-	162* 76.8	14 6.6	29 13.7	6 2.8	-	93* 44.9	88 42.5	9 4.3	17 8.2	-	48 26.1	57 31	44 23.9	21 11.4	14 7.6	374 34.5	14 1.3
Music Therapy	117* 24.2	194 40.2	127 26.3	45 9.3	-	17 8.1	36 17.1	23 10.9	135 64	-	36 17.4	105 50.7	5 2.4	14 6.8	47 22.7	24 13	54 29.3	11 6	81 44	14 7.6	194 17.9	61 5.6

DISCUSSION

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In a study investigating the usage of Complementary and Alternative Medicine (CAM) practices by patients attending the Physical Medicine and Rehabilitation clinic, it was reported that 63.6% of the patients had heard of CAM practices, and 17.8% had tried at least one of these practices. The most well-known CAM practices were acupuncture (74.7%), herbal therapy (73.3%), and cupping therapy (62.7%). The most commonly utilized CAM practices were cupping therapy (71.4%), herbal therapy (23.8%), and phytotherapy (19%).[12] In our study conducted with physiotherapy and rehabilitation department students from the health sciences faculty, the most well-known T&CM methods were phytotherapy (37.5%), hypnosis (32.1%), and chiropractic (29.3%). In comparison, the least known method was larval therapy (23.9%). It was observed that most of the existing studies have been conducted on patients, and there is a lack of studies focusing on the knowledge level of physiotherapy and rehabilitation department students. Thus, our study can be considered the first comparative study conducted between faculties in this regard.

CONCLUSION

The cross-sectional design of our research, being conducted only with students from a single university, measuring attitudes towards T&CM solely with questions included in a scale, and the inability to access detailed information about the participants' knowledge regarding T&CM can be mentioned as the main limitations of this research. However, unlike previous studies, this research stands out by evaluating the attitudes toward T&CM of students from multiple faculties, which represents a strength of this study.

Based on the findings obtained in our study, it has been observed that students in the faculties of medicine and dentistry, as well as in the nursing and physiotherapy and rehabilitation departments of the faculty of health sciences, have a positive attitude toward T&CM applications but lack sufficient knowledge. Revisions to the current educational curriculum and training on T&CM applications will contribute significantly to guiding patients and their families in the integrated and proper use of these applications in conjunction with modern medicine for future healthcare professionals.

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ORIGINAL RESEARCH

Biological Activity of Natural Formulation with Propolis, Lavender and Thyme Oil on *Candida* Species

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Abstract

Objective: *Candida* strains have been frequently associated with nosocomial infections in recent years. On the other hand, the public prefers natural resources rather than synthetic materials. Therefore, it is essential to research the combined effects of natural products. This study aimed to investigate the antifungal and antibiofilm effectiveness of essential oils and propolis-containing samples on *Candida* species and the toxicity of samples.

Materials-Methods: Three different samples were prepared with propolis, lavender oil, and thyme oil. Gas Chromatography-mass Spectrometry (GC/MS) GC/MS was used to determine organic compounds in samples. The antifungal effects of a natural product mix were tested against *C. albicans*, *C. krusei*, and *C. parapsilosis* by disk diffusion method. MIC and MFC tests of the mixture were performed against a standard such as Nystatin. Antibiofilm tests were performed with the microplate system, taking into account the detected concentrations, and finally, the cytotoxicity test was investigated in the L929 cell line with the WST-1 kit.

Results: *C. albicans* was determined as the most sensitive species (MIC50: 90 ppm, MIC90: 750 ppm MFC: 3120 ppm). In the antibiofilm tests, it was determined that the sample was more effective on *C. albicans*, and it inhibited biofilm formation. Also, it was determined that the IC50 value was 5052 ppm.

Conclusion: The natural product mixture, which has a scientifically proven antifungal effect, has a feature that has high added value and contributes to the economy. However, of course, it has to investigate by further studies. The findings of this study may lead to new therapeutic approaches with standardized natural products in the future.

Keywords: Antifungal, Antibiofilm, *Candida*, Propolis, Lavender Oil, Thyme Oil.

INTRODUCTION

Candida species are widely distributed in nature. They are mostly commensal fungi but can also be opportunistic pathogens in human microbiota.[1] Also, *Candida* is the most common causative agent among fungi that cause disease in humans. Candidiasis develops with the excessive proliferation of *Candida* species in the natural flora of humans. The most important reason for that is the inappropriate use of antibiotics and the weakening of the immune system.[2,3] Some of the most common *Candida* species that cause disease are *C. albicans*, *C. krusei*, and *C. parapsilosis*. *C. albicans* is a fungus often isolated from skin, gastrointestinal tract, and esophagus infections. Skin and mucous infections of *C. albicans* include thrush, *Candida* esophagitis, non-oesophageal gastrointestinal candidiasis, *Candida* vaginitis, and cutaneous candidiasis. *C. krusei* is associated with systemic candidiasis and is mainly found in immunosuppressive individuals. Further, *C. krusei* treatment is challenging because it has resistance to many antifungal drugs. Mucocutaneous infections caused by *C. parapsilosis* in cases of immune deficiency can also be chronic in humans.[4]

All *Candida* strains have been frequently associated with nosocomial infections in recent years. In order of prevalence, they are in the top five in invasive yeast infections worldwide.[5] For the ability to switch

from commensal to pathogen, properties such as having a wide range of virulence factors and the ability to change morphology and create biofilms are among the essential characteristics of *Candida* species. Biofilm formation provides highly resistant infections in the host due to their structural nature. *Candia* biofilms are resistant to antimicrobial therapy. Therefore, available therapeutic agents are not sufficient to eliminate *Candida* biofilms.[6] Therefore increased research for new compounds to show the anti-*Candida* effect are essential.[1] On the other hand, consumers prefer natural resources over synthetic materials in food and alternative treatment processes.[7] Therefore, taking advantage of the combined effects of natural products prepared differently is essential. Because, in this way, it is possible to produce powerful natural antifungal agents' alternative treatment methods are also being developed by using these natural extracts directly.[8]

Among these natural products can be the best choice is propolis. Propolis is a resinous substance obtained by collecting from leaves, shoots, and buds of different plant sources and adding enzymes and beeswax by honey bees. Although the propolis content varies depending on the region, climatic conditions, and the collection season, the essential and aromatic oil ratio generally does not exceed 10%. This 10% slice contains phenolic substances, flavonoids, and terpenoids.[9] Due to this special propolis content, it is used as an antimicrobial, antifungal, and antioxidant agent.[10] Combining this activity of propolis with various natural products can create more positive effects. In this case, lavender and thyme, belonging to the *Lamiaceae* family, are critical medicinal plants cultured worldwide due to their high-quality essential oils. The essential oils of these plants have been used for therapeutic and cosmetic purposes for centuries. In addition, it has been the reason for its use in industry and agricultural applications due to the biologically active components. Propolis, lavender, and thyme extracts are also an important branch of natural resources. All of them are products brought to the literature as natural preservatives and antifungal agents.[11] However, when it comes to a natural product, it is wrong to say that it is good in any case. It does not make sense unless it is determined that natural mixtures do not cause toxic effects and if they are not antagonistic to each other. For this reason, cell viability tests are essential. Therefore, standardization of natural products is difficult. For this reason, the microbial inhibition concentration of each natural product should be determined separately. The aim of this study is to investigate the antifungal and antibiofilm efficacy and cell viability of samples containing herbal oil and propolis on *Candida* species.

MATERIALS AND METHODS

Microorganisms

Candida albicans and *C. krusei*, which are clinical isolates from previous studies and were obtained from the culture collection of Duzce University Traditional and Complementary Medicine Applied and Research Centre. Also, C. *parapsilosis* ATCC 22019 was used in the study.

Chemicals and materials

RPMI-1640 medium containing %1 streptomycin and penicillin 10% inactivated fetal bovine serum (all purchased from Sigma) was used for the L929 mouse fibroblast cell line. WST-1 (2-(4-iodophenyl) -3- (4-nitrophenyl) - 5- (2,4-disulphophenyl) -2H-tetrazolium) cell proliferation assay kit (Takara Bio Inc) was used for the viability test. Potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and, sabouraud dextrose broth (SDB), Mueller Hinton agar (MHA) were purchased from Merck for antifungal tests and fungal growth. PEG400 (Polyethylene glycol 400 from Sigma) was obtained from Merck. The ethanolic propolis extract, lavender oil (*Lavandula intermedia*), and thyme oil (*Origanum onites*) were purchased from Duzce University Beekeeping Center and Traditional and Complementary Medicine Applied and Research Centre at Duzce University.

Preparation of natural products

First, ethanol in commercially available propolis was removed with the help of an evaporator, and 0.455 grams of pure propolis was used. Propolis dissolved in 3.46 grams of PEG-400, and sample 1 was obtained. To obtain sample 2, 0.455 grams of commercially available lavender oil was added to sample 1 and mixed homogeneously. To obtain sample 3, 0.455 grams of commercially available thyme oil was added to sample 2. All obtained samples were stored at 4 ^{o}C.

Gas chromatography/mass spectroscopy (GC/MS) analysis of essential oils

Gas Chromatography-mass Spectrometry (GC/MS) GC/MS was used to determine organic compounds. The GC/MS instrument consisted of an Agilent 7890 gas chromatograph with an on-column auto-injector and Agilent mass selective 5975c MS detector. The GC column was HP-innowax column (60 m, 250 μm, 0.25 μm). The GC/MS was operated under the following conditions; an ion source, transfer line

temperature 60-260°C; carrier gas He (≥99.99% purity); flow rate 1 mL/min. 5 µL of the sample solution was injected into GC using splitless injection mode. The GC oven temperature was the program as follows: 100°C for 0 min, then the temperature increased by 1°C/min to 150°C for 0 min, and then with an isothermal hold at 240°C for 10 min. Agilent software was used for data characterization. The compounds were identified with their retention time, and the mass spectra were compared with internal reference library data.

Anti-*Candida* effect of samples

Disk diffusion test was used to compare the anti-*Candida* effects of all three samples.[12] *Candida* cultures in PDA medium were prepared the day before and then adjusted to a turbidity of 0.5 on the McFarland scale in saline. Yeast solutions spread on MHA. Test samples were absorbed into blank disks (Bioanalyase, blank disk, 6mm) and placed on MHA. Test plates were incubated at 35° C for 48 hours. Nystatin (Bioanalyase, NY 100U disk) was used as a positive control. Each sample was tested in triplicate. After then, the sample with the biggest inhibition zone according to the disk diffusion method was chosen for further research.

Determination of minimal inhibitory (MIC) and fungicidal (MFC) concentrations

The sample with the most inhibition zones from the disk diffusion test was considered the most effective. Then MIC and MFC concentrations of the most effective sample were determined for all three *Candida* species by the modified broth dilution method.[1,13] For MIC, a product dilution and SDB medium were added in the dose range of 100000 to 20 ppm for a total of 120 µl per well of the 96 well plate. Each yeast (at 0.5-2.5x 103 cell/ml) was inoculated into the medium containing the medium and test product and then incubated at 35° C for 48 hours. For MFC, 50 µl of non-growth MIC value and above concentrations were added to the SDA. Colonies were counted and compared with control. MFC was defined as the lowest dose that killed more than 99.9% of inoculated *Candida* cells.[1,14] Experiments were conducted three times for each strain on different days.

Antibiofilm assay

The effect of the most effective sample on biofilm formation was investigated. 40 µl SDB, 80 µl yeast (prepared with 0.5 Mcfarland turbidity), and 80 µl different doses of product or water were added to each well of 96 well plate. After 24 hours of incubation at 35°C, the absorbance value (OD) was read at 630 nm by using a microplate reader (Biotek BT 800, USA). AfAfterwardthe contents of all wells were poured and washed three times with distilled water. After drying at room temperature, 125 µl 0.4% crystal violet was added to each well and waited 30 minutes. Then the dye was poured, washed three times, and left to dry again at room temperature. 100 µl of 95% ethanol was added to remove the cells forming biofilms and trap the dye and transferred it into a clean well. OD measured at 490 nm. The ratio of OD value at 490 nm and 630 nm (= OD 490/OD630) was used to assess biofilm formation. Results were evaluated.[1,15] All experiments were performed three times.

WST-1 assay

The cytotoxicity of the active sample was determined in the L929 mouse fibroblasts cell line by the WST-1 cell viability test. Ten different dilutions of the sample between 90 and 50000 ppm were performed. When the cells reached the appropriate concentration, they were inoculated into 96-well culture dishes. It was added 5x104 cells per well and serial dilutions and incubated at 37°C (in a 5% CO2 atmosphere) for 24 hours. After incubation, 10 µl of WST-1 solution was added to each well and incubated for 4 hours. At the end of the incubation, each well's absorbance value (OD) was read at 490 nm wavelength and 630 nm reference range using the microplate reader. All assays were performed three times, and the viability (%) was calculated as the following equation.

Viability (%) = (OD in herbal sample group/OD in control group)×100

Statistical analysis

Inhibition data are given as mean (± SD). Data are given as mean (± SD). The statistically significant differences of each group compared to the control group were compared with Mann Whitney U using SPSS 15.0.

The percentage of cell inhibition x log of the concentration was recorded, and their IC50 and respective confidence intervals (IC 95%) were recorded from non-linear regression. Analyzes and graphs were elaborated using the GraphPad Prism version 9.0 software (San Diego, CA, USA).

RESULTS

GC-MS analysis of essential oils of thyme and lavender used in samples

By GC MS analysis of commercially available lavender and thyme oil, preliminary information was obtained about its chemical components. Microbiological studies were carried out on the products that were brought together in line with the

information obtained. Linalool, linalyl acetate, 1,8-cineole, and camphor accounting for 40.11, 25.23, 6.13, and 5.88%, respectively, were also characterized as major chemical compounds in lavender essential oils. Also, the thyme essential oil was characterized by high amounts of Carvacrol (57.65%) and linalool (15.64%) (Table 1).

Antifungal effects of samples

In the antifungal effect test performed using the disc diffusion test, the effects of lavender oil, thyme oil, and propolis extract (sample 3) combined in equal mass ratios, as well as the effects of only propolis extract (sample 1) and propolis extract-lavender oil (sample 2) mixture were also examined and compared.

It has been determined that the first planned triple mixture is more effective. The results were determined by measuring the zone diameters formed by the impregnated discs at that point. The highest inhibition sites were detected at S3. Considering CLSI guidelines, all three *Candida* strains appeared to be sensitive to Nystatin used as a positive control in the study (Table 2).

Table 1. GC/MS analysis results of examined essential oils

Entry	RT (min)	Compound Name	Thyme Oil % of Total	RT (min)	Compound Name	Lavender oil % of Total
1	9.504	Alfa-pinene	0.34	9.749	Alfa-pinene	0.43
2	16.908	Beta-myrcene	0.92	11.563	Camphene	0.35
3	17.851	Alfa-terpinene	0.89	17.403	Beta-myrcene	0.94
4	23.196	Gama-terpinene	3.13	19.830	Limonene	1.10
5	25.458	Orto-cymene	3.31	20.794	1,8-cineole	6.13
6	56.725	Linalool	15.64	23.132	Beta-ocimene	1.35
7	59.672	Beta-caryophyllene	1.57	24.676	Trans-beta-ocimene	2.30
8	61.478	Terpinene-4-ol	1.77	51.765	Camphor	5.88
9	72.054	Borneol	3.13	58.154	Linalool	40.11
10	74.680	Beta-bisabolene	3.78	58.915	Linalyl acetate	25.23
11	109.955	Thymol	2.97	61.080	Trans-caryophyllene	1.33
12	110.881	Carvacrol	57.65	63.651	Lavandulyl acetate	1.51
13	-	-		73.166	Borneol	4.43
14	-	-		89.433	Geraniol	0.61
15	-	-		107.194	Triacetin	0.77
16	-	-		111.139	Levamenol	0.42

The inhibition region of S3 was significantly larger than the positive control disk. Sample 3, the most effective formulation, was used for further experiments. MIC and MFC tests were performed for sample 3, and the lowest effective doses were determined. In addition, it was determined that the most sensitive species was *C. albicans* (Table 3).

Table 2. Zone diameters of formulations for *Candida* spp.

Yeast	Zone Diameter (mm) (±SD)			
	Formulation-1	Formulation-2	Formulation-3	Nystatin (100µg)
C. albicans	10 (±0.57)	13 (±1)	>30	25
C. krusei	8 (±0.57)	10 (±0)	30 (±0.57)	20
C. parapsilosis	R*	R*	17 (±0.57)	15

**Resistant*

Table 3. MIC and MFC tests for Sample 3

Yeast	Sample 3				
	MIC_{50}, ppm	MIC_{90}, ppm	MFC, ppm	NY (MIC), ppm	NY (MFC), ppm
C. albicans	90	750	3120	4	8
C. krusei	370	1500	6250	8	16
C. parapsilosis	180	1500	6250	8	16

NY:Nystatin

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According to the MIC assay, it was determined that *C. albicans* was more sensitive than other *Candida* species for cidal concentration (Figure 1). The inhibition rates of S3 against *Candida* strains depending on the concentration are given in Figure 1. Each concentration showed a significant difference for *C. albicans* compared to the control group ($p<0.05$). There was no significant inhibition against *C. krusei* at 90 ppm and 180 ppm, while 90 ppm of S3 against *C. parapsilosis* was not significant ($p>0.05$).

Antibiofilm effects of samples

Since S3 was selected as the most effective sample in previous tests, only the antibiofilm effect of S3 was investigated. The biofilm ability of each strain was also tested in control groups not treated with S3 for each bacteria. It was observed that *C. parapsilosis* produced weak biofilms, while *C. albicans* and *C. krusei* produced strong biofilms. Therefore, the strain most susceptible to biofilm formation against S3 was *C. parapsilosis*. It was determined that the biofilm formation ability of *C. krusei* was stronger and more resistant to S3 treatment groups. 75 ppm of S3 almost eliminated the formation of biofilms for *C. albicans* and *C. parapsilosis* while inhibiting more than 75 ppm for *C. krusei*. Inhibition rates of all concentrations against *C. albicans* and *C. parapsilosis* were statistically significant compared to control groups ($p<0.05$). 9 ppm of S3 was not a significant difference against *C. krusei* compared to the untreated control group. The inhibition rates of different concentrations of S3 on biofilm formation against *Candida* strains are given in Figure 2.

Cytotoxicity of sample-3

Cytotoxicity assays were performed at ten different concentrations, and the results were given in % viability. According to the results of the cell viability test conducted with the WST-1 kit, and IC50 value of the S3 is given in Figure 3. IC50 has been defined as the dose that kills half of the cells in the medium.

Data are presented as the mean ± standard error of the mean or as half-maximal inhibitory concentration (IC50) values. The 95% confidence intervals were obtained through nonlinear regression. Results are expressed as mean±S.E. Data was analyzed using GraphPad Prism version 9.0 software (San Diego, CA, USA). The percentage viability data varied with concentration. As the concentration increased, the vitality decreased. The best viability was determined at a dose of 90 ppm. Cell viability was about 90% at 180, 370, and 750 ppm concentrations. After 3120 ppm of S3, viability was severely reduced. The IC50 value at the 95% confidence interval is between 4238 and 6018. According to the analysis results, the IC50 value was 5052 ppm.

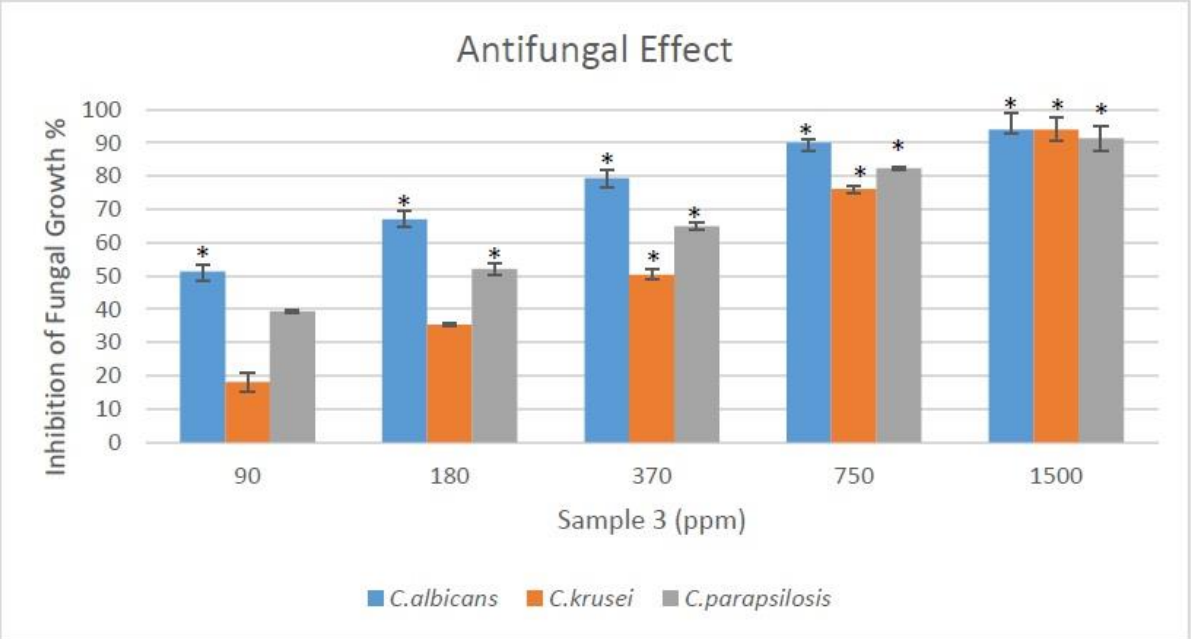

Figure 1. The effect of sample 3 on fungal growth: The normal growth of the control group for each yeast (untreated *Candida* strains) was considered 100%. The inhibition rates were calculated for each concentration. Since the positive control showed 100% inhibition against all yeasts after 16 ppm, it was not included in the graph.

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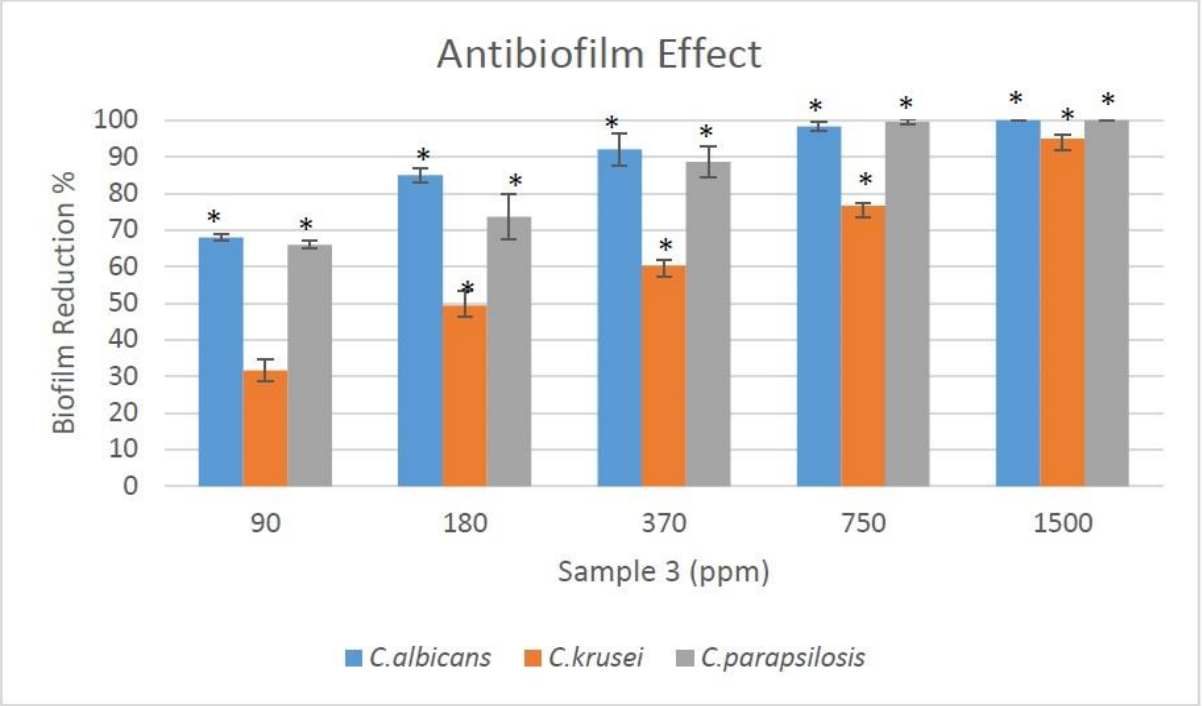

Figure 2. Antibiofilm effect of sample 3: The normal growth of the control group for each yeast (untreated *Candida* strains) was considered 100%. The inhibition rates were calculated for each concentration. Since the positive control showed 100% inhibition against all yeasts after 16 ppm, it was not included in the graph.

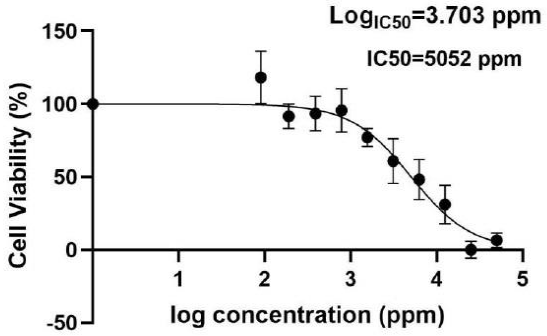

Figure 3. IC50 value of sample 3

DISCUSSION

The use of natural products in microbial treatments is becoming more popular day by day. Nowadays, cures containing very different mixtures of natural products are becoming widespread. Natural ingredients contain quite a lot of phenolic components. Phenolic components, on the other hand, are beneficial for microbial treatment.

It might be more effective when the combined antimicrobial effects of natural products are used together on pathogenic microorganisms. Because it is already known that medicinal plants have some pharmacologically important features via their component. In their combined use, the amount of phenolic components increases, and they turn into more effective antifungal agents due to the variety of phenolic components contained in the product.[16] In our study, Carvacrol (%57.65), Linalool (%15.64) in thyme oil, and Linalool (%40.11), linalyl acetate (%25.23) in lavender oil were found highly. Also, there are many minor ingredients such as gamma-terpinene, orto-cymene, beta-caryophyllene, borneol, beta-bisabolene, thymol in thyme oil, and alfa-pinene, camphene, beta-myrcene, limonene, beta-ocimene, trans-beta-ocimene, lavandulyl acetate, in lavender oil. Yılmaz[17] indicated that major components were Linalool (24.97-2.52-43.86-39.43 %), linalyl acetate (3.4-0.29-9.37-15.76 %), eucalyptol (33.81-43.81-18.47-12.08 %), camphor (13.12-15.91-8.72-9.21 %), and alpha-terpineol (2.84-2.47-1.28-3.86 %) in different parts of (stem, leaf, flower, and mix) *Lavandula x intermedia* Emeric cultivated in Türkiye (Bismil-Diyarbakır). In another study, Linalool (36.801%–28.486%) and Linalyl acetate (33.087%–4.648%) were found in Super A and Grey Hedge cultivar of *L. x intermedia* in Türkiye (Western Anatolia).[18] Some previous studies determined similar Linalool, linalyl acetate, camphor, and eucalyptol values as dominant components in L. intermedia cultivated in Southeast

Spain[19] and Italy[20]. In the chemical profile of the thyme essential oil, Carvacrol and p-Cymene were found as the major components, with an abundance of 74.749% and 9.464, respectively (Table 1). Similarly, it was observed that the oil from samples collected from Türkiye showed mainly Carvacrol, Linalool, and p-Cymene.[21,22] Differences in plant essential oil content are due to genetic factors and cultivation practices. They even differ depending on the period of development during which the plants are harvested and depending on it.[23] Additionally, the propolis used in this study was collected from Duzce (in Türkiye). The propolis is native to that region and is rich in phenolic components of more than 100mg/g for each component, such as quercetin, p-coumaric acid, ferulic acid, trans-cinnamic acid, benzoic acid, emodin, hesperidin, gallic acid, rosemarinic acid.[24]

The samples created in the study are based on propolis. Because it has already known that propolis is an effective natural bee product to control *Candida* infection.[25] In this study, it was aimed to increase its effect with other effective natural ingredients. The thyme and lavender essential oils are herbal products thought to have antifungal effects.[26,27] In previous studies, Lavender oil has been shown to have anti-candidal effects on vulvovaginal candidiasis isolates.[28] It has also been shown to have fungistatic and fungicidal activity against oropharyngeal and vaginal *Candida* strains.[29] In this study, the antifungal efficiency was investigated for three different samples. However, the most effective formula in this study was S3 contains thyme oil. S3 was considered the most effective formula because the zone diameter was the largest. The largest zone diameter may not mean the most effective, but on the other hand, natural products do not work with specific receptor-like antimicrobials. It was interpreted as causing more cell inhibition because essential oils directly change the cell composition of microorganisms. Hence, it has been thought that the antifungal effects of thyme essential oil stand out. Thyme oil is considered a more effective natural product than the other two natural products. There are many studies in which thyme oil has an antifungal effect on clinical *Candida* strains isolated from different body parts, such as skin, urine, wound, and vagina. Due to the biological activity of thyme essential oil, its industrial and medical use has come to the fore in recent years.[30] Zhang et al.[31] reported that MIC was 65 ppm, MFC was 100 ppm for thymol, while MIC was 120 ppm, and MFC was 140 ppm for carvacrol. Also, some combinations of natural products create a stronger antimicrobial effect via their combined effect. It is thought that; these combinations can be a natural therapeutic agent for candidal mycoses, among the important fungal infections today. Considering the chemical composition of essential oils and propolis, except for the major components, there are other components in trace amounts. It is thought that these minor components may be effective due to their combined interaction with other components.[32] In general, phenolic components disrupt the lipids and hydrophobic structure found in the cell membrane of microorganisms, making them more permeable. The amount of these phenolic components determines the MIC and MFC values. In addition to the bacteriostatic effect, the product's concentration and duration of action determine the bactericidal effect. In another study, it has been reported that the kinetic curves of *Litsea cubeba* oil (antibacterial) are 6250 ppm, and the cells are completely destroyed.[33] They also reported that the delay of *E. coli* cells could prolong the phase growth to about 12 hours it was killed at 1250 ppm within 2 hours. In this way, it can be tried that the factors determining the activity of natural mixtures are functional groups with active components in the composition and their synergistic interactions.[34]

Although sample 3 showed a high antifungal effect in the study, fungi have some escape routes from antifungal agents. The effectiveness of natural products on fungi can be eliminated by some metabolites of fungi. The biofilm formation, which is an escape for microorganisms especially in antimicrobial treatment, is common in *Candida* species.[7] The present study showed that the strain most susceptible to biofilm formation of fungal cells against sample-3 was *C. parapsilosis*. There are many reports about the antibiofilm effects of propolis, lavender oil, and thyme oil separately. In those studies, it has been shown that propolis reduces the formation of *Candida* species biofilm formation.[35] Similarly, it has been revealed by many researchers that essential oils disrupt the biofilm structure of different microorganisms.[36] Moreover, lavender and thyme oil have been shown to have antibiofilm effects on different *Candida* species, similar to this study's results.[28]

It is important that natural products are pharmacologically effective. However, on the other hand, combining natural products with no toxic effects when used together is so important. Phenol components in natural products can be toxic due to

increased doses and combined use. In vitro cytotoxicity tests used for this purpose are measurement methods performed in cell culture to evaluate substances with drug characteristics or whose toxic profile has been investigated. There are many tests applied to determine cell viability, and the WST-1 assay is one of them. It is a more sensitive method compared to the MTT test because it does not require additional thawing, and the absorbance is recorded at various times during the experiment. In this study, % viability data varied depending on concentration. As concentration increased, vitality decreased. The best viability was determined at a concentration of 90 ppm. After a dose of 3120 ppm, vitality decreased significantly. The IC50 value was determined as 5052 ppm. Tamfu et al.[37] determined the IC50 value of three different propolis, whose MIC value was 250-500 µg/mL against *Candida,* and it was found to be >100 ppm in the NIH-3 T3 fibroblast cell line. In the previous investigations, the effects of thymol, one of the major components of *T. vulgaris*, on Chinese hamster lung fibroblast (V79) cells MTT assay[38], and the effects of two thyme oils on human keratinocytes with MTT assay[39] were investigated. In addition, there is a cytotoxicity study on murine fibroblasts with 12 plant oils, including lavender oil.[40] Our study results are consistent with other studies. The concentrations required to eliminate yeasts have been shown to show no toxicity or very low toxicity.

CONCLUSION

Candida infections are the most common systemic and superficial mycoses in recent years. Moreover, *Candida* species resistant to various antifungal drugs are frequently reported. The research for natural product samples has increased due to resistance development. This is the first study in which the combined effects of propolis, lavender oil, and thyme oil were investigated by antibiofilm and antifungal assays. In this study, the efficacy of three different combinations was tested, and it was determined that the most effective sample was the S3 sample. Hence, the antifungal activity of the S3 in three different *Candida* species was investigated with other test methods, and its inhibition on biofilm formation was determined. Also, the biological activity of S3 was investigated with further experiments. It has been found that it can be as effective as an antifungal agent at appropriate concentrations. It was thought that the clinical use of these concentrations may be important because they do not show toxicity. Using lavender, thyme essential oils, and propolis combinations can be a practical and safe approach to treating *Candida* infections. But of course, it has to investigate by further studies. Especially considering the increased resistance of antimicrobial agents, the necessity to development of effective natural products is quite high. The findings of this study may lead to new therapeutic approaches with standardized natural products in the future.

Author contributions: Conceptualization: [NS, HG]; Study Design: [PAY, NS]; Investigation/Data collection: [PAY, NS, PGR, HG]; Writing: [PAY, NS, PGR]; Editing: [PAY, NS, PGR].

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ORIGINAL RESEARCH

Preparation of *Centella asiatica* (L.) and *Hypericum perforatum* (St. John's Wort) Plant Extracts and Development of Anti-Aging Herbal Cream Formulations

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Abstract

Objective: The study aims to use two different plant extracts, St. John's Wort (*Hypericum perforatum*) and *Centella asiatica* (L.), in the cream formulation and determine the new formulation's anti-aging effect.

Materials-Methods: The plants used in the study were obtained commercially, and plant extracts were obtained using the classical extraction techniques in the literature. The active ingredients in the obtained extracts were determined by the HPLC method. Physical, protective efficacy, microbial analysis, and anti-aging tests were carried out for cream formulations obtained with extracts.

Results: Plant extraction studies were carried out in the study. *Centella asiatica* (L.) was extracted with a 20.8% yield at 20 mL of methanol and 60°C. As a result of HPLC analysis, it was determined that there were 1740 mg/kg asiatic acid and 4380 mg/kg madecasic acid in the extract. In the extraction studies performed on *Hypericum perforatum*, the expected active ingredient, hypericin, was not found. For this reason, a commercial extract containing 2.5% hyperforin was obtained, and the studies were continued on these extracts, and final cream formulations were obtained.

Conclusion: pH, viscosity, density, protective effectiveness tests, and microbial analysis tests of the final formulations were performed. In addition, in vitro, anti-aging studies have been carried out in accredited laboratories. In-vitro anti-aging tests determined that the level of collagen 1A increased more in the formulation where both extracts were used together.

Keywords: *Hypericum perforatum*, *Centella asiatica* (L.), *In Vitro*, Anti-aging Herbal Cream.

INTRODUCTION

Hypericum perforatum and *Centella asiatica* (L.) have rich contents in terms of polyphenols, flavonoids, and terpenes in terms of active ingredients. This feature emphasizes the power of the antioxidant property of the content. In addition, both ingredients will inevitably have a substantial effect thanks to their anti-inflammatory effect, antioxidant effect, and some triggered reactions in the metabolic pathway. For this reason, after the extracts of both plants were prepared, the types and amounts of active ingredients were determined, and cream formulation studies were carried out. Accredited laboratories have tested appropriate formulas for pH, viscosity, density, protective activity, and microbial analysis. Afterward, in-vitro anti-aging tests were carried out.

For this reason, after the extracts of both plants were prepared, the types and amounts of active ingredients were determined, and cream formulation studies were carried out. For suitable formulas, pH, viscosity, density, protective activity tests, and microbial analyses were performed. In-vitro anti-aging tests were carried out. According to the results, it has been demonstrated that its anti-aging feature is vital. *C. asiatica* (L.) and *H. perforatum* are highly beneficial herbs in traditional medicine and cosmeceuticals.

C. asiatica (L.) contains flavonoids, phenolic acids, steroids, amino acids, vitamins, and essential oils. Madecassoside, asiaticoside, and their sapogenin triterpene acids (madecassic and asiatic acid) are the components used as biomarkers to evaluate the quality of the *C. asiatica* (L.) plant and responsible for most of its pharmacological activity (Figure 1).[1,2] These compounds are considered active ingredients in the pharmaceutical industry. In addition, *C. asiatica* (L.) is a rich source of antioxidants as it contains many phenolic compounds such as quercetin, catechin, luteolin, rutin, kaempferol, myricetin, naringin, and naringenin.[3]

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C. asiatica (L.), known as Cica, has become popular in cosmetics in skin care products, antiaging medications, moisturizers, anti-inflammatory drugs, and for treating scars, scars, and atopic dermatitis.[4]

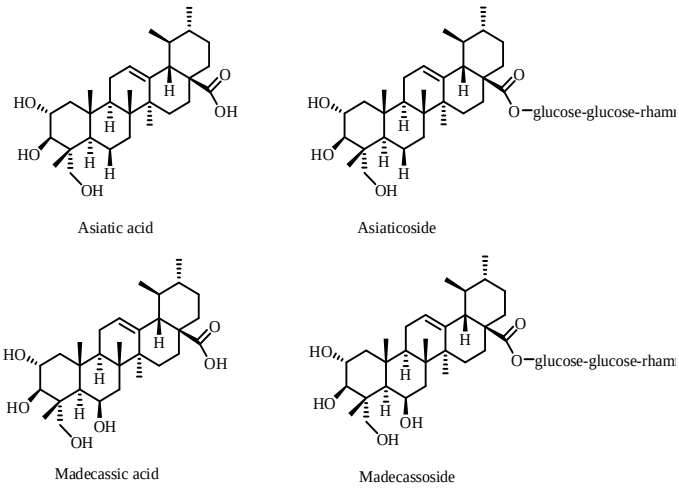

Figure 1. Important compounds for *Centella asiatica* (L.) plant.

Clinical studies have also shown that these effects are due to the triterpenoids in the CA content.[5] Especially *C. asiatica* (L.) has been used in treating skin disorders, and research has focused on this. In scar tissue, asiaticoside, and madecassoside are thought to increase glycosaminoglycan synthesis, hydroxyproline, and collagen content (Figure 1). Besides wound healing in cosmeceuticals, they are also used for anti-wrinkle and anti-cellulite effects as they improve fibronectin production in human skin fibroblasts and increase collagen synthesis[1,6]. Madecassoside, a triterpene in *C. asiatica* (L.), has also been reported to inhibit UV-induced hyperpigmentation in human skin cells.[2] Apart from this, it has been shown to exhibit an antiaging effect[7], memory-enhancing effect against Alzheimer's[8], anti-hypertensive effect[9], diabetes, anti-cancer[10], cardioprotective effect[11], antibacterial effect[12], anti-inflammatory effect[13], antioxidant[14] and anti-ulcer properties.[1,15]

C. asiatica (L.) has been suggested in traditional medicine for treating various skin conditions such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, and fever.[16] It has also been used in traditional medicine to treat neurological diseases such as dementia, Alzheimer's, anxiety, and depression.[17]

H. perforatum, also known as St John's Wort, contains a variety of bioactive substances such as hypericin, chlorogenic, caffeic acids, flavonoids, quercetin, biapigenin, procyanidin, and hyperforin[18] (Figure 2.). Although hypericin and hyperforin, the main components of *H. perforatum*, are the focus of attention due to their antidepressant effects, they also have an essential place in anti-aging studies.[19-22] However, Hypericins are unstable and are easily converted to their different derivatives.[23] It has been shown to strengthen the analgesia caused by morphine with the hypericin it contains.[24] Furthermore, it has been shown in the literature to significantly affect wound healing, acne treatment, and skin disorders such as eczema and psoriasis.[25] Moreover, very positive feedback has been received for the extract of this plant, which has been used in cosmetic products recently. Apart from these, it has been shown that *H. perforatum* extracts can exhibit multiple bioactivities, including anti-inflammatory[26], antioxidant[27], antibacterial[28], antifungal[18], and anti-cancer.[29] It is also effective in digestive problems.[30]

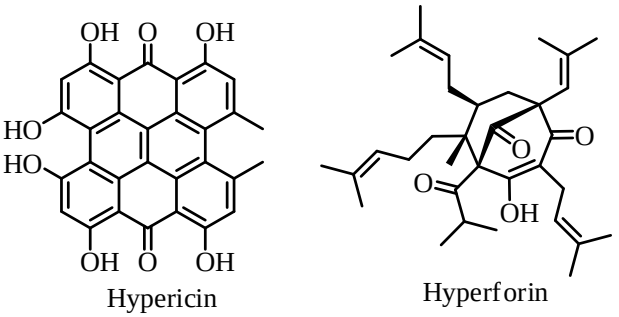

Figure 2. Two important compounds in *Hypericum perforatum*; Hypericin and Hyperforin.

Skin aging is a biological process and is inevitable. Collagen fibers give the skin tensile strength; elastin fibers contribute to its flexibility and durability. The decrease in protein synthesis, which affects Type I and III collagen, is also very effective in this process. Furthermore, skin aging is how these properties change depending on intrinsic and extrinsic aging factors[31]. Skin aging increases due to sun, toxins, air pollution, inflammation, gravity effect, muscle loss, decrease in collagen content, DNA damage, telomere loss, and oxidative stress.[32] However, some applications can delay this process based on inhibiting the function of degrading enzymes such as collagenase, hyaluronidase, and elastase. Specially synthesized molecules and some plant extracts are used for this purpose. Active substances, frequently used in cosmetic products and food supplements, also take their place in medical applications. The most accessible and most practical of anti-aging applications is anti-aging creams.

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MATERIALS AND METHODS

Sample preparation

Centella asiatica and *Hypericum perforatum* plants used in the study were supplied as dry from Aksuvital Natural Products Food Industry and Trade Inc.

Solvents such as hexane (96%), ethanol (99.9%), acetone (99.5%), and methanol (99.8%) were obtained from ISOLAB and used without any processing.

The extraction method of *Centella asiatica* (L.)

1 g of plant was mixed vigorously in 40 ml of methanol at 60 ℃ for 3 hours under reflux in a magnetic stirrer. After the mixing process was completed, filtration was carried out with the help of filter paper without cooling the mixture. After the solvent in the filtrate was removed with the aid of an evaporator, the remaining solid was weighed.[33] The solid part was stored at +4 ℃ for later use.

Extraction method of Hypericum perforatum

200 ml of methanol was added to 5 g of plant and mixed vigorously in a magnetic stirrer under reflux for 6 hours at 60 ℃. After the mixing process was completed, filtration was carried out with the help of filter paper without cooling the mixture. After the solvent in the filtrate was removed with the aid of an evaporator, the remaining solid was weighed.[34] The solid part was stored at +4℃ for later use.

Instruments for HPLC

For HPLC/DAD analysis, the Shimadzu LC 20AT HPLC system is equipped with the SPD-M20A photodiode array detector (DAD). The samples were separated on a C18 250*4.6 mm column.

HPLC method for *Centella asiatica* (L.)

C18 250*4.6 mm column, 1.0 ml/min flow rate, 30℃ column temperature, with 210 nm UV detector, acetonitrile/pure water (contains 0.1% 85% o-phosphoric acid) (v/v= 1/1) HPLC analysis was carried out in the) solvent mixture and for 20 min18.

HPLC Method for *Hypericum perforatum*

C18 250*4.6 mm column, 1.0 ml/min flow rate, 30ºC column temperature, 210 nm UV detector, acetonitrile/pure water (0.1% formic acid contains (v/v=1/1) in the solvent mixture and HPLC analysis was performed for 20 min18.

Cream formulation studies

All excipients and chemicals used in cream production were obtained from Türkiye, Sigma-Aldrich® (Glycerine) and Basf companies (Sodium polyacrylate, Dicaprylyl carbonate, Polyglyceryl-3 caprate Coco caprylate, Sodium Stearoyl Glutamate, Sorbitan Caprylate, Propanediol, Benzoic acid, fragrance agent). *Hypericum perforatum* extract was obtained from Aksu Vital Company.

Anti aging herbal cream

After the extracts and ratios were determined for cream production, cream formulations were created (Table 1 and 2). Cream containing *Hypericum perforatum* and *Centella asiatica* (L.) extract was prepared according to the formulation given in Table 1.

Table 1. Formulation for cream production.

	Ingredient	Amount (%) Experiment I	Experiment II	Experiment III
Phase I	*Hypericum perforatum* Extract	-	0.07-0.13	0.07-0.13
	Centella asiatica Extract	0.07-0.13	-	0.07-0.13
	Glycerine	2.5 –3.5	2.5 –3.5	2.5 –3.5
	Sodium Polyacrylate, Dicaprylyl Carbonate, Polyglyceryl-3 Caprate	0.625 – 2.2	0.625 – 2.2	0.625 – 2.2
	Fragrance Agent	1.25 – 2.5	1.25 – 2.5	1.25 – 2.5
	Water	70-80	70-80	70-80
Phase II	Coco Caprylate	5 – 7.5	5 – 7.5	5 – 7.5
	Sodium Stearoyl Glutamate	0.3 – 0.7	0.3 – 0.7	0.3 – 0.7
	Sorbitan Caprylate, Propanediol, Benzoic Acid	0.5-1.3	0.5-1.3	0.5-1.3

The data in Table 1 were prepared for 400 g of plant extract cream. Experiment I is a formulation made with Centella asiatica (L.) extract. Experiment II is a formulation made with *Hypericum perforatum* extract. Experiment III is a formulation with both plant extracts (Table 1). These three experiments were carried out to compare the anti-aging effect.

For each trial (Experiment I, II, and III), the material of each phase was mixed in different containers. After homogenizing each phase mixture, Phase I and II were combined. The phases were mixed until a homogeneous and dense cream consistency. Three separate trials have been formulated for anti-aging studies. Experiment III was determined as the final formulation as it was demonstrated by anti-aging studies that it was more adequate than the others (Experiments I and II).

In Table 2, the plant species used and the major active

ingredients in it, the amounts in the extract are given. These rates in Table 2 are presented by comparing them with previous studies in the literature.[20,31]

Table 2. The plant species used, the ratio of active ingredient in the extract.

Plant Type	Active Substance	Amount in Extract
Centella asiatica (L.)	Asiatic acid	0.17
	Madecasic acid	0.44
Hypericum perforatum	Hyperforin	2.5

In addition to extracts, different natural ingredients such as thickeners, emulsifiers and preservatives are used in cream formulations. In the formulation, glycerin is an effective ingredient both for its moisturizing properties and as a solvent. The mixture of sodium polyacrylate, Dicapryl carbonate, Polyglyceryl-3 caprate components were used as thickening agents. Coco caprylate is used to support glycerine and as a substrate moisturizer. At the same time, it contributes to the dissolution of the extract containing the active substance. Sodium Stearoyl Glutamate was used as an emulsifier to combine the oil and water phase. Ingredients containing Sorbitan Caprylate, Propanediol, Benzoic acid are also included in the formulation for preservative purposes (Table 1).

***In vitro* skin antiaging cell culture studies test material**

Human skin fibroblast cell line HS68 (ATCC CRL-1635) obtained from ATCC was used in all experiments within the scope of the study. Cell culture study was carried out for Anti Aging Herbal Cream (sample 10g) formulated with 0.5-4% *Hypericum perforatum* and *Centella asiatica* (L.) extract.

Anti-aging cell culture studies on the cream produced with *Hypericum perforatum* extract (Experiment I)

Test material information (TM)

Hypericum perforatum Extract Herbal Cream Content Information: Available in Table 1 (*Hypericum Perforatum* Flower/Leaf Extract Active Ingredient Ratio: (St. John's Wort Extract) 0.5-3%).

Negative Control (NC): Ultrapure water

Test Material Application doses (w/v): 25, 50 and 100 µg/ml.

Anti-aging studies on cream produced with *Centella asiatica* extract (Experiment II)

Test material information (TM)

Centella asiatica (L.) extract *Herbal Cream Ingredients Information:* Available in Table 1(*Centella asiatica* (L.) Flower /Leaf/Stem Extract, Substance Ratio: (*Centella asiatica* (L.) Extract) 0.5-3%).

Negative Control (NC): Ultrapure water

Test Material Application doses (w/v): 25, 50 and 100 µg/ml

Anti aging studies on cream produced with both extracts (Experiment III)

Test material information (TM)

Centella asiatica Extract Herbal Cream Ingredients Information: Available in Table 1 (*Centella asiatica* (L.) Flower /Leaf/Stem Extract, *Hypericum perforatum* Flower/Leaf Extract Active Ingredient Ratio: (*Hypericum perforatum* and *Centella asiatica* (L.) Extract) 0.5-4%).

Negative Control (NC): Ultrapure water

Test Material Application doses (w/v): 25, 50 and 100 µg/ml

After the cream, was exposed to certain conditions, the soluble part was taken and anti-aging studies were continued. The test material was dissolved in DMEM medium containing 0.05% DMSO. It was incubated in an oven at 37 ℃ for 24 hours. At the end of the period, it was passed through a 0.22 µm membrane filter and used at test concentrations.

***In vitro* skin antiaging cell culture conditions**

Cells were grown in DMEM (ATCC Cat No: 30-2006) growth medium supplemented with 10% FBS and 2% glutamine and incubated at 37°C in an oven with 5% CO2. A mixture of 0.25% trypsin and 0.03% EDTA was used for trypsinization of cells as recommended by ATCC. Cells were divided into 6-well plates at 2x105 cells per well. The amounts of Collagen αI (Col I) released from the medium from HS68 cells were determined using the Human Collagen αI ELISA Kit after 48 hours of incubation. Before starting the experiment, each well was washed four times with 300 µL of 1x wash buffer. 50 µL of the test material was taken from the experimental and control groups at the determined doses and added to these wells. It was incubated on a shaker at 200 rpm for two hours at room temperature. Each well was washed four times with 300 µL of 1x wash buffer. Then, 100 µL of Human Col I antibody was added to each well. It was incubated for one hour at room temperature with a shaker. Each well was washed four times with 300 µL of 1x wash buffer. 100 µL of Avidin-HRP A (Avidin Peroxidase A) solution was added to each well and incubated for 30 minutes at room temperature with a shaker. Each well was washed five times with 300 µL of 1x wash buffer. 100 µL of Substrate F (high sensitivity TMB) solution was added to each well and incubated for 10 minutes

at room temperature and in the dark. Afterward, the blue color formation was observed depending on the amount of Col I bound to the wells. The reaction was stopped by adding 100 µL of stop solution to each well, and the color changed from blue to yellow. The absorbance values of the samples were read in an Eliza kit reader (Thermo Fisher, Multi Scan FC Microplate Reader) at 450 nm.

RESULTS

Extraction and analysis studies

Hypericum perforatum and *Centella asiatica* (L.) plant were extracted at different temperatures and in a polar solvent such as methanol. The extractions performed and their results are available in the table below (Table 3).

Based on the yields in the table, HPLC analysis of the solid extracts were made and the active ingredient contents and amounts were tried to be determined.

According to the results of HPLC analysis; asiatic acid and madecasic acid were determined as the active substances we expected in the *Centella asiatica* (L.) plant (Table 4).

If the results in Table 3 are to be interpreted; The amount of solid extract obtained from 0.5 grams of *Centella asiatica* (L.) with a yield of 20.8% was 0.104 grams (Table 3, Entry 3). When matched with the HPLC results in Table 4, it is understood that there are 0.18 mg of asiatic acid and 0.45 mg of made cacid acid in 0.5 grams of *Centella asiatica* (L.) plant.

Table 3. *Hypericum perforatum* and *Centella asiatica* (L.) plant extraction results.

Entry	Solvent	Amount of Solvent	Temperature ℃	TimeH	Weight	*Centella asiatica* (L.) Yield, %	*Hypericum perforatum* Yield, %
1	MeOH	20 mL	25	3	0.5 gr	19.5	18.8
2	MeOH	20 mL	40	3	0.5 gr	19.7	19.6
3	MeOH	20 mL	60	3	0.5 gr	20.8	19.3

Table 4. *Centella asiatica* (L.) HPLC Result (Quantitative).

Analysis name	Conclusion	Unit	Method
Asiatic acid	1740	mg/kg	HPLC-DAD
Madecasic acid	4380	mg/kg	HPLC-DAD

Cream production studies

For cream formulations, tests such as biosafety and physical tests must be performed before they are made available. pH, viscosity, density, protective activity tests and microbial analysis tests were performed for the final formulation (Experiment III) (Table 5-8).

Cell culture studies

Cream formulation studies were carried out successfully. Our products have been tested in-vitro. In-vitro tests conducted for 3 different formulations revealed that the anti-aging effect was strengthened in the product mixture.

In this cosmetic formulation; *Staphylococcus aureus, Pseudomonas aureginosa, Escherichia coli, Candida albicans and Aspergillus brasiliesis* correspond to "Criteria A" defined according to ISO 11930:2012.

In summary, Table 6 indicates that the product is protected against the growth of the bacteria described above, that is, against microbial growth.

Table 5. Microbiological Test Results for Anti-aging Herbal Cream.

Parameter	Unit	Microbiological Analysis Result	Standarts Nu	Limit
Total Aerobic Mesophilic Microorganisms*	kob/g – cfu/g	<10	ISO 21149	<100
Staphylococcus aureus *	/1g- ml	Absence/1g-ml	ISO 22718	Absence/1g-ml
*Pseudomonas aureginosa**	/1g- ml	Absence/1g-ml	ISO 22717	Absence/1g-ml
*Escherichia coli**	/1g- ml	Absence/1g-ml	ISO 21150	Absence/1g-ml
*Candida albicans**	/1g- ml	Absence/1g-ml	ISO 18416	Absence/1g-ml
Total Yeast and Moulds*	kob/g – cfu/g	<10	ISO 16212	<100

(*) Parameters column which are covered by the accreditation. According to the results of microbiological analysis, the sample complies with the provisions of the "Guideline on Microbiological Control of Cosmetic Products" (Table 5).

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Table 6. Antimicrobial Protective Efficacy Test Results.

Microorganism	0.Day	7.Day		14.Day		28.Day	
	*N0 (Log)	*N7 (Log)	Log reduction	*N14 (Log)	Log reduction	*N28 (Log)	Log reduction
Staphylococcus aureus NCTC 10788/Lot 030520029	6.04	1.00	5.04	1.00	5.4	1.00	5.04
Pseudomonas aureginosa ATCC 9027/Lot 3270513	6.77	1.00	5.77	1.00	5.77	1.00	5.77
Escherichia coli ATCC 8739/Lot 4835151	6.77	1.00	5.77	1.00	5.77	1.00	5.77
Candida albicans NCPF 3179/Lot 040920028	4.69	1.00	3.69	1.00	3.69	1.00	3.69
Aspergillus brasilinesis NCPF 2275/Lot 020620065	3.30	Not performed		1.00	2.30	1.00	2.30

(*) Number of microorganisms by day.

Table 7. Stability Test Results (First Day Results)

First Day Results	Color	Smell	Appearance	pH	Intensity	Packaging	Microbiological Analysis	Phase Separation
	Light brown	Characteristic	Cream	5.19	1.080	Suitable	Suitable	Not observed

Table 8. Stability Test Results (Following Days)*

Parameters	**1 Week**		**1 Month**		**2 Month**		**3 Month**	
temperature	**25°C**	**45°C**	**25°C**	**45°C**	**25°C**	**45°C**	**25°C**	**45°C**
Color	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown
Odor	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics	Characteristics
Appearance	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
Ph	5.23	5.18	5.28	5.11	5.19	5.02	5.35	5.03
Density	1.082	1.085	1.084	1.082	1.081	1.081	1.085	1.083
Package	Ok	Ok	Ok	Ok	Ok	Ok	Ok	Ok
Microbiological Analysis	Ok	Ok	Ok	Ok	Ok	Ok	Ok	Ok
Phase Separation	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed	Not Observed

(*) Chemical Result: Density changes at a level that can be ignored. Microbiological Result: The possibility of microbiological growth was not observed. Physical Result: About color change was observed. About odor change was not observed. Color change was observed in the sample from the 1 st month 45°C.

DISCUSSION

The yields obtained in Table 3 are not the active substance yields. The yields here are the methanol solvent and the plant extraction yields obtained at different temperatures. In other words, it is the mixing efficiency with different components as well as active substance. Determination of the amount of active substance was obtained by HPLC analysis and is given in Table 4.

HPLC spectra corresponding to the quantitative results of asiatic acid and madecasic acid in Table 4 are shown in Figure 3. When the plant extracts were compared with the asiatic acid and madecasic acid standards, madecasic acid was found in 6.29 minutes and asiatic acid was obtained in 9.54 minutes.

However, the expected Hypericin could not be detected in *Hypericum perforatum* extract obtained by the methods mentioned in the literature. HPLC data are shown in Figure 4. *Hypericum perforatum* extract is likely to contain some glycoside derivatives other than Hypericin. In this case, *Hypericum perforatum* extract was obtained directly from Aksu Vital. The amount of active substance in the provided extract, hyperforin, HPLC results are clear. There is 2.5% hyperforin in the extract. The product report is provided and attached.

In order to confirm this result, analysis was performed using the HPLC technique in the literature.[18] However, Hypericin could not be detected both in the extract obtained by experimental methods and in the commercially available extract.

Accordingly, there has been a trend towards commercially available extract containing hyperforin active ingredient. Hypericin and hyperforin are two major active ingredients for *Hypericum perforatum* Hyperforin acts as an antimicrobial and anti-inflammatory agent.

The hyperforin molecule is a biologically active molecule and it is supported by the literature that it is an an active component in cream formulations and even in anti-aging studies.[20,33]

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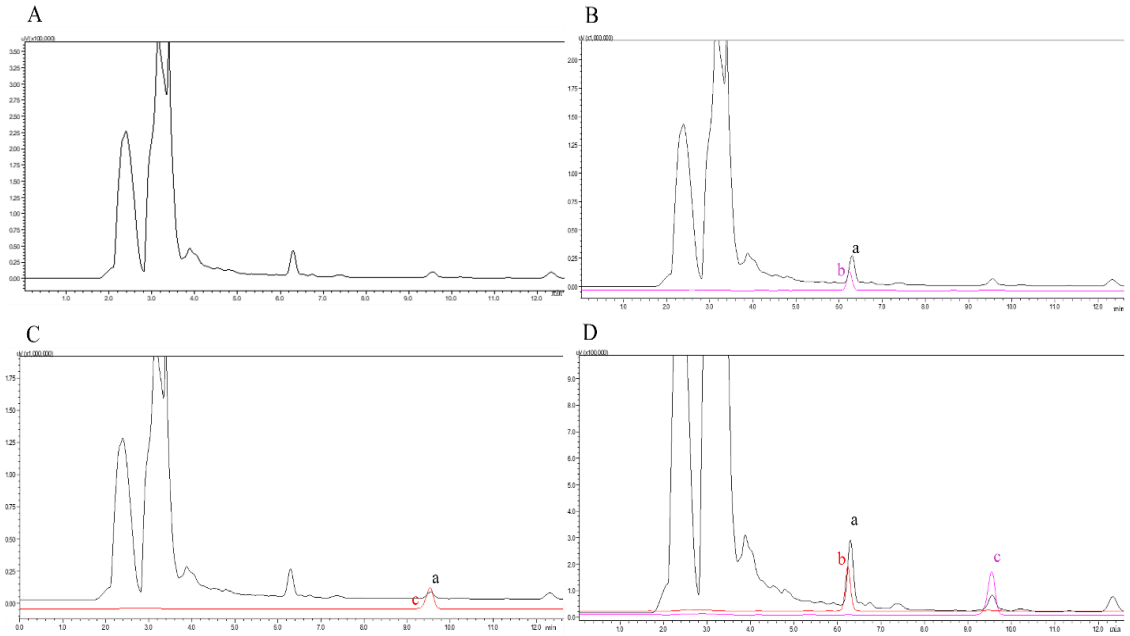


Figure 3. A) *Centella asiatica* (L.) extract Data Spectrum. B) *Centella asiatica* (L.) extract (a-Black) and Madecassic acid Spectrum (b-Pink). C) *Centella asiatica* (L.) extract (a-Black) and asiatic acid Spectrum (c-Red). D) *Centella asiatica* (L.) Extract (a-Black), asiatic acid Spectrum (c-Pink), and madecassic acid Spectrum (b-Red).

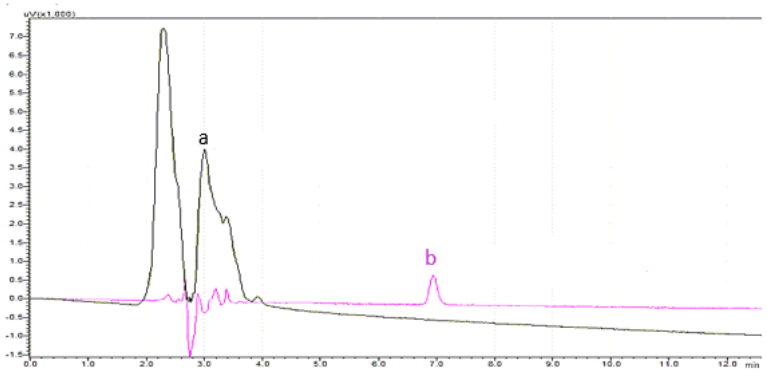


Figure 4. *Hypericum perforatum* extract a-Black data sample and b-pink data hypericin standard.

Extracts were prepared for cream production. For cream production, cream formulations were created after the ratios of *Centella asiatica* (L.) prepared with methanol and commercially available *Hypericum perforatum* extract were determined. In the formulation, besides the extracts, different natural ingredients such as thickeners, emulsifiers and preservatives were used. The contents other than the extract were prepared with the experimental experience and literature support of our group in previous studies, and the extracts were formulated considering the toxicology studies in the literature. The concentrations used in toxicology studies in the literature on *Hypericum perforatum* plant extract were evaluated as the upper limit and formula studies were carried out at values below this limit. In addition, in anti-aging studies, it has been proven that the most effective content is obtained by extraction with methanol.[20,31] Similar literature studies were also carried out for the *Centella asiatica* (L.) plant and the formulation was adapted at values below the working concentrations.[34,35]

Three different cream formulations were prepared for In vitro cell culture study to reveal the anti-aging effect. Experiment I is a formulation made with *Centella asiatica* (L.) extract. Experiment II is a formulation made with *Hypericum perforatum* extract. Experiment III is a formulation created with both plant extracts (Table 1). First of all, after Experiment III was created, after positive results were obtained from the tests made, Experiments I and II were prepared (Table 5-8). As expected, Experiment III with both extracts proved to be a more effective formulation than the others (Experiments I and II).

The most important problem while preparing the

formulations was the homogeneous distribution of the extracts. For this purpose, the extract and/or extract mixtures were primarily dissolved in a mixture of glycerin, coco caprylate, sorbitan caprylate, propanediol, benzoic acid, and a cream formulation was created after a homogeneous mixture was obtained. We have also confirmed with the tests performed that there is no solubility problem as expected.

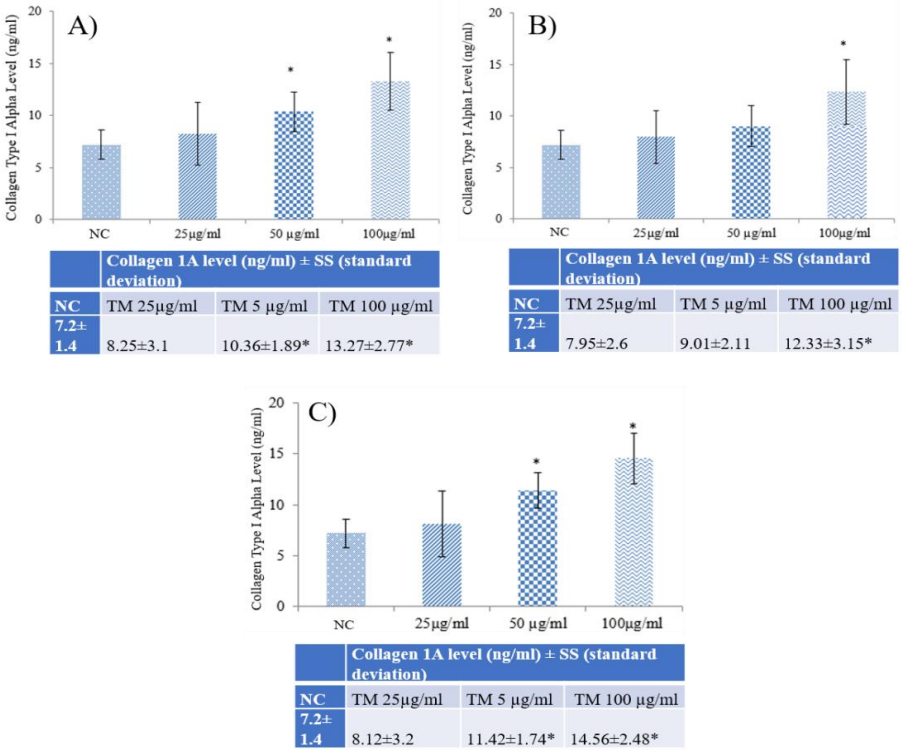
Stability test results are observed in Tables 7 and 8. Microbiological tests must be performed before stability tests. Table 7 presents the analysis of the cream on the first day it reaches the laboratory. In the tests, it has been determined that the cream color is brown, has a characteristic odor, has a pH of 5.19 (this pH is within the desired range), the density is 1.080, the packaging and microbiological analysis are suitable, and most importantly, no phase separation is observed.

In Table 8, when the same measurements were made by exposing to different temperatures (25 °C, 45 °C) in 1 week, 1 month, 2 months and 3 months periods, different results from the first day were not encountered. These results reveal that the product is stable.

The most abundant protein found in all vertebrates is Type I collagen. Collagen, synthesized mainly by fibroblasts, myofibroblasts, osteoblasts and chondrocytes, is a simple and fibrillar scleroprotein found in significant amounts in tendons, cartilage, organic matrix of bones, and cornea of the eye. Anti-aging creams can be used against decreases in the amount of collagen I due to aging. Thus, anti-aging creams can increase the amount of collagen I. Therefore, the amount of collagen I was measured in the test.[36,37]

First of all, anti-aging studies have been carried out on the cream produced only with *Hypericum perforatum* extract. Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney tests. According to these tests, differences with a p value of 0.05 and small were considered statistically significant (*p<0.05).

According to the test results, it was determined that the collagen type I alpha level of *Hypericum perforatum* Extract Herbal Cream at 50 and 100 µg/ml concentrations was statistically increased compared to the control group. According to these results, *Hypericum perforatum* extract Herbal Cream is an effective anti-aging product (Figure 5A).



	Collagen 1A level (ng/ml) ± SS (standard deviation)		
NC	TM 25µg/ml	TM 5 µg/ml	TM 100 µg/ml
7.2± 1.4	8.25±3.1	10.36±1.89*	13.27±2.77*

	Collagen 1A level (ng/ml) ± SS (standard deviation)		
NC	TM 25µg/ml	TM 5 µg/ml	TM 100 µg/ml
7.2± 1.4	7.95±2.6	9.01±2.11	12.33±3.15*

	Collagen 1A level (ng/ml) ± SS (standard deviation)		
NC	TM 25µg/ml	TM 5 µg/ml	TM 100 µg/ml
7.2± 1.4	8.12±3.2	11.42±1.74*	14.56±2.48*

Figure 5. A) Demonstration of dose-dependent changes in the amount of Collagen Type I Alpha from the cream produced only with *Hypericum perforatum* extract in skin fibroblast cells at the end of the 48th hour; B) Dose-dependent change of Collagen Type I Alpha amounts in skin fibroblast cells at the end of 48th hour in cream produced only with *Centella asiatica* (L.) extract; C) The dose-dependent change of Collagen Type I Alpha amounts for the cream prepared by both extractions at the end of the 48th hour in skin fibroblast cells.

Secondly, anti-aging studies were conducted on the cream produced only with *Centella asiatica* (L.) extract. Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney tests. According to these tests, differences with a p value of 0.05 and small were considered statistically significant (*p<0.05).

It was determined that there was a statistical increase in collagen type I alpha level at 100 µg/ml concentration compared to the control group. According to these results, *Centella asiatica* (L.) extract Herbal Cream is an effective anti-aging product (Figure 5B).

Finally, anti-aging studies were carried out on the cream produced with both extracts (mixture of *Hypericum perforatum* extract and *Centella asiatica* (L.) extract). Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between groups were made with the Kruskal-Wallis and Mann-Whitney tests. According to these tests, differences with a p value of 0.05 and small were considered statistically significant (*p<0.05).

As a result of the test, it was determined that there was a statistical increase in collagen Type I alpha levels at 50 and 100 µg/ml concentrations of anti-aging Herbal Cream compared to the control group. According to these results, Anti-Aging Herbal Cream is an effective anti-aging product (Figure 5C)

When the results in Figure 5 are compared within themselves and with each other, the results when we do not have a cream product are weaker than the results after applying the cream product. In addition, Trial III with both extracts shows better results. Considering the cell line used (ATCC CRL-1635), these are the results that prove the absorption of the active ingredients in the extract from the skin.

CONCLUSION

The aim of the study is to bring together *Hypericum perforatum and Centella asiatica* (L.) plant extracts, which contribute partially to anti-aging studies independently, in the production of a new cosmetic product and with a new formulation. The mixture created is a first both scientifically and commercially. *Hypericum perforatum* and *Centella asiatica* (L.) plant were extracted at different temperatures and with methanol. Active ingredient contents and amounts were determined by HPLC analysis of solid extracts. Asiatic acid and madecasic acid were compared with standards in HPLC spectra. Asiatic acid 9.54 and madecasic acid 6.29. detected in minutes (Figure 3). In addition, the active ingredients of Asiatic acid (0.17%) and Madecasic acid (0.44%) were determined in the *Centella asiatica* (L.) plant (Table 2).

Hypericin could not be detected in the *Hypericum perforatum* (L.) extract in HPLC (Figure.6). For this reason, the processes were continued with *Hypericum perforatum extract* obtained from Aksu Vital company with a hyperforin ratio of 2.5%. The active ingredient ratios in the cream formulation were applied according to the literature. An cream formulation was created with *Hypericum perforatum* and *Centella asiatica* (L.) extracts (Table 1). The ultimate formulation was determined as a result of pH, viscosity, density, protective activity test, and microbial analysis tests.

We wondered if the combination of *Hypericum perforatum* and *Centella asiatica* (L.) showed comparable effects to Hypericum alone or *Centella asiatic* (L.) alone. Cell culture studies were carried out to show the anti-aging effect of the cream formulation. Our data showed that *Hypericum* and *Centella asiatica* (L.) had greater anti-aging effects when combined. This shows us that the combination of the anti-aging effect with *Hypericum perforatum* and *Centella asiatica* (L.) for the cream formulation is possible with lower doses than when they are used individually. It can be concluded that the anti-aging effect is greater than expected based on single factor studies. In in-vitro anti-aging tests, it was revealed that the level of Collagen 1A was higher in the cream formulation where both extracts were used together.

Author contributions: Conceptualization: [HG]; Design: [HG, EA]; Writing: [HG, EA]; Investigation/Data collection: [HG, EA, BD].

Conflict of interest: There is no potential conflict of interest relevant to this article.

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CASE REPORT

Combination Therapy of Major Autohemotherapy and Ozonated Bagging in a Case Diagnosed with Buerger's Disease (Thromboangiitis Obliterans)

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Abstract
It is well known that ozone therapy provides positive results as a complementary method in peripheral obstructive arterial diseases alongside medical treatment. This article presents a case diagnosed with Buerger's disease, where rapid improvement was observed after a combination of major autohemotherapy (AHT) and ozonated bagging.
Keywords: Buerger's Disease, Thromboangiitis Obliterans, Ozone Therapy, Ozonated Bagging

INTRODUCTION

Buerger's disease (thromboangiitis obliterans) is a chronic and occlusive vasculitis that affects small to medium-sized vessels, mainly in the extremities, causing partial inflammatory involvement without atherosclerosis. The most significant factor contributing to its development is tobacco use.[1]

Atherosclerosis, or even a small occlusion in the lower extremity arteries due to conditions like diabetes mellitus or Buerger's disease, significantly reduces blood flow to peripheral tissues. Ischemia and minor traumas in the tissue create challenging ulcerations since the necessary oxygen, growth factors, and nutrients for repair are lacking, making major autohemotherapy an appropriate treatment option.[2]

Ozonated bagging is an effective treatment method for managing infections, ulcerations, and local skin lesions in the extremities. The procedure involves covering the treated area with a bag, evacuating the air inside, delivering ozone gas for a specific duration, and then removing the ozone gas through vacuuming3. Properly combining major AHT and ozonated bagging has been shown to prevent a significant portion of foot amputations caused by peripheral obstructions, especially in cases of diabetic foot.[2]

This article presents a case diagnosed with Buerger's disease who underwent a combination therapy of major AHT and ozonated bagging.

CASE

The patient was a 46-year-old male with no known systemic diseases, smoking approximately[2] packs of cigarettes per day for 30 years and having a history of social alcohol use. In 2008, he sought medical attention for an ingrown toenail in his left big toe at an orthopedic clinic. After examination and tests, he was diagnosed with Buerger's disease, and a plastic surgeon partially amputated the left big toe. Despite continued smoking, the patient developed ischemic changes in his right toe starting in 2018, leading to the amputation of the right foot's first, second, and third toes by a cardiovascular surgeon at another center. Due to wound infection, the patient was referred to our infectious diseases service and, upon discharge, directed to our center for Major AHT and ozonated bagging treatments (Figure 1). The patient was provided with smoking cessation counseling and advised on lifestyle changes. However, he currently reports smoking 3-4 cigarettes per day. Nevertheless, three weeks after starting sessions of ozone therapy, along with Major AHT, the infected-looking wound on the right foot appeared cleaner and healthier, with reduced discharge and necrotic appearance (Figure 2).

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Figure 1. Appearance at the beginning of ozone therapy

Figure 2. Appearance after three weeks of 5 sessions of ozone eans Major AHT and ozone bagging treatment

DISCUSSION and CONCLUSION

This article presents a case of Buerger's Disease with a history of toe amputations and an active necrotic foot ulcer that showed visible improvement with the combination therapy of Major AHT and ozonated bagging. Existing literature indicates positive results when ozone therapy is used as a complementary method alongside medical treatment for peripheral obstructive arterial diseases.[2,4] However, most studies of studies in the literature focus on patients with diabetic foot ulcers, and there is a lack of sufficient research regarding Buerger's disease. Therefore, further extensive studies in this context are necessary.

Author contributions: Conceptualization: [SÜ, AK, AAHA]; Design: [AK, SÜ, AAHA]; Writing: [AK, SÜ, AAHA]; Investigation/Data collection: [SÜ, AK, AAHA]
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REVIEW

The Medicinal Plants Traditionally Used Against Cardiovascular Diseases in Türkiye

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Abstract
Since immemorial, humankind has benefited from plants as nutritient, medicine, cosmetic, or to make tools and shelters for itself. Historically, the trial-and-error method gathered traditional knowledge; practices were developed and sustained. It has been passed on from generation to generation to reach our day. This study aims to gather information on medicinal plants used traditionally against cardiovascular diseases in Türkiye before it is lost forever. In the library of Istanbul University, in the first place, the approachable sources have been scanned thoroughly. Plants' scientific name, Turkish name, family, used parts, method of use, and effects has been put together in a table. As a result, we composed a report of 129 plants from 43 families used for cardiovascular diseases. It has been concluded that of these, the most outstanding herbs are *Crataegus monogyna* Jacq., *Lavandula stoechas* L., *Melissa officinalis* L., *Tribulus terrestris* L., *Urtica dioica* L. and *Viscum album* L. Rosaceae, Lamiaceae and Asteraceae were the most used plant families. It has been detected that people mainly benefit from the leaves, aerial parts, fruit, and flowers of medicinal plants as infusion or decoction. Although the biological activity studies on the plants most used by the people support the use in cardiovascular diseases, more studies are needed to provide scientific evidence for folklore use, especially for *L. stoechas*. It is thought that this study may be a resource for developing future herbal medicines or treatments.
Keywords: Cardiovascular System, Ethnobotany, Medicinal Plant, Traditional Medicine, Türkiye

INTRODUCTION

The cardiovascular system consists of a closed system of organs and blood vessels that transports blood, oxygen, and nutrients throughout the body. It comprises the heart, blood vessels (arteries, veins, and capillaries), and the blood itself. The heart circulates blood through the blood vessels, which transport oxygen and nutrients to the tissues and organs of the body. Moreover, the blood eliminates waste materials and carbon dioxide from the tissues and organs, returning them to the heart and lungs for oxygenation.[1] The circulatory system affects almost every cell, tissue, organ, and system in the body. The term covers coronary heart diseases, cerebrovascular diseases, hypertension, peripheral arterial disease, rheumatic heart disease, congenital heart diseases, heart failure, and cardiomyopathies. Tobacco smoking, physical inactivity, an unhealthy diet, obesity, or diseases such as diabetes, hypertension, or dyslipidemia are significant factors that influence susceptibility to many diseases.[2]

By the turn of the 21st century, chronic diseases continued to plague the global population, while infectious diseases became less of a concern.[3] World Health Statistics indicate that an estimated 41 million deaths occurred due to noncommunicable diseases (NCDs), accounting for 71% of the total 57 million deaths in 2016. Most of these deaths were caused by the four main NCDs: cardiovascular disease, cancer, chronic respiratory disease, and diabetes. With 17.9 million records, deaths due to cardiovascular diseases account for 44% of all NCD deaths. If present trends persist, this number will increase to almost 23,6 million by 2030, and the studies indicate that the situation is especially severe in middle-income countries.[4] As a developing country in the Eastern Mediterranean, Türkiye, chronic diseases are

increasing due to the aging population and changing lifestyle.[5] According to the statistics from the Turkish Statistical Institute, cardiovascular diseases, which are the first among six diseases causing death, have been the leading cause of all deaths in Türkiye 36.8% in 2019, 37.8% in 2018, and 39.5% in 2017.[5,6]

Traditional medicine has been an essential part of healthcare in Türkiye for centuries, and numerous individuals continue to use traditional remedies to treat various health conditions, including cardiovascular diseases.[7,8] In a study conducted in Türkiye, researchers discovered that 51.3% of 193 hypertensive patients utilized herbal remedies, predominantly when feeling ailing.[9] Despite the need for additional research to evaluate these remedies' safety and efficacy, traditional medicine plays an essential role in Türkiye's healthcare system. However, urbanization and changes in lifestyle have pushed people away from nature, and traditional medical knowledge is vanishing. Therefore, transmitting this value to the scientific literature before its extinction is essential. Traditional medicine knowledge is valuable and merits scientific study before it is lost forever. As known, nearly three-quarters of the new medications that have been approved since 1981 were derived directly or indirectly from natural sources, and especially plants used in traditional medicine are potential sources for the modern pharmaceutical industry.[10,11]

MATERIALS AND METHODS

Study area

This study represents a compilation of ethnobotanical studies since 1991 conducted within Türkiye's borders.

Data collection

This study was prepared by searching theses at the National Higher Education Center and ethnobotanical surveys and related studies conducted in various parts of Türkiye with the goal of selecting regional plants used for cardiovascular diseases. Published research, books, doctorate dissertations, and master's theses were searched using scientific literature databases (Pubmed, Scopus, Google Scholar, Web of Science, SciFinder, Springer, and Elsevier). The International Plant Names Index (https://www.ipni.org/) was used to verify the scientific names of all taxa and families identified. Since synonym names were given in some published research, these alternative scientific names were also given in brackets. Related information about medicinal plants, such as botanical, family, and vernacular names; used parts; preparation methods; and administration, is presented in Table 1. Moreover, the most cited plant families, the most cited medicinal plants, and the most used plant parts are presented in graphics. Finally, pharmacological studies were reviewed to confirm the traditional use of the most cited plant taxa.

Table 1. List of the medicinal plants traditionally used against cardiovascular diseases in Türkiye.

Plant species	Vernacular name (in Turkish)	Family	Plant part(s) used	Preparation	Use
***Achillea crithmifolia* Waldst. et Kit.**	Civanperçemi, Mayasıl otu	Asteraceae	Aerial part	Infusion/ Internal	Heart diseases[20]
***Achillea millefolium* L.**	Ayvadene, Kedi Tırnağı	Asteraceae	Whole plant	Infusion/ Internal	Heart diseases[21]
			Leaf, Flower	Crushed, Decoction/ Internal	Cardiovascular diseases[22]
***Achillea nobilis* L.**	Ayvadana, Aslanbıyığı, Civanperçemi	Asteraceae	Leaf	Kept in olive oil/ Internal	Heart diseases[23]
			Aerial part	Infusion, Decoction/ Internal (A teacup)	Cardiotonic[24]
***Achillea santolinoides* subsp. *wilhelmsii* (K.Koch) Greuter (Syn*: Achillea wilhelmsii* C. Kocu)**	Kedicırnağı, Keditırnağı, Tilki otu	Asteraceae	Aerial part	Infusion/ Internal	Atherosclerosis[25]
***Adiantum capillus-veneris* L.**	Pore fatme, Şaar, Cıbbar	Pteridaceae	Aerial part	Infusion/ Internal	Heart diseases[26]
***Aegopodium podagraria* L.**	Baldıran, Mendek, Mide otu	Apiaceae	Leaf, Petiole	Cooked, Decoction, Pickled/ Internal	Cardiovascular diseases[22]
***Alchemilla caucasica* Buser**	Dokuztepe, Aslan pençesi	Rosaceae	Flower, Leaf, Whole plant	Decoction, Infusion, Raw/ Internal	Cardiovascular diseases[22]
***Allium cepa* L.**	Soğan	Amaryllidaceae	Bulb	Maceration/ Internal	Blood-thinner, Good for heart[27]
***Allium sativum* L.**	Sarımsak	Amaryllidaceae	Bulb	Crushed/ Internal	Heart diseases[28]
***Odontarrhena pateri* (Nyár.) Španiel, Al-Shehbaz, D.A.German & Marhold (Syn: *Alyssum pateri* Nyár.)**	Keselmehmut	Brassicaceae	Aerial part	Decoction/ Internal	Heart diseases[29]

***Prunus dulcis* (Mill.) D.A.Webb (Syn*: Amygdalus communis* L.)**	Tatlı badem	Rosaceae	Seed	Raw/ Internal	Heart diseases[30,31]
***Anthemis cretica* L.**	Papatya	Asteraceae	Flower	Infusion/ Internal	Vasodilator[32]
***Asparagus acutifolius* L.**	Tilki kuyruğu, Kedikuyruğu, Kediyan, Mıcık, Sarmaşık, Kuşkonmaz, Melcü	Asparagaceae	Aerial part	Roasted, Cooked/ Internal	Heart diseases[23]
				Boiled/ Internal	Heart diseases[33]
				Decoction/ Internal	Heart diseases[34]
***Asparagus officinalis* L. (Syn: *Asparagus longifolius* Fisch. ex Steud)**	Gırguni	Asparagaceae	Root	Infusion/ Internal (A cup in the morning on the empty stomach)	Heart diseases[35]
***Astragalus gummifer* Labill.**	Günizer, Kitre, Türk Kitresi	Fabaceae	Root	Decoction (After peeling)/ Internal	Heart diseases[35]
***Bellis perennis* L.**	Mayıs papatyası	Asteraceae	Flower	Decoction, Infusion/ Internal	Cardiovascular diseases[22]
***Berberis crataegina* DC.**	Karamuk, Kızamuk, Karamık	Berberidaceae	Leaf	Raw/ Internal	Good for heart[36]
				Marmalade/ Internal	
***Sinapis nigra* L. (Syn: *Brassica nigra* L.)**	Ardal, rapisa, radika	Brassicaceae	Seed	Wrapped in a cloth and put into grape juice, wait 1-2 months, a glass per day/ Internal	Heart diseases, Vascular diseases[37]
***Capsella bursa-pastoris* (L.) Medik.**	Çoban Çantası	Brassicaceae	(Flowering) Branch	Decoction/ Internal	Arteriosclerosis[38]
Centaurea calcitrapa L.	Twelu	Asteraceae	Aerial part	Decoction/ Internal (A teaglass, two times a day)	Heart diseases, Embolism[33]
***Prunus avium* (L.) L. (Syn: *Cerasus avium* (L.) Moench)**	Kiraz, Napolyon Kirazı	Rosaceae	Fruit stalk	Decoction (Dried form)/ Internal	Heart diseases[23]
***Ceratonia siliqua* L.**	Keçiboynuzu	Fabaceae	Broad bean	Infusion/ Internal	Atherosclerosis[39]
***Cichorium intybus* L.**	Mavi çiçek, Çıtımık, Hindiba	Asteraceae	Aerial part	Decoction/ Internal	Heart diseases[40]
***Cichorium pumilum* Jacq.**	Sütlü Ot, İndiba Otu	Asteraceae	Flowering branch	Infusion/ Internal (3 times a day, before meals)	Cardiotonic[41]
***Cornus mas* L.**	Kızılcık	Cornaceae	-	-	Heart diseases[43]
***Cota tinctoria* (L.) J. Gay**	Giyayezer	Asteraceae	Aerial part	Infusion/ Internal	Heart diseases[26]
***Crataegus ambigua* C.A. Mey. ex A.K. Becker**	Gunc	Rosaceae	Fruit	Infusion/ Internal (A cup on empty stomach in the morning)	Heart disorder, Embolism[33]
***Crataegus azarolus* var. *aronia* L. (Syn: *Crataegus aronia* (L.) Bosc. ex DC.)**	Alıç, Ardıç, Andırın, Sarı Alıç, Ahlat	Rosaceae	(Young) Leaf, (Flowering) Shoot, Flower	Infusion/ Internal	Heart diseases[25,40,44,45]
			Fruit	Decoction/ Internal	
				Raw/ Internal	
***Crataegus azarolus* L.**	Alıç, Kızılalıç, Sinz, Sez, Risok, Roğık	Rosaceae	Leaf, Fruit, Flower	Infusion, Decoction/ Internal (2-3 times a day for 3-5 days)	Cardiovascular diseases, Heart diseases[46,47]
				Fruit/ Internal (Handful for 3-8 days)	
			Flower, Fruit	Infusion, Decoction/ Internal (A teaglass two times a day or a cup in the evening)	Heart diseases[33]
			Fruit	Raw/ Internal	Heart diseases[28]
***Crataegus x bornmuelleri* Zabel ex K. I. Chr. & Ziel**	Alıç	Rosaceae	Fruit, Flower, Leaf	Decoction/ Internal	Heart diseases[48]
***Crataegus meyeri* Pojark.**	Alıç	Rosaceae	Leaf, Trunk bark	Decoction/ Internal	Cardiovascular diseases[25]
			Fruit, Wood	-	Vasodilator[49]
***Crataegus monogyna* Jacq.**	Alıç, Yemişen, Kızılcık, Kızılalıç, Kırmızı Alıç, Adi Alıç, Dikenli Alıç, Yemiş, Beyaz Diken, Ekşi Muşmula, Edram, Geviş, Yemişgen, Yemişgen çalısı, Arıç, Cherven glog,	Rosaceae	Flower	Infusion (dried)/ Internal (2-3 teacups per day)	Heart diseases[36,42,44,50]
			Fruit, Flower	Decoction/ Internal (1-2 teacups per day)	Vasodilator, Circulation accelerator[51]
					Cardiovascular diseases[29,42,56,57,58]
			Fruit	Raw (dried)/ Internal (Regularly)	Heart diseases[23]
			(Young) Leaf, (Flowering) Shoot, Flower	Infusion/ Internal	Cardiovascular diseases[25]

	Glog, Kara Alıç, Enişen, Geyikdikeni, Gırgat, Keçialıcı, Sürsülük, Öküzgöbeği, Kocakarıyemişi		Fruit	Raw/ Internal	Vasodilator[32,52]
			Leaf, Flower	Infusion/ Internal	Heart diseases[53,54,55]
			Stem	–	Cardiovascular diseases[59]
			Aerial part	Infusion/ Internal	Cardiovascular diseases[60]
***Crataegus orientalis* Pall. ex M. Bieb.**	Alıç, Dikenli Alıç, Alıç, Alış, Yemiş, Alıç Toburcuğu, Alış Topurcuğu, Sevçik, Sarı Alıç, Beyaz Alıç, Sinz, Sez, Risok, Roğık	Rosaceae	Fruit, Leaf	Decoction (dried)/ Internal	Vasodilator, Heart diseases[23]
			Root	Decoction/ Internal	Heart diseases[25]
			Flower	Infusion/ Internal	Cardiovascular diseases, Heart diseases[25,61]
				Infusion/ Internal (A cup on empty stomach in the morning)	Heart diseases[33]
			Flower, Stem bark	Infusion/ Internal (A cup on empty stomach in the morning)	Heart diseases[33]
			Flower, Shoot	Decoction/ Internal	Cardiovascular diseases[36]
			Fruit	Decoction, Raw/ Internal	Heart diseases[62]
			Fruit	Raw/ Internal	Cardiotonic, Heart diseases[28]
***Crataegus pentagyna* Waldst. & Kit. ex Willd.**	Yemişen	Rosaceae	Fruit	Raw/ Internal	Heart diseases[63]
***Crataegus rhipidophylla* Gand.**	Alıç	Rosaceae	Fruit	Infusion, Raw, Jam/ Internal	Cardiovascular diseases[22]
***Crataegus azarolus* var. *pontica* (Koch) K.I.Chr. (Syn: *Crataegus pontica* K.Koch)**	Bilan	Rosaceae	Fruit	Decoction/ Internal	Heart diseases[64]
***Crataegus monogyna* Jacq. (Syn: *Crataegus stevenii* Pojark.)**	Alıç	Rosaceae	Fruit, Flower	Decoction/ Internal (1-2 teacups per day)	Vasodilator, Circulation accelerator[61]
***Crataegus orientalis* subsp. *szovitsii* (Pojark.) K.I.Chr. (Syn: *Crataegus szovitsii* Pojark.)**	Alıç	Rosaceae	(Young) Leaf, (Flowering) Shoot, Flower	Infusion/ Internal	Heart diseases[25]
			Fruit	Raw/ Internal	Heart diseases[25]
			Leaf, Trunk bark	Decoction/ Internal	Heart diseases[25,55]
			Branch, Fruit, Leaf	Infusion/ Internal	Heart diseases[55]
			Flower, Leaf	Infusion/ Internal	Cardiovascular diseases[65]
***Crataegus tanacetifolia* (Lam.) Pers.**	Alıç	Rosaceae	Flower	Infusion/ Internal (A teacup two times a day)	Atherosclerosis[66]
***Cuscuta babylonica* Aucher ex Choisy**	Iqşut	Convolvulaceae	Aerial part	Decoction/ Internal	Heart diseases[26]
***Cynodon dactylon* (L.) Pers.**	Ayrık, Ayrık Otu	Poaceae	Aerial part	Infusion/ Internal	Atherosclerosis[25]
			Root	Infusion, Decoction/ Internal	Arteriosclerosis[67]
***Cydonia oblonga* Miller**	Ayva	Rosaceae	Seed	Decoction/ Internal	Heart diseases[68]
			Fruit	Raw/ Internal	Cardiovascular diseases[65]
***Cyperus rotundus* L.**	Şembelik	Cyperaceae	Tuber	Raw/ Internal	Heart diseases[47]
***Ephedra major* Host.**	Deniz üzümü	Ephedraceae	Aerial part (without flowers)	Tea/ Internal (A cup three times a day for 1-3 weeks)	Cardiotonic[24]
***Eriolobus trilobatus* (Labill. ex Poir.) Roem.**	At Elması	Rosaceae	Fruit	Decoction/ Internal	Cardiovascular diseases[40]
***Erodium cicutarium* (L.) L'Hér.**	İğnelik Otu, Filket Otu, Siğil Otu, Dönbaba, İğnelik	Geraniaceae	Stem	Infusion/ Internal (warm)	Heart diseases[70]
***Equisetum arvense* L.**	Kırkkilit, Gırkkilit, Kırkkilit Otu, Gırkkilit Otu	Equisetaceae	Leaf	Infusion/ Internal	Heart palpitations[23]
***Equisetum ramosissimum* Desf.**	Kirkkilit otu, Ulu	Eqisetaceae	Aerial part	Infusion/ Internal (A glass a day regularly)	Atherosclerosis[66]

***Fumaria officinalis* L.**	Şahtere	Papaveraceae	(Flowering) Branch	Tea/ Internal (warm)	Arteriosclerosis[70]
***Glycyrrhiza glabra* L.**	Mekik Kökü, Meyan, Piyan, Payam, Meyan Kökü, Bıyan	Fabaceae	Root	Decoction/ Internal	Heart diseases[29]
***Hedera helix* L.**	Hebil, Sarmaşık, Yabani Tevek	Araliaceae	Leaf	Infusion/ Internal	Heart diseases[40]
***Hypericum perforatum* L.**	Binbirdelikotu, Delikli Kılıçotu, Sarı Kantaron Otu, Jalt Kantarion	Hypericaceae	Flower	Infusion/ Internal	Cardiovascular diseases[25,55]
			Aerial part	Decoction (dried)/ Internal	Cardiovascular diseases[42]
***Hypericum triquetrifolium* Turra**	Kızılcık, Kızılcık Otu, Kantoron Otu, Sarı Kantoron, Aran, Ğırsile	Hypericaceae	(Flowering) Aerial part	Infusion/ Internal	Heart diseases[23]
			Aerial part	Decoction/ Internal	Arteriosclerosis[72]
				Infusion/ Internal	Cardialgia[72]
***Juglans regia* L.**	Adi Ceviz	Juglandaceae	Fruit	Raw (dried)/ Internal (regularly)	Cardiovascular diseases[23]
				Raw (dried)/ Internal (on empty stomach)	Heart palpitations[73]
			(Immature) Fruit	Raw/ Internal	Heart diseases[74]
			Seed	Raw/ Internal (3 pieces per day)	Blood-pressure regulator[41]
				Maceration/ Internal	Cardiovascular diseases[65]
			Seed	Raw/ Internal (Five seeds a day)	Heart diseases[69]
***Juniperus communis* L.**	Andız, Adi Ardıç	Cupressaceae	Root	Decoction/ Internal	Heart diseases[75]
***Juniperus drupacea* Labill.**	Andız	Cupressaceae	Fruit	Infusion/ Internal	Heart diseases[76]
***Juniperus excelsa* M. Bieb.**	Andı Adi Ardıç	Cupressaceae	Fruit	Pulverized, added honey/ Internal (A spoonful per 10 hours)	Cardiovascular diseases[52]
***Juniperus oxycedrus* L.**	Mal ardıcı, Akardıç, Ardıç, Kızıl ardıç, Kızılcık	Cupressaceae	Cone	Crushed/ Internal (With honey)	Heart failure[77]
				Crushed/ Internal	Heart diseases[78]
			(Fresh) Fruit	Decoction, Raw/ Internal	Heart diseases[37]
***Lactuca serriola* L.**	Eşek marulu	Asteraceae	Leaf	Infusion/ Internal	Cardiovascular diseases[65]
***Lactuca virosa* L.**	Acı Marul	Asteraceae	Leaf	Raw/ Internal	Heart diseases[79]
***Laurus nobilis* L.**	Defne, Tehnel	Lauraceae	(Fresh) Flower, Leaf	Infusion/ Internal (Once a day)	Vasodilator[53]
			Leaf	Decoction/ Internal (a cup per day)	Heart diseases[58,80]
***Lavandula stoechas* L.**	Karabaş, Karabaş Kekiği, Çalı kekiği, Kekik, Mor Kekik, Karahan, Karabaş Otu, Sargan Otu	Lamiaceae	(Flowering) Branch	Infusion/ Internal	Atherosclerosis[23,39]
					Cardiovascular diseases[21]
			Leaf	Crushed with honey (dried)/ Internal (One spoonful after meals)	Cardiovascular diseases[81]
			Flower, Leaf	Decoction/ Internal	Cardiovascular diseases[27]
				Infusion/ Internal (2-3 times daily)	Tachycardia[82]
			Stem	Tea/ Internal (2-3 times a day)	Vasodilating[70]
			Flower	Infusion/ Internal	Cardiovascular diseases[45]
			Aerial part	Infusion/ Internal (A glass three times a day)	Heart diseases, Atherosclerosis[57,70,83]
				Decoction/ Internal	Heart diseases[84]
***Lycopersicon esculentum* Mill.**	Domat, Domates	Solanaceae	Fruit	Raw/ Internal (regularly)	Cardiac hypertrophy[23]
***Malva neglecta* Wallr.**	Ebegümeci, Doğnuk, Hiru	Malvaceae	Leaf, Root, Stem	Infusion/ Internal	Heart diseases[44]
***Malva sylvestris* L.**	Ebegömeci, Ebegümeci, Gömeç, Ebemgümeci	Malvaceae	Fresh stem	Infusion/ Internal	Atherosclerosis[23]
			Flower	Infusion/ Internal	Heart diseases[21,85]
***Marrubium anisodon* K.Koch**	Elbir otu	Lamiaceae	Aerial part	-	Blood-pressure regulator[49]

***Matricaria aurea* (Loefl.) Sch.Bip.**	Beybunıc, Gayeka, Seva, Gihake Seva	Asteraceae	Aerial part	Decoction/ Internal	Heart diseases[72]
***Matricaria chamomilla* L.**	Papatya, Papatya Otu, Papatça, Pobeş, Koyungözü	Asteraceae	Aerial part	Infusion/ Internal	Atherosclerosis[23]
			Capitulum	Infusion/ Internal	Heart diseases[57]
***Medicago arabica* (L.) Huds.**	Bıtırak	Fabaceae	Aerial part	Infusion/ Internal (regularly)	Cardiovascular diseases[23]
***Medicago minima* (L.) Bartal.**	Gurnik	Fabaceae	Branch (with fruit)	Infusion/ Internal (In the mornings on the empty stomach)	Heart diseases[86]
***Medicago orbicularis* (L.) Bartal.**	Düğmelik	Fabaceae	Seed	Infusion/ Internal	Heart diseases[87]
***Melissa officinalis* L.**	Oğul otu, Saçkıran, Yabanidereotu, Yabanısırganı, Limonotu, Melisa, Turunçcuk	Lamiaceae	Aerial part	Decoction/ Internal	Vasodilator[40,88]
				Infusion/ Internal (Half of a teacup twice a day for 20 days)	Atherosclerosis, Embolism[20,39,40]
					Heart diseases[40]
				Infusion (dried)/ Internal	Heart diseases like palpitations, Heart problems[23,69]
			Leaf, Flower	Infusion (dried)/ Internal	Good for heart[27]
				Decoction/ Internal (3-4 times a day)	Vasodilator[39]
			Leaf	Infusion/ Internal	Heart palpitations[57]
				Decoction/ Internal (before breakfast)	Arteriosclerosis[41,82]
				Decoction/ Internal (A cup of the plant two times a day)	
				Infusion/ Internal (Couple times daily)	Cardiovascular diseases[89]
***Melissa officinalis* subsp. *inodora* Bornm.**	Pung	Lamiaceae	Leaf, stem, flower, aerial part	Decoction/ Internal	Heart diseases[47]
***Mentha x piperita* L.**	Nane, Bahçe Nanesi, Nana	Lamiaceae	Aerial part	Raw, Tea, Spice/ Internal	Heart diseases (Preventive)[23]
***Mentha longifolia* subsp. *typhoides* (Briq.) Harley**	Pung, Su yarpuzu	Lamiaceae	Whole plant	Infusion/ Internal	Heart diseases[90]
***Momordica charantia* L.**	Kudretnarı, Cennet Narı, Çenet Narı, Cenet Narı	Cucurbitaceae	Fruit	Mashed and kept in olive oil/ Internal	Atherosclerosis[23]
***Morus alba* L.**	Beyaz dut	Moraceae	Fruit	Raw/ Internal	Heart strengthener[53]
***Myrtus communis* L.**	Yaban mersini	Myrtaceae	-	-	Heart strengthener[43]
***Nasturtium officinale* R.Br.**	Su Teresi, Gerdeme, Kerdeme, Sumancası, Çakandura	Brassicaceae	Stem (with leaf)	Decoction/ Internal	Heart-rhythm regulator[70]
			Leaf	Raw/ Internal	Heart stress[91]
***Olea europaea* L.**	Zeytin, Zeytin Ağacı, Gemlik Zeytini, Trilya, Eşek Zeytini, Domat, Yasemin, Edremit, Taze Fışkın	Oleaceae	(Young) Leaf, (Flowering) Shoot, Flower	Infusion/ Internal (Twice a day after meals)	Atherosclerosis[23]
			Leaf	Decoction/ Internal	Cardiovascular diseases[27,70]
				Chewed	Heart-rhythm regulator[70]
				Decoction (32 pieces of it)/ Internal	Heart diseases[87]
			Fruit, Leaf	Infusion/ Internal (One cup)	Heart diseases[70]
***Onobrychis megataphros* Boiss.**	Gurnik, Gurnuk	Fabaceae	Aerial part	Decoction (dried)/ Internal (A cup in the mornings on the empty stomach)	Good for heart, Atherosclerosis[92]
***Onosma isauricum* Boiss. & Heldr.**	Adaçayı	Boraginaceae	Aerial part	Infusion/ Internal	Cardiovascular diseases[25,55]
***Origanum onites* L.**	Kırkbaş Kekik, Tokalıkekik	Lamiaceae	Aerial part	Infusion/ Internal	Heart palpitations[23]
***Origanum vulgare* L.**	Kaya Kekiği, Taş Kekik, Kokulu Kekik, Sarı Kekik	Lamiaceae	Aerial part	Infusion/ Internal	Heart palpitations[23]
					Vasodilator[93]
***Paliurus spina-christi* Mill.**	Çaltı, Karaçalı	Rhamnaceae	Fruit	Infusion/ Internal	Heart diseases[57]
				Decoction/ Internal	Blood-pressure regulator[41]

					Heart diseases[20]
					Atherosclerosis[66]
***Papaver argemone* L.**	Gelincik, Gelneli, Gelineli, Lale Otu, Kelemli, Kapurcuk	Papaveraceae	Seed	Infusion (crushed)/ Internal	Cardiovascular diseases[23]
***Papaver bracteatum* Lindl.**	Haşhaş, Adamağusu	Papaveraceae	Seed	Raw/ Internal	Heart diseases[35]
***Papaver rhoeas* L.**	Gelincik, Kamacık, Kağma, Aşotu	Papaveraceae	Root	Roasted/ Internal	Heart diseases[45]
			Flower	Infusion/ Internal	Anti-arrhythmic[41]
			Leaf	Cooked/ Internal	Heart diseases[69]
***Papaver somniferum* L.**	Haşhaş, Haşeş, Haşgeş	Papaveraceae	(Fresh) Leaf	Raw/ Internal	Vasodilator[79]
***Phlomis armeniaca* Willd.**	Adaçayı	Lamiaceae	Aerial part	Infusion/ Internal	Cardiovascular diseases[25,55]
***Phyllitis scolopendrium* L.**	Geyikdili eğreltisi	Aspleniaceae	Aerial part	Decoction/ Internal	Arteriosclerosis[94]
***Alkekengi officinarum* Moench (Syn: *Physalis alkekengi* L.)**	Altın Çilek, Güveyfeneri	Solanaceae	Leaf, Fruit	Infusion/ Internal	Heart diseases[44]
***Pinus brutia* Ten.**	Çam, Şam, Kızılçam	Pinaceae	Mucilage	Boiled with olive oil and applied on the desired area with massage/ External	Arteriosclerosis[75]
***Pinus pinea* L.**	Fıstık çamı	Pinaceae	Branch tip	Infusion/ Internal	Vasodilator[39]
***Plantago lanceolata* L.**	Pel hewes, Pelonbaş, Omulwaş, Omınwaş	Plantaginaceae	Leaf	Infusion/ Internal (A cup on empty stomach in the morning)	Embolism[33]
			Leaf	Decoction, Fresh, Crushed/ Internal	Cardiovascular diseases[22]
***Plantago major* L.**	Sinir otu, Damarlıot, Damarotu, Balazağa, Çıban Otu, Sinirli Ot, Pel hewes, Pelonbaş, Omulwaş, Omınwaş	Plantaginaceae	Leaf	Decoction (with leaves of Mentha sp. and Urtica sp.)/ Internal	Vasodilator[88]
				Decoction/ External (Compress)	Embolism[33]
			Aerial part	Infusion, Decoction/ Internal	Cardiovascular diseases[67]
***Platanus orientalis* L.**	Çınar	Platanaceae	Leaf	Decoction/ Internal	Cardiovascular diseases, Heart diaeases[22,47]
***Portulaca oleracea* L.**	Pirpirim, Semizotu, Parpar	Portulacaceae	Stem, Leaf	Raw/ Internal	Heart diseases[44]
			Aerial part	Infusion, Decoction/ Internal (A teaglass after the meal, drink 1 cup on empty stomach in the morning)	Heart diseases[33]
***Prunus cerasifera* Ehrh. (Syn: *Prunus divaricata* Ledeb.)**	Delierik, Dağ Eriği	Rosaceae	(Raw) Fruit	Raw/ Internal (Handful daily)	Cardiovascular diseases[23]
***Pyrus elaeagnifolia* Pall.**	Ahlat, Ağlat, Yaban armutu, Dağ armutu	Rosaceae	Flower	Decoction/ Internal (A glass once a day)	Atherosclerosis[66]
***Punica granatum* L.**	Nar	Lythraceae	Fruit	Juice/ Internal (A glass daily)	Heart-rhythm regulator[75]
					Atherosclerosis[66]
***Quercus brantii* Lindl.**	Dara berru, Berru	Fagaceae	Leaf, Branch, Flower, Seed	Decoction, Fresh/ Internal	Good for heart[47]
***Rheum ribes* L.**	Ribes, Kap, Işgın	Polygonaceae	Aerial part, Root	Infusion/ Internal (A cup on empty stomach in the morning)	Heart diseases[33]
***Rosa canina* L.**	Kuşburnu, İtburnu, İtgülü, Yaban Gülü, Kozalak, Kozalak Ağacı, Yabani Gül, Gül Bubusu	Rosaceae	Fruit	Decoction/ Internal	Heart diseases[23]
				Decoction (with hawthorn)/ Internal	Heart diseases[70]
				Infusion/ Internal	Heart diseases[53]
			Hypanthium	Decoction/ Internal	Arteriosclerosis[41]
			(Flowering) Branch	Infusion/ Internal	Cardiovascular diseases, Embolism[70]
***Rosa foetida* Herrm.**	Menderis	Rosaceae	Fruit	Decoction/ Internal	Cardialgia[72]
				Infusion/ Internal	Cardialgia[72]
***Salvia rosmarinus* Schleid. (Syn: *Rosmarinus officinalis* L.)**	Biberiye, Biberiye Otu, Kuşdili, Kuşdili Otu	Lamiaceae	Leaf	Infusion/ Internal (Mornings, after a cup of warm lemon juice)	Heart diseases[88]
			Branch with leaves	Infusion/ Internal	Heart diseases[57]

***Rubus canescens* DC.**	Böğürtlen, Diken, Karamuk, Mora, Hamdüspara, Garantı, Garaltı,	Rosaceae	Branch	Decoction (With *Plantago sp*., *Urtica sp*. and *Melissa officinalis*)/ Internal	Atherosclerosis[88]
			Flowering shoot	Decoction/ Internal	Atherosclerosis[39]
***Rubus idaeus* L.**	Çitir, Temel üzümü	Rosaceae	Root	Decoction/ Internal	Heart diseases[61]
***Rubus sanctus* Schreb.**	Orman, Orman Üzümü, Orman Gülü, Gür, Kür, Kür Üzümü, Gür Üzümü, Kocakız Kürü, Gocagız Gürü, Böğürtlen, Böğürtlen çalısı, Gülleğen Dikeni	Rosaceae	Fruit	Raw/ Internal	Atherosclerosis[23]
					Cardiovascular diseases[25,34,55]
			(Thin and fringe) Root	Infusion/ Internal	Cardialgia[44]
					Heart diseases[34]
***Rumex crispus* L.**	Labada	Polygonaceae	Lower leaf	Infusion/ Internal	Heart diseases[44]
***Rumex* sp.**	Kalmık çayı, Çerkez cayı	Polygonaceae	Aerial part	Decoction/ Internal	Cardiovascular diseases[60]
***Salix alba* L.**	Söğüt	Salicaceae	Leaf	Decoction/ Internal	Arteriosclerosis[55]
***Salvia cryptantha* Montbret & Aucher ex Bentham**	Ada çayı, Ballık Otu, Çalba, Kayışkıran, Kokulu Ot, Sarı Şabla, Şabla	Lamiaceae	Aerial part	Infusion/ Internal	Cardiovascular diseases[25,55]
***Salvia aethiopis* L.**	Karakoyun Teği	Lamiaceae	Aerial part	Infusion, Decoction/ Internal	Heart diseases[67]
***Salvia hypargeia* Fisch. & C.A.Mey.**	Iccak otu	Lamiaceae	Aerial part, Sap	Infusion/ Internal	Heartstroke[95]
***Salvia multicaulis* Vahl.**	Çaya çiyan	Lamiaceae	Leaf, flower, aerial part	Decoction/ Internal	Reducing heart attack risk[47]
***Sambucus ebulus* L.**	Yir otu, Ayıboğan	Adoxaceae	Seed	Raw/ Internal	Atherosclerosis[40]
			Fruit	Kept in sugar for 40 days, drunk the resulting water on the empty stomach in the mornings.	Heart diseases[42]
***Satureja cuneifolia* Ten.**	Dağkekiği, Karakekik, Arı kekiği	Lamiaceae	Aerial part	Tea/ Internal (A teacup three times a day for 1-2 weeks)	Cardiotonic[24]
***Satureja spicigera* (K. Koch) Boiss.**	Kekik, zımpara	Lamiaceae	Leaf	Infusion/ Internal (A cup in the mornings on the empty stomach)	Heart diseases[96]
***Sideritis libanotica* subsp. *linearis* (Benth.) Bornm.**	Dağ çayı	Lamiaceae	Aerial part	-	Vasodilator[49]
***Sisymbrium altissimum* L.**	Yabani hardal	Brassicaceae	Flower, Leaf	Raw/ Internal	Vasoconstrictive[79]
***Torminalis glaberrima* (Gand.) Sennikov & Kurtto (Syn: *Sorbus torminalis* (L.) Crantz)**	Geyikelması, Dağelması, Kegelması	Rosaceae	Fruit, Leaf	Decoction/ Internal	Heart diseases[31]
			Fruit	Raw/ Internal	Heart diseases[63]
***Aria umbellata* (Desf.) Sennikov & Kurtto (Syn: *Sorbus umbellata* (Desf.) Fritsch)**	Geyikelması	Rosaceae	Leaf	Decoction/ Internal	Arteriosclerosis[77]
***Spinacia oleracea* L.**	Ispanak	Amaranthaceae	Leaf	Raw/ Internal	Heart diseases[79]
			Leaf	Cooked/ Internal	Cardiovascular diseases[22]
***Stachys sylvatica* L.**	Hamsırgan	Lamiaceae	Aerial part	Tea/ Internal Infusion/ Internal (A teacup, twice a day)	Heart diseases[48,102]
***Styrax officinalis* L.**	Ayı Fındığı, Günlük	Styracaceae	Fruit seed	Raw/ Internal	Heart diseases[70]
***Tamarix smyrnensis* Bunge**	Ilgın	Tamaricaceae	Stem bark	Decoction/ Internal	Cardiovascular diseases[78]
***Tamus communis* L.**	Acı ot, Sarmaşık, Vicirne, Mıcır, Mıcık, Bıcık, Bıcık Otu, Kapırcık, Kapurcuk, Kedien, Kediyen	Dioscoraceae	Flower	Cooked (in olive oil)/ Internal	Atherosclerosis[23]
***Teucrium chamaedrys* L.**	Kırmızı Ot, Kalp Otu, Dağ kekiği	Lamiaceae	Whole plant	Decoction/ Internal (Half of a teacup, 3 times per day)	Cardiovascular diseases[50]
			Aerial part	Decoction/ Internal	Heart diseases[86]
				Infusion/ Internal	Arteriosclerosis[97]

					Heart diseases[78]
***Teucrium polium* L.**	Oğul Otu, Anam Babam Kokusu, Koyun Otu, Yavşan	Lamiaceae	Root	Infusion/ Internal	Heart diseases[21,76]
***Thymbra spicata* L.**	Karabaş otu, Dağ çiçeği, Zahter	Lamiaceae	Aerial part	Infusion/ Internal	Heart diseases, Arteriosclerosis, Cardiac deficiency[83,97]
			Leaf, Flower	Infusion/ Internal	Tachycardia[82]
***Thymus longicaulis* C. Presl**	Kekik otu	Lamiaceae	Whole plant	Decoction/ Internal	Blood-pressure regulator[98]
***Thymus sipyleus* Boiss.**	Kekik	Lamiaceae	Aerial part	Infusion/ Internal	Cardiovascular diseases[25,55]
***Tilia platyphyllos* subsp. *corinthiaca* (Bosc ex K.Koch) Pigott (Syn: *Tilia rubra* DC.)**	Ihlamur	Malvaceae	Flower, Bract	Infusion/ Internal	Tachycardia[82]
***Tragopogon reticulatus* Boiss. & A. Huet**	Sinza	Asteraceae	Aerial part	Raw/ Internal	Cardialgia[99]
***Tribulus terrestris* L.**	Diken, Çoban Çökerten, Dadaş Otu, Demir Dikeni, Bitirak, Pıtırak, Yeşilpıtrakotu, Deve çökerten, Çakır dikeni, Gwerçal, Gerçal, Çobankalkıtan, Çobankalgıdan, Çökelek	Zygophyllaceae	Whole plant	Infusion, Decoction/ Internal (Kept a night long in the cold before use)	Atherosclerosis, Heart diseases[50]
				Decoction/ Internal (A glass once a day)	Cardiovascular diseases, Atherosclerosis[66]
			Aerial part	Decoction/ Internal	Cardiovascular diseases[25,34,54,60]
					Atherosclerosis[25,38]
					Heart attack[38]
				Infusion/ Internal	Cardiovascular diseases[55]
			Aerial part, Fruit	Decoction/ Internal (3 times a day) Infusion/ Internal	Heart diseases[37]
			Leaf, Fruit	Infusion/ Internal (A teacup, twice a day)	Cardiovascular diseases, Cardialgia[33,44]
			Fruit	Decoction/ Internal	Heart diseases, Atherosclerosis[73]
				Infusion/ Internal	Heart diseases, Blood-thinner[68,100]
			Seed	Infusion, Decoction/ Internal	Vasodilator[57,67]
			Leaf, Flower	Decoction/ Internal	
***Tripleurospermum elongatum* (Fisch. & C.A.Mey.) Bornm.**	Papatya	Asteraceae	Flower	Infusion, Decoction/ Internal	Cardiovascular diseases[22]
***Tripleurospermum heterolepis* (Freyn & Sint.) Bornm.**	Papatya, Yabani Papatya	Asteraceae	Flower	Tea/ Internal	Heart diseases[36]
***Tripleurospermum monticolum* (Boiss. & A.Huet) Bornm.**	Papatya, Yabani Papatya	Asteraceae	Flower	Tea/ Internal	Heart diseases[36]
***Tripleurospermum sevanense* (Manden.) Pobed.**	Sarı papatya	Asteraceae	Flower	Infusion/ Internal	Heart diseases[44]
***Urtica dioica* L.**	Isırgan Otu, Erkek Çakır, Eşek Çakırı, Deli Çakır, Deli Eşek Isırganı, Alaısırgan, Deve Gicirgeni, Dalagan, Dalıgan, Dalak, Sırgan, Isırgı	Urticaceae	Leaf, Whole plant	Infusion, Decoction/ Internal (Especially in the mornings, on empty stomach)	Atherosclerosis[50]
					Heart diseases[34]
			Aerial part	Infusion/ Internal	Heart diseases[23]
					Atherosclerosis[25,81]
				Decoction/ Internal	Heart diseases[72]
					Blood-purifier[101]
			Leaf	Infusion/ Internal	Blood-thinner[53]
				Decoction/ Internal (Once a day)	Arteriosclerosis[41]
				Infusion/ Internal	Blood and circulation system diseases[82]
			Seed	Decoction/ Internal	Cardiovascular diseases[21]
				Internal (With honey or pekmez)	Blood-purifier[101]

***Verbena officinalis* L.**	Mine çiçeği	Verbenaceae	Aerial part	Decoction/ Internal	Heart diseases[40]
***Viola ermenekensis* Yild. & Dinç**	Menekşe	Violaceae	Flower	Decoction/ Internal (1 or 2 cup)	Heart diseases[81]
***Viola odorata* L.**	Bınevşok	Violaceae	Whole plant	Decoction/ Internal	The pain in the veins[29]
***Viscum album* L.**	Ökseotu, Çakum, Ökse, Yapışkanotu, Burç, Buruç, Armut Burçu, Ahlat Burçu, Ahlat Puruncu, Çam Purucu, Çam Puruncu, Çam Puruşu, Çam Burcu, Güvelek, Puruç, Puruş, Ahlak, Öksü Otu, Göğelek, Gevele, Çekem, Gökçe, Çekem	Viscaceae	Aerial part	Maceration/ Internal	Atherosclerosis[23]
			Whole plant	Decoction/ Internal (A cup per day)	Heart diseases[81]
			Leaf	Infusion (dried)/ Internal	Vasodilator, Heart disaeses[69,93]
			Whole plant	Infusion (dried)/ Internal	Heart diseases, Cardiovascular diseases[60,70]
				Decoction/ Internal	Heart diseases[78]
					Atherosclerosis[48]
			Branch, Leaf	Decoction/ Internal	Atherosclerosis, Heart diseases, Vasodilator[53,67,75]
				Infusion, Maceration/ Internal	Vasodilator[67]
***Viscum album* L. subsp. *abietis* (Wiesb.) Janch.**	Govelek	Viscaceae	Whole plant	Decoction/ Internal	Cardiovascular diseases[60]
***Viscum laxum* Boiss. & Reut. (Syn: *Viscum album* L. subsp. *austriacum* (Wiesb. ex Dichtl) Vollm.)**	Çeküm, Ökseotu, Gökçeotu	Viscaceae	Fruit, Leaf, Seed	Decoction, Infusion, Crushed, Raw/ Internal	Cardiovascular diseases[22]
***Vitex agnus-castus* L.**	Hayıt, Beşparmakağacı	Lamiaceae	Seed	Infusion/ Internal	Atherosclerosis[78]
			Fruit	Infusion/ Internal	Cardiovascular diseases[60]
***Vitis vinifera* L.**	Üzüm, asma, ba, loza, grozde	Vitaceae	Fruit	Raw/ Internal	Blood-forming, Cardiovascular diseases[42]
***Xanthium spinosum* L.**	Gurnık	Asteraceae	Branch	Decoction/ Internal (A cup two times a day	Heart diseases, Embolism[33]
***Ziziphus jujuba* Mill.**	Hünnap, Günnap	Rhamnaceae	Fruit	Raw/ Internal	Heart diseases[23]

RESULTS

As a result of a detailed study of the literature, it was found that, in parallel with the expected incidence of cardiovascular diseases in our society, the use of medicinal plants for their treatment is quite common. One hundred twenty-nine plants from 43 families have been reported as being used to treat the group of diseases, including high blood pressure, cardiac disease, and blood vessels (Table 1). Cardiovascular diseases and/or effects of plants are classified as heart diseases, cardiovascular, atherosclerosis, vasodilator, arteriosclerosis, embolism, heart palpitations, cardialgia, cardiotonic, and blood pressure regulator, as taken precisely from the related ethnobotanical study (Figure 1).

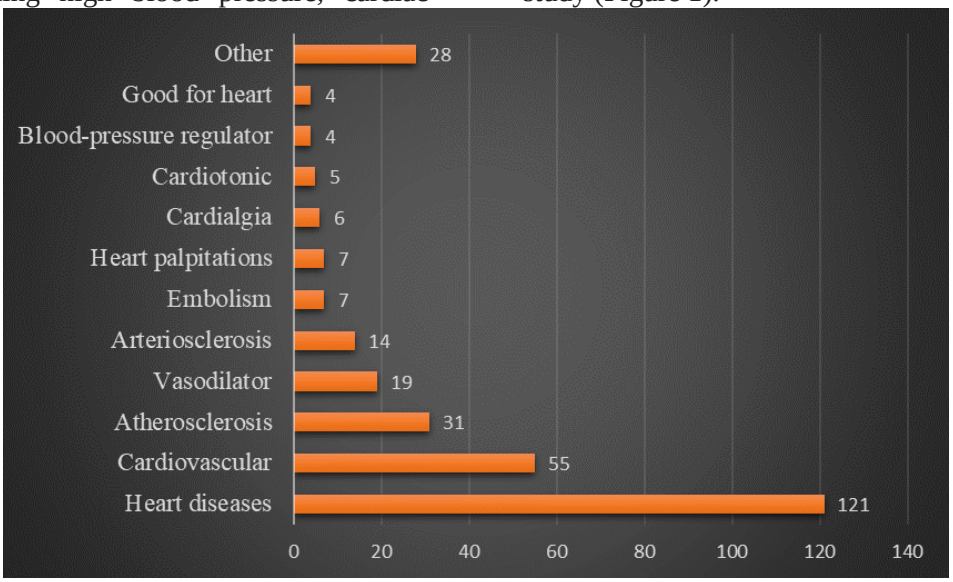

Figure 1. Classification of cardiovascular diseases.

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Over fifty percent of the plants belong to four families: Rosaceae, Lamiaceae, Asteraceae, and Fabaceae. According to the percentage distribution of plants on a family basis, Rosaceae tops the list with 17.8%. In this family, 23 species have been determined to be used against cardiovascular diseases among Turkish people. It is followed by Lamiaceae, with 18 species (14%). The family Asteraceae ranks third with 17 species (13.1%), and Fabaceae follows it with 7 species (5.4%) (Figure 2). *Crataegus monogyna* Jacq., *Lavandula stoechas* L., *Melissa officinalis* L., *Tribulus terrestris* L., *Urtica dioica* L., and *Viscum album* L. are the most frequently cited medicinal plants. When the used parts were analyzed, it was observed that the leaf, aerial parts, fruit, and flower were consumed the most (Figure 3). The consumption is usually in the form of herbal tea prepared as an infusion or decoction (Figure 4).

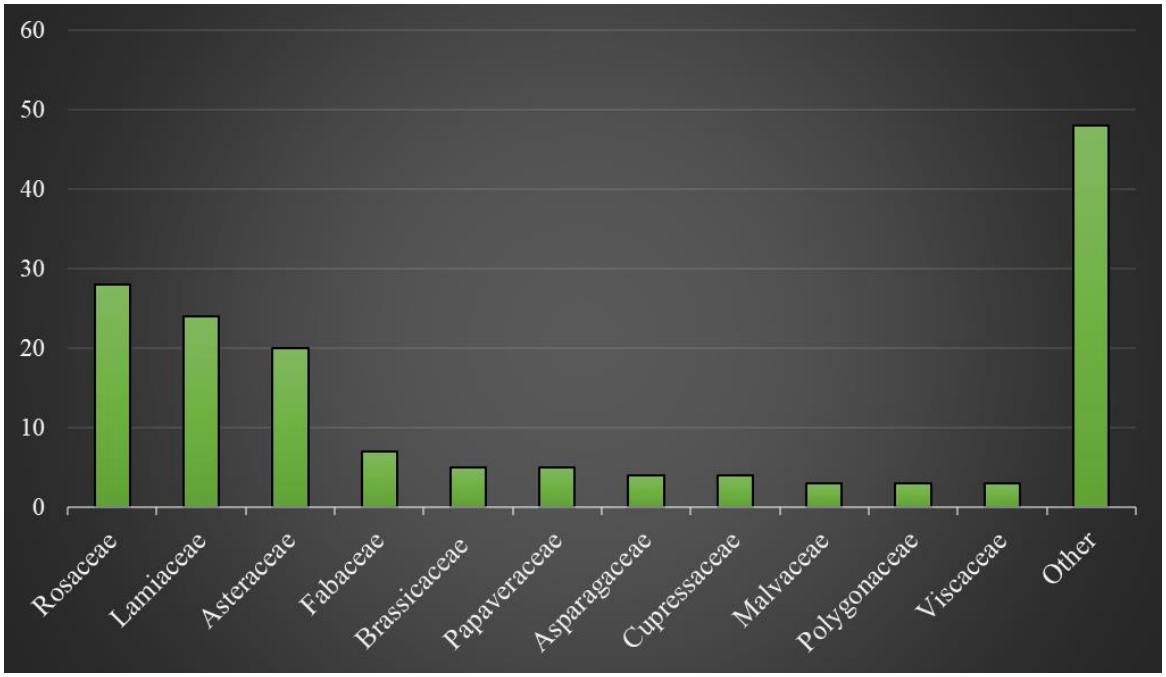

Figure 2. The most cited plant families.

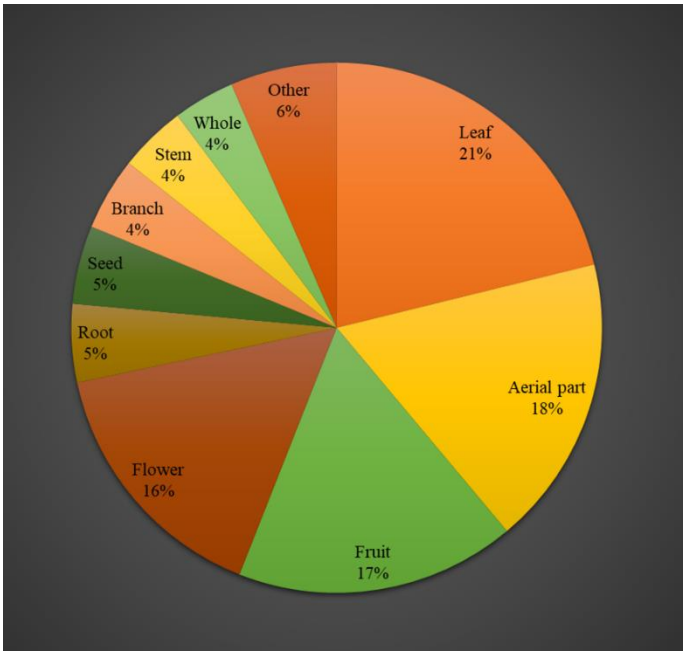

Figure 3. Plant parts used against cardiovascular disease ranked by frequency of use.

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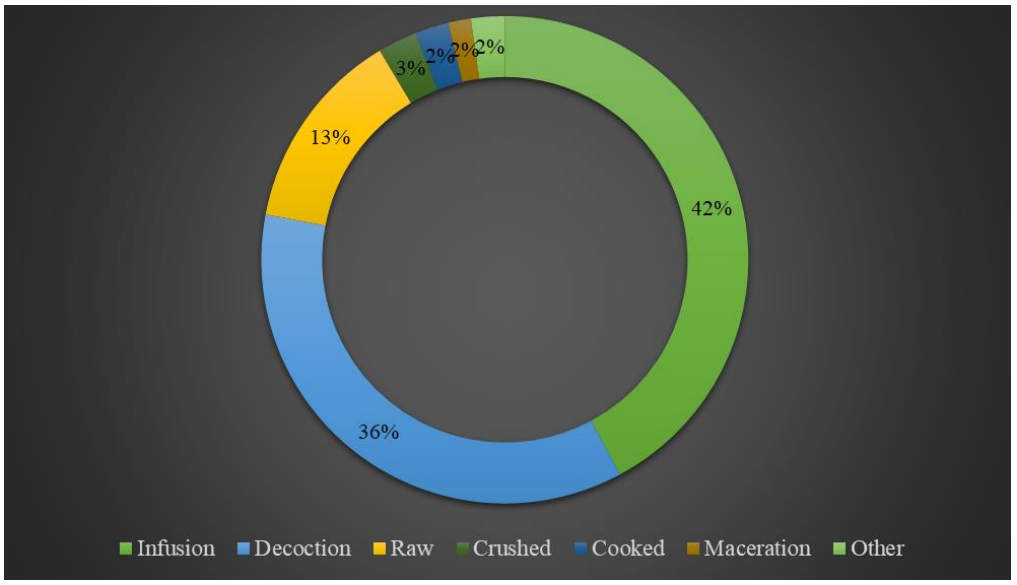

Figure 4. Preparation methods of the plants used against cardiovascular disease ranked by frequency of use.

DISCUSSION

Several medicinal plants have shown experimental cardiovascular activity *in vitro/ in vivo* or clinical studies. We present pharmacological studies that have directly or indirectly evaluated the most cited medicinal plants used to treat cardiovascular diseases.

Crataegus monogyna

The main active compounds found in the flowers, leaves, and fruits of *Crataegus* sp. are sugars and sugar alcohols, phenolic acids, terpenes, essential oils, phenylpropanoids, essentially hydroxycinnamic acids, lignans, and flavonoids. Especially flavonoids (hyperoside, vitexin, etc.) and oligomeric proanthocyanidins are responsible for their protective activity in cardiovascular disease. Numerous studies are directed at the action of *Crataegus monogyna* in preventing atherosclerosis and vascular diseases. It is thought that *Crataegus* sp. increases the ability of cardiomyocytes to transfer calcium via Na+/K+-ATPase.[12] It has been reported that a unique extract of *Crataegus* leaves with flowers induced an endothelium-dependent, NO-mediated vasorelaxation via eNOS phosphorylation at serine 1177. Moreover, fruit extracts of Crataegus reduced the mitochondrial membrane potential by 1.2–4.4 mV.[13]

Lavandula stoechas

Lavandula stoechas contains flavonoids, catechic tannins, sterols, coumarins, leucoanthocyans, and mucilages. The essential oil's major terpenic compounds are fenchone, camphor, and 1,8-cineole. Although various research has proven the pharmacological effects of essential oils and extracts, including antibacterial, antifungal, insecticidal, anti-leishmanial, antioxidant, and anti-inflammatory activities, the cardiovascular activity of *L. stoechas* still needs to be evaluated.[14]

Melissa officinalis

According to ancient sources, Avicenna (980-1037) recommended *Melissa officinalis* to strengthen the heart in the Middle Ages. The plant's bioactive compounds are phenolic compounds, L-ascorbic acid, carotenoids, flavonoids, and terpenoids. In addition to *in vitro* and *in vivo* studies, clinical studies have shown that the plant remarkably affects cardiovascular diseases. In a study investigating the effect of the plant on mild palpitations, leaf extract was given to 28 patients at a dose of 2 × 500 mg/d for 14 days. A decrease in the rate of palpitations by 36.8% was observed.[15] It has also been shown that the aqueous extract is associated with significant electrocardiogram alterations in rats after one week (50, 100, 200 mg/kg) of application. In a double-blind study, 80 people with stable angina were given 3 g of lemon balm herb extract per day or a placebo for three months. According to the study results, a higher nitric oxide concentration, a higher cardiac ejection fraction, a lower lactate dehydrogenase concentration, and reduced systolic and diastolic blood pressure were determined in the study group compared to the control.[16]

Tribulus terrestris

Several chemical studies have identified various compounds in *Tribulus terrestris*, such as saponins,

flavonoids, alkaloids, and other nutrients. The steroidal saponins (terrestrosins A-E, desgalacto-tigonis, etc.), found in high amounts, especially in aerial parts, are responsible for biological activity. In a clinical trial, isolated saponins were given to 406 patients with coronary heart disease. The results indicated that the overall effective remission rate in angina pectoris was 82.3 percent. The total efficacious rate of ECG improvement (52.7%) was higher than that of the control group (35.8%). Researchers have pointed out the positive effects of the plant in the treatment of angina pectoris, owing to the saponins that have the activity of dilating the coronary artery and improving coronary circulation.[17]

Urtica dioica

The chemical constituents of *Urtica dioica* are phytosterols, saponins, flavonoids, tannins, sterols, greasy acids, carotenoids, chlorophylls, proteins, amino acids, and vitamins. The crude aqueous and methanolic extracts of the plant roots were tested on preparations of the aorta with or without prior vasoconstriction of the endothelium. Extracts and the purified fractions possess a vasodilatory activity, and the inotropic activity was recognized in guinea pigs. It was stated that *U. dioica* has a notable antiplatelet action thanks to the flavonoids it contains. Furthermore, the aqueous extract induced strong bradycardia through non-cholinergic and non-adrenergic pathways *in vivo*.[18]

Viscum album

Lectins, viscotoxins, lignans, amines, flavonoids, and polysaccharides are the principal components of the *Viscum album*. Flavonoids and phenolic acids have significant antioxidant activity, which can be beneficial in preventing diseases, including cardiovascular diseases. Research has intensified the antihypertensive activity of different extracts. The aqueous extract exerted an antihypertensive effect on salt-induced hypertension in male rats without alteration in heart rate, possibly involving sympathetic mechanisms. Various extracts of *V. album* stem on arterial blood pressure were performed in Wistar rats. The ethanol extract revealed a significant effect even at the lowest administered concentration (3.33×10^{-5} mg kg^{-1}) and significantly reduced the blood pressure after an administered concentration of 1.00×10^{-3} mg kg^{-1}.[19]

CONCLUSION

It was found that the plants can be used for different purposes in different locations. We determined the six most frequently cited medicinal plants. As there is a lack of scientific validation for *Lavandula stoechas*, further phytochemical and pharmacological studies are recommended to confirm efficacy. Further studies are needed to base the collected information on clinical evidence.

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REVIEW

Pimpinella Species (Anise): Traditional Use, Mineral, Nutrient and Chemical Contents, Biological Activities

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Abstract
Plants, which are natural materials of great importance to humans, are currently utilized for various purposes. The use of plants with nourishing properties for treating diseases has a long history. This study compiled the general characteristics, usage areas, mineral and nutrient contents, biological activities, and chemical compositions of *the Pimpinella* species reported in the literature. The literature review reveals that the plant is widely used in traditional medicine. Furthermore, it has been determined that they may serve as significant natural resources regarding their mineral, nutritional, and chemical content. Furthermore, it has been observed, based on reported data in the literature, that *Pimpinella* species exhibit high levels of antioxidant and antimicrobial activity. Within this context, it has been observed that *Pimpinella* species can be significant sources not only in terms of their nutritional properties but also from a medicinal perspective.
Keywords: Antimicrobial, Anise, Antioxidant, Medicinal Plants, Traditional Medicine.

INTRODUCTION

Various natural materials, such as fungi, animals, and plants, are utilized in traditional medicine.[1] Among these natural products, plants are significant materials. Plants are utilized for various purposes such as food, spices, combating diseases, heating, and shelter in different cultures.[2] Plants constitute significant components of many human diets. They possess highly nutritious properties because they contain vitamins, minerals, and essential nutrients.[3] Plants that stand out with their nourishing properties are also significant materials from a medical perspective.[4,5] Numerous studies have reported that plants possess various activities such as antioxidant, anticancer, antimicrobial, anti-inflammatory, hepatoprotective, anti-aging, anti-allergic, and DNA protective properties.[6-13] Determining the biological potential of plants is of great importance within this context. This study compiled the reported usage areas, general characteristics, nutritional and mineral contents, biological activities, and chemical compounds of Pimpinella species from the literature.

GENUS *PIMPINELLA* AND USAGE AREAS

Pimpinella species belonging to the Apiaceae family are plants with a distribution range of 150 species across Europe, Asia, and Africa. The types refer to annual and perennial plants. From a morphological perspective, the plant possesses cordate-ovate or rectangular-ovate leaves and fruits that are slightly compressed laterally, each with five filiform veins.[14-18]

Pimpinella species are favored in alternative medicine in countries such as China, Egypt, Iran, Lebanon, and Palestine. It has been reported that there is still usage even in countries such as England and Italy, where the use of herbal medicines is low. Türkiye is the country where *Pimpinella* is most commonly utilized and preferred. The species of *Pimpinella* are commonly preferred for their above-

ground, root, and seed parts. The above-ground parts of the plant are utilized for their medicinal properties, including their ability to alleviate gastrointestinal distress, bronchial asthma, insomnia, persistent cough, renal colic, flatulence, expectorant, sedative, antidepressant, antiseptic, antispasmodic, analgesic, diuretic, estrogenic, and pectoral stimulant effects.

Table 1. Mineral and Nutritional Contents of *Pimpinella* species.

Nutritional Composition	Values (%)
Protein	13.35-28.73 %
Lipids	2.66-9.02 %
Carbohydrate	9.87-61.09 %
Crude fibers	2.83-33.50 %
Moisture	4.9-80.42 %
Ash	5.69-19.14 %
Mg	6.45-478.6 mg/kg
K	93.13-6332 mg/kg
Na	5.44- 365.10 mg/kg
Fe	0.15-1512 mg/kg
Ca	12.64-3141 mg/kg
Zn	0.12-14.31 mg/kg
Cu	0.029-0.035 mg/kg

The seed components exhibit various pharmacological properties such as abdominal pain relief, gastrointestinal disorder management, carminative effects, expectorant properties, sedative effects, antidepressant properties, and antiseptic properties. Insomnia, persistent cough, carminative, stomach upset, stomach ache, calming, colic, tranquilizer, diuretic, flu, and various alcoholic beverages such as "pastis" and "sambuca" are commonly used for their therapeutic properties as hypnotics, antispasmodics, expectorants, and in the treatment of epilepsy. The root parts, on the other hand, are utilized in the treatment of asthma, bronchitis, and menstrual disorders.[19-26]

NUTRITIONAL AND MINERAL CONTENTS

Plants are an essential component of human diets and cannot be substituted. The food products that many individuals prefer to consume during each meal are commonly observed.[27] In this study, the nutritional and mineral contents of *Pimpinella* species reported in the literature are presented in Table 1.

BIOLOGICAL ACTIVITY

In the literature, various extracts such as ethanol, water, acetone, methanol, essential oil, aqueous, hexane, methylene chloride, hydroalcoholic, benzene, ethyl acetate, and n-butanol have been reported to be utilised *in vitro* and *in vivo* biological activity studies on *Pimpinella* species. The biological activity studies conducted on *Pimpinella* species reported in the literature are presented in Table 2.

Table 2. Biological activity of *Pimpinella* species.[32, 35-54]

Plant species	Biological activities	Extractions
P.anisoides V.Brig.	Antioxidant, anti-inflammatory	Ethanol
P. anisum L.	Antioxidant, antimicrobial, cytotoxicity, antiproliferative, anticancer, antispasmodic, antidiabetic, antiviral, insecticidal	Ethanol, water, methanol, essential oil, aqueous, hexane, methylene chloride, hidroalcoholicbenzene, ethylacetate, n-butanol
P. aurea DC.	Antioxidant, antimicrobial	Methanol
P.brachycarpa (Kom.) Nakai	Antioxidant, antimicrobial	Ethanol, methylene chloride, ethylacetate, hexane, methanol
P. candolleana Wight & Arn.	Antioxidant, α-glucosidase inhibitory	Ethanol, methanol
P. puberula (DC.) Boiss.	Antimicrobial	Essential oil
P. saxifraga L.	Antioxidant, antimicrobial	Essential oil
P. stewartii Nasir	Antioxidant, acetylcholinesterase	Acetone, water, aqueous, ethanol, ethylacetate
P. thellungiana H. Wolff	Antioxidant	Essential oil

Antioxidant activity

Living organisms synthesize numerous compounds with oxidizing properties due to their metabolic activities. While compounds of this particular book do not exhibit harmful effects at low levels, they can cause cellular damage as their levels increase.[55] The antioxidant defense system plays a role in suppressing the formation of oxidizing compounds, such as reactive oxygen species, in the presence of oxidizing agents. In cases where the antioxidant defense system is insufficient, oxidative stress occurs.[56] Numerous diseases such as Alzheimer's, Parkinson's, multiple sclerosis, cancer, and cardiovascular disorders may manifest due to oxidative stress.[57,58] Supplementation with antioxidants can be utilized to reduce the effects of oxidative stress.[59] Plants are considered significant natural sources of supplementary antioxidants.[60]

The literature reports the values of protein (13.35-28.73%), lipids (2.66-9.02%), carbohydrate (9.87-61.09%), crude fibers (2.83-33.50%), moisture (4.9-80.42%), and ash (5.69-19.14%) for Pimpinella species.[28-32] Furthermore, it has been reported that Pimpinella species contain Mg (6.45-478.6 mg/kg), K (93.13-6332 mg/kg), Na (5.44-365.10 mg/kg), Fe (0.15-1512 mg/kg), Ca (12.64-3141 mg/kg), Zn (0.12-14.31 mg/kg), and Cu (0.029-0.035 mg/kg).[30, 32-34] Within this context, it is believed that Pimpinella species may serve as a natural source of nutrients and minerals.

According to the literature, the antioxidant effect of the ethanol extract of *P. anisoides* collected from Italy was reported using the DPPH assay, with an LC50 value of 3.02 mg/mL.[35] The antioxidant activities of *P. anisum* extracts obtained from water and ethanol sourced from Türkiye were reported using various methods, including reducing power, superoxide anion scavenging, free radical scavenging, metal chelating, scavenging of hydrogen peroxide, and total antioxidant activity. According to the test results, it has been reported that plant extracts exhibit high antioxidant activities.[37] According to a study conducted in Egypt, the ethanol and aqueous extracts of *P. anisum*'s seed and aerial parts exhibited DPPH activities ranging from 13.7% to 91.3% at concentrations of 0.05-0.3 mg/mL.[46] It has been reported that the volatile oil of *P. anisum* collected from Greece exhibited antioxidant activity with DPPH and ABTS assay values of 48% and 18.6%, respectively.[44] According to reports, the IC50 values of the methanol, volatile oil, polar subfraction, and non-polar subfraction of *P. aurea* collected from Iran were found to be between 108-549 µg/mL in the DPPH test. Furthermore, it has been reported that the LC values of β-carotene/linoleic acid test results vary between 5.98% and 65.87.[47] It has been reported that the ethanol extract of *P. brachycarpa* collected from South Korea exhibits a DPPH anion scavenging activity value was 8.80 mg/g, ABTS cation scavenging activity value was 63.53 mg/g, and a reducing power test result was 0.85.[49] According to reports, the IC50 value of *P. candolleana* extracts collected from China, including petroleum ether, methanol, and ethanol, varied between 25.46-27.20 µg/mL in the DPPH test, 9.23-21.69 µg/mL in the ABTS test, and 138.88-919.84 µmol/g in the FRAP test.[50] It has been reported that the IC50 value of the volatile oil of *P. saxifraga* collected from Tunisia for DPPH radical scavenging activity is 6.81 µg/mL, the IC50 value for β-carotene bleaching inhibition test is 206 µg/mL, the EC50 value for ferric reducing power test is 35.20 µg/mL, and the total antioxidant activity test result is 213.96 µmol/mL.[52] The DPPH values of aqueous and acetone extracts of *P. stewartii* collected from Pakistan were reported as 61.08% and 62.39%, respectively, ferrous ion chelating values were reported as 61.16% and 50.76%, and hydrogen peroxide scavenging values were reported as 25.18 and 52.59%, respectively. In addition, the hydroxyl radical scavenging value was reported 0.091 and 11.70%, respectively, the phosomolybdenium complex assay value was 56.55 and 86.26 µM/100 g, and the ferric ion reducing antioxidant power test result was 23.28 and 14.24 µM/100 g, respectively.[32] According to a study conducted in Kazakhstan, the volatile oil obtained from the root and aerial parts of *P. thellungiana* exhibited anti-radical activity ranging from 2.9% to 12.7% at concentrations between 0.1-1 mg/mL.[54] Upon reviewing the literature on antioxidant activity studies conducted on *Pimpinella* species within this scope, it is observed that *Pimpinella* species exhibit high antioxidant properties. It is believed that Pimpinella species may serve as a natural source of antioxidants within this context.

Antimicrobial activity

There is an increasing trend in the incidence of diseases caused by microorganisms.[61] The emergence of resistant microorganisms due to improper medication use renders the antimicrobial drugs used insufficiently. In this context, researchers are directing their attention toward novel antimicrobial sources.[62] The potential side effects of synthetic drugs have led individuals to turn towards natural antimicrobial agents. Within this context, plants are significant sources of natural antimicrobial

agents.[63,64] In this study, the antimicrobial potential of *Pimpinella* species was compiled based on literature data (Table 2). It has been reported that the water and ethanol extracts of *P. anisum* collected from Türkiye exhibit inhibition zones ranging from 7-11 mm against *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Citrobacter koşeri, Enterobacter aerogenes, Staphylococcus aureus, Streptococcus pneumoniae, Micrococcus luteus, Staphylococcus epidermidis*, and *Candida albicans*.[37] It has been reported that the volatile oil and methanol extracts of *P. anisum* collected from Iraq exhibited the most favorable outcomes against *Staphylococcus aureus, Bacillus cereus*, and *Proteus vulgaris* at a concentration of 62.5 µg/mL.[39] According to a study conducted in Egypt, ethanol and aqueous extracts of *P. anisum*'s seed and aerial parts exhibited inhibition zones ranging from 2.7-21 mm against *Bacillus cereus, Staphylococcus aureus, Salmonella typhimuriu*m, and *Escherichia coli* at concentrations ranging from 1.25-5 mg/mL.46 It has been reported that silver nanoparticles produced using aqueous extracts of *P. anisum* collected from Saudi Arabia exhibit minimum and average diameter values ranging from 3.2 to 16 nm against *Acinetobacter baumannii, Klebsiella pneumoniae, S. typhi*, and *P. aeruginosa*.[43] According to the literature, *P. anisum* collected from Iraq effectively against *S. aureus, B. cereus*, and *E. coli* at a concentration of 31.2 µg/mL. Additionally, it was effective against P. mirabilis and *K. pneumonia* at a concentration of 62.5 µg/mL, against *Candida albicans* at a concentration of 500.0 µg/mL, and against *Pseudomonas aeruginosa* at a concentration greater than 500.0 µg/mL.[40] It has been reported that the volatile oil of P. aurea collected from Iran exhibits inhibition zones ranging from 10-16 mm against *K. pneumoniae, P. vulgaris, S. dysenteriae*, and *S. aureus.*[47] It has been reported that the ethanol extract of *P. brachycarpa* collected from South Korea exhibits antimicrobial effects against *S. aureus* and *B. subtilis*.[49] A study reported from South Korea indicates that P. brachycarpa has MIC and MFC values of 0.25 mg/ml and 0.5 mg/ml, respectively, against *Aspergillus niger*.[48] It has been reported that the inhibition zone values of *P. aeruginosa, Bacillus cereus, Micrococcus luteus, S. aureus, Yersinia entrocolitica*, and *C. albicans* vary between 3-36 mm when exposed to the volatile oil of *P. puberula* collected from Iran.51 It has been reported that the volatile oil of *P. saxifraga* collected from Tunisia has MIC values ranging from 0.78 to 3.125 mg/mL against *E. coli, S. typhimurium, P. aeruginosa, B. cereus, M. luteus*, and *L. monocytogenes*.[52] Upon examination of the studies reported in the literature regarding *Pimpinella* species, it is evident that these plants possess significant antimicrobial potential. Within this context, it is believed that *Pimpinella* species may serve as natural antimicrobial agents.

Other activities

The literature has reported that *Pimpinella* species exhibit various biological activities in addition to their antioxidant and antimicrobial activities (Table 2). The ethanol extract of *P. anisoides* collected from Italy was utilised to investigate its anti-inflammatory effect on murine monocytic macrophage cell line RAW 264.7. It has been reported that the working outcome possesses an IC50 value of 72.7 µg/mL *in vivo*.[35] It has been reported that silver nanoparticles produced using aqueous extracts of *P. anisum* collected from Saudi Arabia showed minimal negative effects on cell proliferation, without any changes in cell viability, when tested against a skin stromal cell line (hSSC) and a cancer cell line (HT115) at various concentrations of less than 10 µg. Furthermore, it has been reported to lead to increased cytotoxicity at doses exceeding 10 µg.[43] It has been reported that the EC50 values of the essential oil of *P. anisum* collected from Greece against HepG2, Caco2, MCF-7, and THP-1 cell lines were 0.39, 0.25, 0.3, and 0.11 mg/mL, respectively.[44] A study in Israel reported that the ethanol extract of *P. anisum* exhibited robust proliferation on rat skeletal muscle cell line (L6) and human prostate cancer cell line (PC-3) at an IC50 value of 400 µg/mL.41 The hydroalcoholic extract of *P. anisum* collected from Brazil was utilised to determine its antispasmodic effect on the anococcygeus smooth muscle of rats. According to the study findings, it has been reported that the extract used inhibited the contraction caused by acetylcholine at a concentration of 50 µg/mL.[38] In a study conducted in India, the IC50 values of the highest α-amylase and α-glucosidase effects of the ethyl acetate fraction of *P. anisum* were reported as 0.12 and 0.15 mg/mL, respectively.[42] A study conducted in India reported that the volatile oil of *P. anisum* exhibited inhibitory effects against Potato virus X, Tobacco mosaic virus, and Tobacco ringspot virus at a concentration of 300 ppm.36 It has been reported that the insecticidal effect of the essential oil of *P. anisum* collected from Egypt against the red flour beetle, *Tribolium castaneum*, has an LC50 value of 9.3% v/v.[45] It has been reported that the petroleum ether, methanol, and ethanol extracts of *P. candolleana* collected from China exhibit α-

glucosidase inhibitory activity with an LC50 value ranging from 4.42-68.71 µg/ml, attributed to the chemical compounds present in *P. candolleana*.[50] The IC50 values of the acetylcholinesterase activity of the water and ethyl acetate fractions of *P. stewartii* collected from Pakistan were reported as 72.6 and 30.41 µg/mL, respectively.[53]

CHEMICAL CONTENTS

In plants, numerous biologically active compounds are synthesised within their structures. Numerous studies have reported significant biological activities of compounds found in plants.[65] In this study, the chemical compounds identified in *Pimpinella* species in the literature have been compiled. Within this context, it has been observed that studies have been conducted on the aerial, stem, and seed parts of *Pimpinella* species in the literature. The findings obtained were presented in Table 3.

Table 3. Chemical contents of Pimpinella species.[35, 51, 54, 66-78]

Plant species	Geographic regions	Used Parts	Chemical contents
P. acuminata (Edgew.) C.B. Clarke	India, Pakistan	Aerial, stem	β-caryophyllene (12.5%), dill apiole (11.3-20.4%), parsley apiole (39.9-61.8%), myristicin (16.2%), methyl coniine (70.0%), coniine (4.0%), n-pentadecane (4.0%), 1-methyl-2-pentyl piperidene (3.3%), heptadecane (3.0%), apiole (1.5%)
P. anisoides V.Brig.	Italy	Aerial	Trans-anethole (54.5%), limonene (13.5%), sabinene (4.4%)
P. anisum L.	Estonia, Serbia, Iran, Brazil, Algeria, Pakistan, Egypt	Aerial, seed	Trans-anethole (65.6–93.7%), γ-himachalene (0.4–8.2%), trans-pseudoisoeugenyl 2-methylbutyrate (0.4–6.4%), p-anisaldehyde (<1-5.4%), methylchavicol (0.5–2.3%), cis-isoeugenol (1.99%), linalool (1.79%), (E)-anethole (80.7%-90.35%), estragole (1.9-5.6%), eugenyl acetate (3.34-3.92%), α-zingiberene (1.9%), cis-pseudoisoeugenyl 2-methylbutyrate (~3%), o-isoeugenol (1.9%), fenchone (5.6%), camphor (3.1%)
P. aurea DC.	Iran	Aerial, stem, seed	Limonene (8.9-21.4%), viridiflorol (12.8-37.0%), α-pinene (11.5%), kessane (10.5%), germacrene D (4.9%), β-bisabolene (4.2-50.8%), α-zingiberene (3.3%), citronellyl acetate (3.1%), caryophyllene oxide (6.6%), 1,8-cineol (8.9-21.4%), estragol (5.1%), trans-a-bergamotene (72.8%)
P. puberula (DC.) Boiss.	Iran	Aerial, stem, seed	Limonene (21.7-82.4%), pregeıjerene (14.6-55.4%), geijerene (7.2-11.7%)
P. saxifraga L.	Iran	Aerial	Trans-α-bergamotene (20.1%), β-sesquiphellandrene (10.8%), β-bisabolene (10.1%)
P. thellungiana H. Wolff	Kazakhstan	Aerial, stem	Hexenal (0.2-8.9%), β -pinene (0.4-4.1%), undecane (1.5-15.6%), geijerene (0.6-9.2%), 9-Methyl-10-methylenetricyclo[4.2.1.1(2,5)]decan-9-ol (3.4%), cis - β -farnesene (0.3%-12.6%), β -bisabolene (1.6-18.8%), spathulenol (3.3%), caryophyllene oxide (0.7-4.3%), 2-methyl-4-methoxy-2-(3-methoxyoxiranyl)phenylbutanoate (0.4-29.5%)
P. tragium Vill.	Iran	Aerial, stem	Germacrene D (6.2-34.7%), germacrene B (14.1-18.3%), bornyl acetate (4.1-15.8%), β-caryophyllene (4.8-7.3%) β-pinene (4.5-25.3%), hexadecanol (4.7-10.3%), sabinene (13.6%)

In the literature, the main components in the essential oil content of *P. acuminata* species are β-caryophyllene (12.5%), dill apiole (11.3-20.4%), parsley apiole (39.9-61.8%), myristicin (16.2%), methyl coniine (70.0%), Coniine (4.0%), n-pentadecane (4.0%), 1-methyl-2-pentyl piperidene (3.3%), heptadecane (3.0%), and apiole (1.5%) have been reported.[66,67] It has been reported that the main components in the essential oil content of P. anisoides species are trans-anethole (54.5%), limonene (13.5%) and sabinene (4.4%).[35] It has been reported that the main components in the essential oil content of P. anisum species are trans-anethole (65.6–93.7%), γ-himachalene (0.4–8.2%), trans-pseudoisoeugenyl 2-methylbutyrate (0.4–6.4%), p-anisaldehyde (<1-5.4%), methylchavicol (0.5–2.3%), cis-isoeugenol (1.99%), linalool (1.79%), (E)-anethole (80.7-90.35%), estragole (1.9-5.6%), eugenyl acetate (3.34-3.92%), α-zingiberene (1.9%), cis-pseudoisoeugenyl 2-methylbutyrate (~3%), o-

isoeugenol (1.9%), fenchone (5.6%) and camphor (3.1%).[68-74] It has been reported that the main components in the essential oil content of *P. aurea* species are limonene (8.9-21.4%), viridifluorool (12.8-37.0%), α-pinene (11.5%), chestnut (10.5%), germacrene D (4.9%), β-bisabolene (4.2-50.8%), α-zingiberene (3.3%), citronellyl acetate (3.1%), caryophyllene oxide (6.6%), 1,8-cineol (8.9-21.4%), estragole (5.1%) and trans-a-bergamotene (72.8%).[75,76] It has been reported that the main components in the essential oil content of *P. puberula* species are limonene (21.7-82.4%), pregeijerene (14.6-55.4%) and geijerene (7.2-11.7%).[51] It has been reported that the main components in the essential oil content of *P. saxifraga* species are trans-α-bergamotene (20.1%), β-sesquiphellandrene (10.8%) and β-bisabolene (10.1%).[77] It has been reported that the main components in the essential oil content of P. thellungiana species are hexenal (0.2-8.9%), β -pinene (0.4-4.1%), undecane (1.5-15.6%), geijerene (0.6-9.2%),9-Methyl-10-methylenetricyclo [4.2.1.1 (2.5)]decan-9-ol (3.4) %), cis - β -farnesene (0.3-12.6%), β -bisabolene (1.6-18.8%), spathulenol (3.3%), caryophyllene oxide (0.7-4.3%) and 2-methyl-4-methoxy-2-(3-methoxyoxiranyl) phenyl-butanoate (0.4-29.5%).[54] (Suleimen et al., 2017). It has been reported that the main components in the essential oil content of *P. tragium* species are hexadecanol (4.7-10.3%), germacrene D (6.2-34.7%), germacrene B (14.1-18.3%), bornyl acetate (4.1-15.8%), β-caryophyllene (4.8-7.3%) β-pinene (4.5-25.3%), and sabinene (13.6%).[78] In this context, based on the literature data, it has been reported that the 5 highest reported compounds in *Pimpinella* species are trans-a-bergamotene (72.8%), methyl coniine (70.0%), trans-anethole (65.6–93.7%), (E)-anethole (80.7-90.35%), and limonene (21.7-82.4%). In this context, it is thought that *Pimpinella* species may be a natural source for the compounds reported in its body.

CONCLUSION

This study compiles the literature on Pimpinella species reported in previous studies. Within this scope, the general characteristics, mineral and nutrient contents, areas of use, biological activities, and chemical compositions have been compiled. Based on the literature review conducted, it has been determined that Pimpinella species are commonly used in traditional medicine. Furthermore, it is believed that the reported minerals, nutrients, and chemical contents within it could potentially serve as a natural resource. Additionally, it has been reported in the literature that *Pimpinella* species exhibit high antioxidant and antimicrobial activities. As a result, it has been observed that in addition to the nutritious properties of *Pimpinella* species, they could be significant natural materials in pharmacological designs in future studies.

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REVIEW

Acupuncture for Obesity

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Abstract
In this review study, we aimed to show the effects of acupuncture on obesity in light of recent studies. Obesity is a significant health problem that is accepted as a risk factor for many diseases, such as diabetes, hypertension, hyperlipidemia, cardiovascular disease, and fatty liver and has become an epidemic worldwide. Acupuncture is a form of treatment applied by inserting needles into specific points on the body for therapeutic purposes. There are still uncertainties about whether acupuncture practices alone are effective in treating obesity. In addition, there is evidence that it stimulates some anorexigenic hormones, inhibits some orexigenic hormones, and, as a result, suppresses appetite. It has been reported in the literature that these effects of acupuncture are much more effective when applied together with diet and exercise. Acupuncture reduces the appetite of obese patients, increases their compliance with diet and exercise, and reduces the anxiety caused by hypocaloric nutrition with the anxiolysis effect it creates. It also accelerates metabolism by providing homeostasis in the body and enables patients to lose weight. In these multidisciplinary treatment approaches, it would be very beneficial to include acupuncture treatment, one of the complementary and alternative treatment methods for treating obesity.
Keywords: Acupuncture, Obesity, Treatment.

INTRODUCTION

Obesity is a significant health problem that is accepted as a risk factor for many diseases, such as diabetes, hypertension, hyperlipidemia, cardiovascular disease, and fatty liver and has become an epidemic worldwide.[1] According to the data of the World Health Organization, there are 1.9 billion overweight and 650 million obese individuals worldwide. Again, 41 million children under 5 are overweight or obese.[2]

Obesity is not just a health problem caused by the problem of self-control. It occurs due to excessive accumulation of body fat, which is caused by many factors (genetic predisposition, environmental factors, individual behavior) based on obesity and is mainly caused by problems in energy metabolism and appetite regulation.[3,4] There are many different methods used in the treatment of obesity. These treatment methods are applied individually or as a combination treatment. These; can be listed as diet therapy tailored to the individual, providing adequate physical activity, necessary psychological support, appropriate behavioral therapy, pharmacotherapy, complementary/alternative treatments, and bariatric surgery treatment if necessary. The first step in treating obesity is to make the proper lifestyle change. The next step is to question whether the person sleeps enough and report that at least 7 hours of sleep will positively affect weight loss by calculating the amount of water the person should take daily. In addition, acupuncture, one of the oldest treatment methods of Traditional Chinese Medicine, has a significant place in treating obesity. One of the mechanisms of action of acupuncture is; the effects of endogenous opioid peptides. It is known that acupuncture has analgesic effects. After acupuncture application, beta-endorphin (BE), one of the endogenous opioids, rises in the plasma and affects the metabolism. It is known that beta-endorphin, one of the endogenous opioids rising in the plasma with abdominal and ear acupuncture application, affects lipid metabolism by increasing lipase activation and causing lipoproteinemia.[5,6] It is known that acupuncture applied to specific points causes weight loss by controlling the feeling of hunger.[7,8]

History of Acupuncture

Acupuncture is a form of treatment applied by inserting needles into specific points on the body for

therapeutic purposes. Consists of two Latin words (acus, needle, puncture, prick).[9] Acupuncture is one of the methods of complementary and alternative medicine that originated in ancient Chinese medicine. Known as the life energy, Qi circulates in the meridians in the body. Acupuncture is a treatment method based on stimulating some special points in the ear or body to regulate this energy excess or deficiency in diseases. The National Center for Complementary and Alternative Medicine (NCCAM), affiliated with the United States National Institute of Health (NIH), was established in 1998 to investigate the safety and effectiveness of acupuncture practices in the world and to ensure the participation of practices in treatments for diseases whose effectiveness is accepted.[10]

According to Traditional Chinese Medicine, the body's Yin and Yang is the delicate balance of two opposing and inseparable forces that explain the workings of the entire universe. According to this theory, the yin and yang poles are never fixed. Therefore, they are not absolute. On the contrary, they are constantly changing and transforming. Opposites always exist together. No situation does not have an opposite pole. Examples of poles are night and day, light and dark, cold and warm, inside and outside, near and far, short and long. In the darkest hour of the night, the day begins. In most mania state, depression is expected to be triggered. The poles are always in a mutually producing-consuming or supporting-restricting relationship. Poles can consume and constrain each other in the same way they produce support. If yin is increasing in the body, it will harm yang; if yang is increasing, it will harm yin. A yang-influenced disease at the beginning can turn into a yin-influenced disease; A yin-effect disease can also have a yang-effective course. In Chinese Medicine, the yin or yang nature of the diseases is an essential factor in determining the treatment strategy.[11,12]

According to Traditional Chinese Medicine, health is achieved when the body is balanced. The disease occurs due to the internal imbalance of Yin and Yang. This imbalance causes Qi (Vital Energy) to be blocked in pathways known as meridians. According to Traditional Chinese Medicine, the blockage in Qi can be opened by applying acupuncture to specific points associated with the meridians. Many sources define meridians as 14 main channels formed by networking of at least 2000 acupuncture points.[13,14]

It is claimed that the primitive practices of acupuncture in Chinese history date back to much earlier than written periods. It is known that sharp-edged stones called bian were used long before needles. Over time, needles made of bone or bamboo began to be used instead of Bian stones. When the bronze casting technique was developed, the use of bronze needles emerged, and the use of metal needles gained importance. Dabry (1853) and Morant (1927) first introduced acupuncture to the West. The first European countries to use acupuncture are France and Germany. The electro-acupuncture technique, which is based on the stimulation of acupuncture points with electrical instruments, was developed in France in 1825.[14]

Effect Mechanisms of Acupuncture

1965 Melzack and Wall introduced the door control theory (Figure 1). According to this theory, small-diameter nerve fibers carry the pain impulse through a gate mechanism. In contrast, large diameter fibers pass through the same gate to inhibit the signal carried by the smaller ones. Depending on whether the door is open or closed, the brain releases various neurochemicals in response to the pain stimulation it receives. For this reason, pain signals can be prevented by stimulating the periphery of the pain area to stimulate the relevant areas in the brain stem or cerebral cortex, or the nerves in the muscle.[13] The neural plate theory, on the other hand, is a slightly more advanced version of the gate control theory, which includes the effects of opioids. Opioids are a group of endogenous chemicals that bind to opioid receptors and inhibit nerve activity and pain transmission in the substantia gelatinosa part of the spinal cord. Stimulation of peripheral nerves in the muscles sends impulses to the central nervous system and stimulates the release of endorphins from the hypothalamic-pituitary axis. It is thought that due to the increase in endorphins in the cerebrospinal fluid, endorphins increase in the neuromuscular junction or nerve endings and affect the sense of pain with the analgesic effect.[15]

An opiate antagonist of endorphins, naloxane has been used to demonstrate the analgesic effect of acupuncture. It has been shown that administration of naloxane before acupuncture destroys the effect of acupuncture.[15] The present invention demonstrates that the effects of endorphins are through opiate receptors.

The release of beta endorphins, a type of endorphin, is associated with the release of ACTH. ACTH releases cortisol from the adrenal cortex. Some of the anti-inflammatory effect of acupuncture may be related to cortisol. ACTH and cortisol are important

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in substance abuse and substance withdrawal. ACTH and cortisol levels are high in abstinence in substance addicts. Acupuncture may be effective in addiction by reducing the release of these chemicals.[14-16]

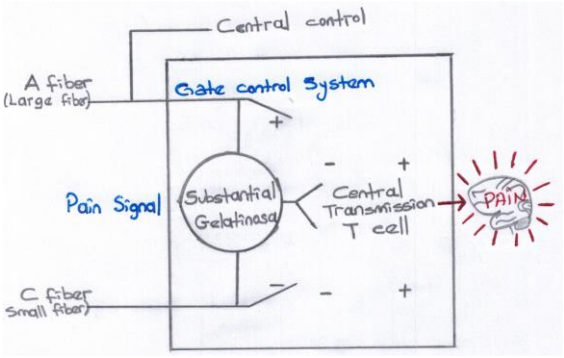

Figure 1: Door control theory

EFFECTS OF ACUPUNCTURE

Immunity enhancing effects

Acupuncture increases body resistance to many diseases. It produces many different changes in the levels of leukocytes, opsonins, kinins, and antibodies. It provides regulation of immune activity. Studies have been carried out on the effect of acupuncture, especially on T-Lymphocytes, and it has been found that it has an increasing impact on the release of interferon from lymphocytes. The leukocyte count increases approximately three hours after acupuncture, and this increase continues for 24 hours. An increase in phagocytic activity is also seen in hepatic cells. In particular, stimulation of the Large Intestine-4 (Li-4) point, which has 360 receptors, and the Stomach-36 (St-36) points, increases the number of T-helper cells.[17,18]

Anti-inflammatory effects

ST 36 is one of the most critical points for balancing Qi and Blood and supporting overall health. In a study investigating how acupuncture at the ST36 acupuncture point regulates inflammation and its underlying mechanisms, acupuncture at ST36 has been found to have clinical benefits in attenuating inflammation through several means, including vagus nerve activation, toll-like receptor 4 (TLR4)/NF-κB signaling, macrophage polarization, and mitogen-activated protein kinase (MAPK).[19]

Sedative effects

Acupuncture therapy also has sedative effects. It'sIts sedative effect can be combined with other techniques in complex conditions such as depression, anxiety, and insomnia. The sedative effect occurs due to changes in brain chemicals such as dopamine and serotonin. Anxiety, believed to be caused by hyperactivation of the sympathoadrenal system, can be relieved with endorphins. Anxiety can be reduced by inhibiting the tone of the sympathoadrenal system by acupuncture and making endorphins secreted.[18-20]

According to the literature, acupuncture is effective in reducing anxiety related to cataract surgery under topical anesthesia.[21]

Homeostatic effects

The autonomic nervous system constantly and automatically controls the internal organs. Sympathetic and parasympathetic nerves mediate this control. With the application of acupuncture, both sympathetic and parasympathetic effects are created. Heart rate, blood pressure, respiratory system, and endocrine system can be affected by acupuncture application. Particular nerves are stimulated when specific skin points suitable for the patient's complaints are produced to heal the disease. Through these, electrical impulses first go to the spinal cord, then to the lower center of the brain, and then to the diseased area. As a result, acupuncture provides homeostasis through the autonomic nervous system, regulates the water and electrolyte balance, and turns hypotension and hypertension into normotension by regulating the vascular system. In addition, it regulates the heartbeat with impaired blood sugar, regulates sweating and body temperature, and regulates urinary and stool excretion.[18]

Uses of acupuncture

Today, acupuncture effectively treats many health problems, such as pain, cardiovascular diseases, neurological diseases, obesity, depression/anxiety, and asthma. It is noteworthy that it is trendy today and has an intense use.[22]

There are many studies in the literature in which acupuncture is used to treat various pains.[23-25] In a meta-analysis of forty-nine randomized controlled studies, it was reported that the positive effects of laser acupuncture in treating musculoskeletal pain were prominent in approximately two-thirds of the studies, and these effects were more evident in long-term follow-up.[23] In a meta-analysis study in which 1218 participants were examined, it was reported that acupuncture was more effective than pharmacological agents in terms of analgesic effect in the treatment of migraine.[26]

In a systematic review investigating acupuncture treatment in cardiovascular diseases, a total of 17 studies from the last 20 years were examined, and it was stated that acupuncture could be a complementary and alternative treatment for many cardiovascular diseases, especially coronary artery

disease and hypertension.[27]

It is known that acupuncture also has positive effects on asthma. In a case report of a 45-year-old male individual being followed up with the diagnosis of asthma, It has been reported that acupuncture treatment applied to the patient for 12 sessions caused a significant reduction in the patient's complaints and contributed to maintaining well-being.[28]

Acupuncture treatment is also an alternative for brain damage or neurological degenerative disorders. Behavioral and neural responses of 10 outpatients with Parkinson's disease were examined before and after 8-week electroacupuncture treatment, and it was reported that the treatment had beneficial clinical effects.[29]

Acupuncture in obesity treatment

There are many studies in the literature regarding the use of acupuncture in treating obesity.[30,31] Various hypotheses have been proposed regarding the effects of acupuncture treatment on obesity. These hypotheses are primarily based on the regulation of the neuroendocrine system. It has been reported that the effects of acupuncture on obesity occur in the form of decreased food intake and body weight loss due to the inhibition of some orexigenic factors, such as Neuropeptide-Y and ghrelin, and the expression of anorexigenic factors such as leptin.[31] Acupuncture may play a role in regulating the effects of the hypothalamus-pituitary-adrenal cortex and sympathetic adrenal cortex; In addition, it has been reported that it can regulate lipid metabolism by reducing serum triglyceride, total cholesterol and low-density lipoprotein (LDL), and reduce bacteroids in the body, thus being effective in the treatment of obesity.[32]

In a systematic review of 12 randomized controlled studies investigating the efficacy of acupuncture and body weight loss in Asian individuals and 1151 participants, it was reported that acupuncture significantly reduced BMI and waist circumference. In addition, it has been reported that the combination of medical nutrition therapy-exercise-acupuncture is more effective than diet and exercise alone and that acupuncture monotherapy or acupuncture-exercise combination does not make a significant difference compared to exercise alone.[33]

In a pilot study in which ten healthy adults were examined, it was reported that weekly auricular acupuncture application for a month caused a significant decrease in body weight and active ghrelin levels in individuals compared to sham acupuncture. In the study, individuals' food intake reductions were evaluated over the meals recorded by digital cameras.[34] Similarly, in another study conducted on 66 postpartum obese individuals, individuals were randomly divided into two groups. Five sessions of laser and sham acupuncture were applied to the groups per week, and no intervention was made on the dietary habits of the individuals; instead, food consumption records were taken. It was reported that the laser acupuncture group's BMI and body fat percentage decreased significantly compared to the sham acupuncture group after twelve sessions of application. However, it was stated that there was no significant difference in waist-hip ratio between the two groups, and there was no significant difference in the daily energy intake of the groups.[35]

Appropriate dietary approaches combined with acupuncture

There are still uncertainties about whether acupuncture practices alone are effective in treating obesity. In addition, there is evidence that it stimulates some anorexigenic hormones, inhibits some orexigenic hormones, and, as a result, suppresses appetite. It has been reported in the literature that these effects of acupuncture are much more effective when applied together with diet and exercise. Acupuncture application provides a positive decrease in the patient's appetite in about one week. The patients who will have acupuncture are calculated according to their weight, and the dietitian gives the appropriate diet.[31,34,36,37]

National and international guidelines or guidelines have yet to be published for appropriate weight loss diets to be applied with acupuncture. When the literature is examined, it is seen that the principles on which the diets applied together with acupuncture are based, the amounts of energy and macro-micro nutrients could be more precise. In a study examining the effectiveness of a combination of electroacupuncture and slimming diet program against diet monotherapy in the treatment of obesity, It has been reported that individuals are given diets containing an average of 1400 kcal of energy, with 55-60% of total daily energy coming from carbohydrates, 25-30% from fat, and 15-20% from protein, taking care not to be below basal metabolic rates.[38] In a study investigating the effectiveness of laser acupuncture and a low-calorie diet in treating postmenopausal visceral obesity, it was reported that the daily energy needed to be calculated for individuals should be reduced by 1000 kcal. Therefore, a diet plan containing an average of 1200-1500 kcal energy was made, with 10-15% of

the total daily energy coming from protein, 25% from fat, and 60-65% from carbohydrates. In addition, individuals have been recommended to eat 5-6 meals daily and consume 3-4 liters of fluid.[39]

In many studies, the characteristics of the diets applied with acupuncture were not explicitly mentioned. Instead, it was stated that a nutritionist or dietitian programmed the diet programs, and expressions such as high fiber, energy-restricted, and low energy were used. As understood from all these studies, in medical nutrition therapy approaches applied together with acupuncture; The amount of power given to individuals is considerably lower than the daily needs of individuals.[39-41]

Safety of acupuncture

The recommendation of acupuncture, a complementary and alternative medicine practice, by clinical practice guidelines and guidelines remains to be determined. The studies have not reported severe adverse effects of acupuncture applications on health. In a systematic review of 105 studies on the safety of acupuncture in pregnancy, Park et al. said that most adverse events were mild to moderate. However, it has been reported that the most common negativity is pain caused by needling, and the relationship between severe adverse events and deaths with acupuncture is not specific. In addition, it has been reported that the incidence of adverse events associated with acupuncture is 1.3%, and acupuncture is safe when applied correctly during pregnancy.[42]

The study has negative aspects of acupuncture: organ and tissue injuries, infections, local side effects, and other complications. The most common organ and tissue injuries seen in patients were reported to be pneumothorax, spinal cord injuries, subarachnoid or intracranial hemorrhages, cardiac tamponade, or cardiac injuries. The most common infections in patients are hepatitis, abscess, tetanus, ear infections, etc. It has been reported that the most common local side effects are contact dermatitis, local allergies, argyria, local bleeding, local pain or tenderness, and local burning or bruising. Other common complications in patients were reported to be dizziness, syncope, nausea, vomiting, and epilepsy. For these reasons, it was emphasized that the education level of the acupuncturist should be at a good level.[43]

CONCLUSION AND RECOMMENDATIONS

Acupuncture is a traditional complementary and alternative medicine method that can be applied in various forms, the history of which dates back to the written periods. While the current literature is convincing that acupuncture is safe, it also mentions the existence of some negativities that can be encountered, albeit rare, during the application. It says that acupuncture practitioners should have sufficient education levels in this regard.

The effectiveness of acupuncture in treating obesity has been demonstrated in many studies. However, uncertainties regarding the mechanism of this activity remain. Against the view that acupuncture can be effective alone in treating obesity, the opinion that it is more effective to be applied together with weight loss diet programs has come to the fore in some studies. Data on the principles and contents of weight loss diet programs applied together with acupuncture in the literature still need to be expanded today. However, according to the data reported by these limited sources, the amount of energy contained in these diets can be pretty low. Deficient calorie diets have many known adverse effects on health, particularly micronutrient deficiencies. Suppose such diets are to be applied for a long time. In that case, regular clinical follow-up of these patients' biochemical parameters and body composition by relevant specialists is essential. For these reasons, medical nutrition therapy is applied together with acupuncture treatment in obese individuals by a physician and a dietitian; it must be controlled with individual-specific diets suitable for adequate-balanced nutrition. In addition, well-equipped teamwork is vital to prevent all kinds of negativity, mild or severe, and to provide the best possible treatment to the patients throughout the process; The role of the attending physician in this team is very critical.

Acupuncture reduces the appetite of obese patients, increases their compliance with diet and exercise, and reduces the anxiety caused by hypocaloric nutrition with the anxiolysis effect it creates. It also accelerates metabolism by providing homeostasis in the body and enables patients to lose weight. In these multidisciplinary treatment approaches, it would be very beneficial to include acupuncture, one of the complementary and alternative treatment methods for treating obesity.

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REVIEW

Fever and the Ageing Immune System, A Review

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Abstract
Whether to treat fever in the elderly, is a question that has not yet been answered. This review examines the available evidence so far, to arrive at a comprehensive picture about this question. Aged population are a special category due to their lower basal body temperature, blunted fever response and existing co morbidities. The aging immune system undergoes many changes in all its faculties, which alters its ability to mount an efficient acute inflammatory response. In such a scenario, fever is mostly absent and there is a need to revise the fever criteria in the elderly. Having said that, the most successfully aging, centenarians, mount efficient febrile response during infections, indicating that aging itself may not be the reason for the reduced febrile response. Holistic medicine, such as homeopathy, views the fever scenario differently and advocates interference only when the immune system is helpless. The 'levels of health theory' explains that the healthiest benefit from fever while those with reduced immunity suffer from it. This theory may prove to be an invaluable clinical tool to arrive at definite clinical guidelines concerning the treatment of fever, especially in the elderly. However, it requires scientific investigation before it can be used as a clinical tool.
There is no conclusive evidence on whether fever must be treated or not in the elderly and whether holistic medicine may have a solution to this dilemma. This is a research gap that needs to be filled with quality studies in the current health scenario.
Keywords: Fever, Agieng, Elderly, Older Adults, Immunosenescence.

INTRODUCTION

Evolutionary mechanisms were not designed for longevity and yet, the world is aging today. The need to understand the aging immune system is more relevant than ever before in history. Fever in the elderly requires special consideration because of the inflammaging and immunosenescence that occur with age. This review examines the literature available regarding the special scenario of fever in the elderly in the context of acute inflammatory response and its modification with changes in the aging immune system.

ACUTE INFLAMMATION AND FEVER

The acute inflammatory response (AIR) was developed and maintained through evolution as a defence mechanism against injury and infection.[1] Galen considered it an essential process in healing, whereas Virchow identified it as a pathological process, that may be detrimental.[2] Immunology has come a long way since then and today we know the truth to be lying somewhere in between these two extremes. For example, it is known that AIR is essential for dealing with infecting pathogens effectively but, when it continues beyond necessary or when the response is stronger than required, it is detrimental to the organism.[3]

One of the cardinal features of AIR is fever.[1] Fever is defined as regulated elevation of core temperature achieved through the integrated behavioural,

physiological and biochemical processes that determine the balance between heat generation and elimination.[4] Heat seeking behaviour appeared 600 million years ago, even before the advent of adaptive immunity. Evolution has developed and maintained fever as a means to mount defence response during tissue injury and conserved it despite the heavy metabolic cost, implying a survival advantage from fever.[5]

With breach in tissue integrity by pathogens, the resident immune cells, recognize the foreign antigen, engulf it, and release pro inflammatory cytokines. IL1, in humans, is a pyrogenic cytokine that stimulates the conversion of arachidonic acid into prostaglandins. Prostaglandin E2 (PGE2), thus produced, acts upon the preoptic area of the hypothalamus through EP3 receptors to generate fever, which has manifold roles to play during AIR.[6] It acts as a systemic alarm whereby all the components of the immune system are primed for inflammation and neutralization of the pathogen.[7–10]

FEVER – THE YAY AND THE NAY

To begin with, febrile temperature was found to be detrimental to most pathogens and immune cells such as macrophages require febrile range temperature for efficient functioning.[11,12] Both the innate and adaptive immune systems of most organisms are programmed to respond with defensive functions at febrile range temperatures.[13] Increased recruitment of lymphocytes to the site of injury or infection occurs and the fever range thermal stress also acts on distinct cell types and regulates the adhesion cascade, necessary for lymphocytes to reach the site of infection. The 'lymphocyte – high endothelial venules - IL6' axis is responsive to thermal element of fever and improves immune surveillance in secondary lymphoid organs.[8] The lymphocytes neutralize the pathogens through aerobic and anaerobic killing, thus completing the task.[2]

Many studies have shown the benefit of survival from fever in an infected host.[2] Pathogen clearance is enhanced by fever and hindering fever during infections with antipyretic drugs has been shown to increase mortality in a trial.[13,14] Many researchers have used fever induction as a therapy for cancer and it has been observed, albeit not definitively, that children with autism improved considerably in their behaviour during a fever.[2,15,16]

Evidence suggests that the most important sequel to inflammation, viz. resolution of inflammation requires an efficient acute inflammatory response to be activated.[17] Studies in the last two decades have shown that the return of the tissue environment and the immune system to the normal state is an active process and does not passively result from removal of the pathogen.[18,19] If this fails, the tissue environment fails to return to its normal state as the pro inflammatory factors remain in the tissue environment, leading to the activation of chronic inflammatory phenomenon.[20,21] The acute inflammatory response itself has various checkpoints that it must pass through for the downstream resolution to be stimulated.[17,22] For example, PGE2, a *proinflammatory* cytokine, responsible for fever generation as stated, after the neutralization of bacteria, turns *anti-inflammatory* and signals for the phagocytosis of apoptotic neutrophils and their efferocytosis from the tissue environment. This ensures onset of the resolution.[23] In addition, febrile temperature of >38ºC inhibits further production of pro inflammatory cytokines but not anti-inflammatory cytokines, ensuring smooth resolution and return of homeostasis.[24]

Many theories have proposed that interfering with the acute inflammatory process may result in the activation of chronic inflammatory diseases, which may contribute to the increasing trend of non-communicable diseases today.[25–27] The Immune system is constantly reorganizing itself to identify optimal functioning points.[28] When its efforts are thwarted by drugs, its response is subnormal, which may increase the inflammatory stimulus and in a deeper tissue than otherwise would have occurred.[21] This may lead to chronic inflammation in the deeper/more vital organ systems.

Despite evidence of fever as a beneficial phenomenon, there is widespread practice of suppressing fevers.[29–32] However, while exaggerated, or sometimes misplaced due to anxiety, putative damage from fever does exist.[33,34] Fever causes heavy metabolic burden and may cause death in people with compromised cardiorespiratory reserve such as the frail elderly.[13,31,35,36] In children, it is known to cause febrile convulsions and behavioural alterations.[10] Organ damage due to cell death and protein synthesis impairment is a real danger in high fevers.[37,38] Even though pathogen load decreased, febrile mice could still die from high fevers and fever in post-operative scenario was fatal.[13,39] In many conditions of decreased cardiorespiratory respiratory reserve, such as cardiac arrest and ischemic stroke, therapeutic hypothermia is practiced to avert major organ damage.[40,41] In fact,

in many situations, naturally occurring hypothermia was observed during infections which seemed to confer a survival advantage.[42]

Such opposing and equally strong evidence for and against fever behoves immunologists to investigate this further. Bhavani et al., have shown that fever patterns are not uniform in sepsis, and each pattern has a different outcome. The basic state of the individual seems to have a great bearing on the temperature developed, the effect on pathogen and on the host.[43] Therefore, many researchers believe that fever must be considered in each individual case before deciding the therapy.[44,45] The benefit seems to lie in a balance between pathogen clearance and tissue injury.[46]

IMMUNOSENESCENCE

The evolutionary theory of aging posits that evolution never really programmed for longevity. Its focus was on the continuity and survival of the species.[47] Therefore, many adaptive genes selected for optimal functioning and defence such as acute inflammatory response with high metabolic cost are required for the preservation of the individual until the reproductive age.[48] Post reproductive age, the same genes turn maladaptive and aging sets in as a chronic inflammatory process, termed as *antagonistic pleiotropy*.[48,49] This implies a regulated progressive decline of the organism. However, immunological studies have shown that it may not be as simplistic as that.[28] There are many other theories that propose different factors as instrumental in aging. For example, the *network theory*, considers the aging process to be controlled by various defence functions and their influence on the organism.[28,50] Each organism is subject to various stressors, physical, chemical, biological, and radiational. The network of defence against these stressors in an organism includes antioxidants (e.g., superoxide dismutase), heat shock proteins, Poly ADP Ribosyl Polymerase (PARP), DNA repair enzymes and other stress proteins. Genes that regulate these factors are stimulated by stress and the organism copes with stress. These stresses are beneficial to the organism at low levels and maintain the immune system in shape (hormetic effect). However, when they become overwhelming, they have a detrimental effect.[28] When the organism is unable to cope, macrophage activation occurs, resulting in a subclinical chronic inflammatory state. The theory further suggests that the outcome of longevity is a balance between the hormetic and detrimental effects of stress. Better coping capability implies increased longevity as seen in centenarians.[28] Chronic inflammation itself may not be responsible for frailty or decreased life span as it is present in both healthy and the diseased elderly. However, the ability to cope with pro-inflammatory status may make all the difference. There is a genetic component that decides the capacity to cope with stress. The theory suggests there are two such genetic factors involved – one that is responsible for inflammatory response – adding up over time and the second that confers robustness or frailty. This implies a combination of high inflammatory status and frailty gene will result in unsuccessful aging.[28]

Other theories of aging consider metabolic activity itself as responsible for aging - Effect of accumulation of cellular debris and telomere shortening over the years. Similarly, cellular oxidation products accumulating in the cells with increased reactive oxygen species are responsible for aging.[28,50,51]

They all seem to consider facets of a complex phenomenon and it appears that all of them are probable. A common thread running through all these theories is the existence of chronic inflammation in the process – a cause, or result of aging, known as *inflammaging*.[28,49,50,52-54] This is evidenced by changes in the immune system that is seen commonly in the elderly.[44,55,56] There is a global reduction in the activity of the innate, cellular, and humoral immunity. All elderly, whether healthy or with the disease, demonstrate increased IL6 – an indicator of inflammation. Thymic involution with age implies a decrease in lymphocytes.[57] Lymphocytes are reduced in number and changed in composition so that naïve T cells are lacking but activated T cells are increased in proportion in the elderly.[55] Cytokine production is predominantly Th2, and there is an increased expression of Cellular Adhesion Molecules (CAM).[49,55,56] Almost all immunoglobulins are expressed more in the elderly (except IG4) and so are non-organ-specific autoantibodies, while organ-specific autoantibodies are decreased. B lymphocytes, Natural Killer cells, and tissue-resident macrophages are decreased. Somatic cells reach a cell proliferation exhaustion, and lymphocytes cannot undergo clonal expansion during a pathogen challenge.[52,55]

These changes are relevant clinically as the elderly are shown to be more susceptible to infections with a lack of optimal response leading to serious complications of infections and increased mortality. This is especially so with novel pathogens. While the elderly maintain a good defence against known

pathogens, the inability for clonal expansion of T cells translates to subnormal defence to novel pathogens. Other factors such as predominant Th2 response and lack of febrile response also contribute to the severity of infections. The existence of comorbidities in the aged is associated with an increased risk of infections.[55] However, it was demonstrated through studies in centenarians that the process of aging is not uniform. Those with a better genetic endowment, the centenarians, and their offspring, showed better adaptive capability.[28,58]

FEVER IN THE ELDERLY

Body temperature in the elderly also adapts to the changed need according to the above-mentioned changes in the immune system. Basal body temperature, like the basal metabolic rate, is lower in the elderly.[59] This has been shown to confer survival advantage from the decrease in metabolic demand.[59] While successful aging is associated with adaptive hypothermia, it renders detecting infections in the aged difficult, as the body does not readily raise the temperature to the established febrile range. With every decade increase in age, a temperature drop of 0.15ºC was observed during the first 3 days of infections, emphasizing the lack of robust febrile response and the danger of missing a diagnosis in the elderly.[59,60,61] Many researchers have recommended a reduction of febrile range for the elderly to avoid missing an infection in diagnosis.[62] Most elderly seem to have a basal body temperature of <98.6ºF. Fever criteria for the elderly, therefore, have been recommended to be: even a single temperature reading of ≥2ºF above baseline or oral temperature of ≥99ºF or rectal temperature of ≥99.5ºF.[63]

Fever seems to be ambivalent in its effect on this age group. While many studies have shown the increased mortality from high fever, due to the extra cardiopulmonary stress and metabolic demand, many studies have also shown increased mortality from lack of fever, especially in septic patients. Ahkee et al. showed that lack of fever and leucocytosis, both characteristics of efficient AIR, was associated with increased mortality in community-acquired pneumonia scenarios in the elderly.[31,63,64,65,66] In the latest COVID 19 pandemic, the clinical presentation of severe dyspnoea and tachypnoea in the elderly was associated with decreased survival while those who presented with fever and headache, tended to survive.[54] While it is true that there have not been definitive studies to investigate the effect of fever in this age group, the elderly have been a majority population in many studies investigating the effect of fever.[67,68] Schulman et al. endeavoured to study the effect of reducing temperature with drugs in septic patients and had to abort their study due to the unacceptably high mortality in the treatment group when compared to those who were not treated for fever.[14] However, epidemiological studies of dengue have demonstrated that the elderly are more prone to develop severe dengue fever and dengue shock syndrome although, they did not present with the classical signs of dengue fever as described by the WHO for diagnosis, including fever.[68,69] Most of the elderly did not develop a high fever during infections but presented with discomfort or altered sensorium.[63] Over 60% of infected elderly did not mount a fever in response to even severe infections. Such blunted fever response was shown to be associated with increased mortality.[62,65] Hypothermia, a defence response to infection seems to confer a survival advantage in some elderly while being associated with increased mortality in others.[42,70] While therapeutic hypothermia is practiced[40,41] in ischemic stroke and myocardial infarction, in the infections scenario, there is ambiguity over the benefit/damage from fever.[40,41,31,33-35,42,70-75]

Antipyresis is practiced due to fear in the general population regarding harm from fever. While known to reduce discomfort in the febrile patient, the effect of these drugs on the immune response is ambiguous.[14,76-82]

IT IS A SPECTRUM REALLY!

Febrile response, as stated above requires effective cytokine response from the innate immunity, to which the adaptive immunity must react with downstream stimulation of the appropriate cells, cytokines, and eventually, the anti-inflammatory factors.[2,6,7,83] However, the effect of aging on these systems implies poor cytokine release, so that fever is not generated efficiently.[63,68,84] With activation of pro-inflammatory status, the anti-inflammatory response may be late or absent, causing undue damage, even end organs failure as seen in many dengue cases of the aged.[68] However, centenarians, who have ideal immune systems show febrile responses as efficient as young adults, indicating that unsuccessful aging is associated with damage caused by fever and successful aging implies efficiency of the AIR.[55,58] Two homeostatic mechanisms are shown to be at work during inflammation – linear and nonlinear.[47] The linear path involves a dose-response relationship to an

inflammatory stimulus. As the inflammatory stimulus increases, so does the immune response, establishing homeostasis. The nonlinear path involves an unpredictable chaotic path, where small changes in the initial conditions stimulate an amplified response, characterized by an undue explosion of inflammation and organ dysfunctions clinically.[47] The aged, with multiple co-morbidities and inflammaging may often develop the nonlinear response. This was demonstrated in the current pandemic of Corona Virus Disease – cytokine storm that occurred in the aged caused increased morbidity.[54,85,86]

As demonstrated, multiple factors, including genetics, oxidation, exposure to stress, influence the efficiency of the immune system in the aged.[28,47,51,58] In this context, the theory propounded by Vithoulkas may explain these confounding observations.[87] The *levels of health theory* states that each person is born with a certain possibility for health, determined by genetic and epigenetic influences at the moment of fertilization.[87] This is the potential that one may achieve if one lives right. Living right implies a healthy lifestyle, minimal psychological stress, relatively less exposure to infections, and least use of drugs/medications of any kind. Under such circumstances, a person ages successfully and preserves the ability to put up an efficient AIR well into old age. However, any of these conditions not being optimal tends to reduce the efficiency of the organism and bring down the 'level of health'.[87] These levels are relative and make up a spectrum with the healthiest people with a potential for the lifespan of over ninety years at the top and those born with severe genetic disorders and pathologies causing them to die in infancy at the bottom. Everyone else makes up the spectrum in between. The healthiest are characterized by the ability to mount efficient AIR and a lack of chronic inflammatory tendency. As we go down, there are people who develop recurrent infections, followed by those in whom chronic inflammations have set in and AIR is impaired. In these people, we may not perceive efficient AIR, but an altered, chaotic AIR which is detrimental to the being. The AIR which was beneficial to the 'higher level' people, will kill in the 'lower levels' due to the magnitude of the response and the inability of the body to control it.[87] Fundamentally, the level of health, decided by the influence of genetic inheritance and allostatic load, decides the ability to mount and the outcome of an AIR, especially fever.

This, to a great extent, explains the widely different outcomes from fever observed in the elderly. If a person has a well-preserved immune system, fever is beneficial, and it is advisable to let the fever run and achieve its ends. If the immune system is compromised in any way, then fever is detrimental.[58] This is supported by many immunological studies as well. It is seen that when there is a subnormal response to inflammatory stimulus in the local tissue, the inflammatory stimulus becomes stronger and attacks a deeper tissue than would have happened in the healthier systems.[21] Further, as shown in a study, fever patterns differed in the infection scenario and each pattern was shown to have a different outcome. Those who quickly mounted a high fever and showed quick resolution of fever were the least likely to die or suffer during sepsis. However, those who did not mount fever or developed hypothermia during sepsis were most likely to die.[43]

This means that every individual's response to an inflammatory stimulus may be peculiar to that individual. Mechanisms have been detected that help the body in this defence. It has been suggested that the preoptic hypothalamus may be involved in sensing the inflammatory stimulus and judging the energy resources available for mounting a response. It may decide whether a fever or hypothermia was more adaptable in that individual.[6] Further, glutathione, an antioxidant in the cells has been shown to direct the ability to raise fever in response to inflammatory stimulus.[88] This research is in the same vein as the levels of heath theory. Here, the researchers found that the level of glutathione in the cells is determined by the presence of chronic inflammation in the environment. In very healthy individuals without chronic inflammation, glutathione is very high and during AIR, there is barely any fever recognized as the process of inflammation is carried out with barely any cost to the individual. When the chronic inflammation is strong and deep and has been established, the glutathione is used up for combatting the oxidative stress and is very low in the cells. In such conditions, the body adapts to the absence of defence and develops hypothermia instead of fever. The people in between these two extremes are shown to be able to raise a high/efficient fever response during AIR as in them, the glutathione is available in moderate quantities.[88,89]

Thus, we see that while evolution developed fever as a defence, in the current scenario of increased longevity of organisms (humans and their domesticated animals), a new outlook and

understanding is necessary to interpret the AIR and fever response. Indiscriminate use of fever suppressive drugs has been proven to be detrimental, while fever suppression is necessary in many. The levels of health theory may have the answer to this and investigating the real-life truth behind this theory is warranted. If practical clinical diagnostic parameters may be developed to detect the level of health of the person developing fever, a more logical and beneficial approach may be advised to the physicians, leading to preservation of the efficiency of the immune system.

CONCLUSION

Fever has evolved as a defence against pathogenic invasion. However, in the scenario of increased longevity, it is shown to cause both increased survival and increased morbidity/mortality especially in the elderly. So far, there is no conclusive evidence for or against the administration of antipyretic drugs in the special scenario of the aging immune system. Individualised approach may be the solution with levels of health theory providing the tools for such an assessment. Until such tools are developed, each case may have to be clinically assessed before prescribing.

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