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ORIGINAL RESEARCHES

ORIGINAL RESEARCH

Use of Natural and Traditional Medicine in Cuba: Results from the National Health Survey 2018-2019

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Abstract
Objective: To analyze the prevalence of Natural and Traditional Medicine (NTM) use in the last 12 months, according to the report from the National Health Survey (NHS), Cuba 2018-2019.
Material-Method: It was an observational, descriptive cross-sectional study that was part of the referred NHS. Socio-demographic characteristics of the survey respondents were collected (living area, gender, age, skin color, marital status, educational level, and occupation). The main variable selected for this study was the prevalence of NTM use by the Cuban population in the previous 12 months.
Results: More than one out of five Cubans used NTM therapies in the previous 12 months to the NHS, Cuba 2018-2019, with a higher prevalence of use by people living in rural areas, females, the age group of 65-74 years old, a co-habited marital status, university educational level and those in retirement. Phytotherapy was the most reported NTM therapy.
Conclusion: This study analyzed for the first time the prevalence of NTM use by the Cuban population. It demonstrated that more than one out of five Cubans used NTM therapies in the previous 12 months, according to the NHS, Cuba 2018-2019. Cuba The data from the CNHS and the information from this particular study would contribute to strengthening the integration of these treatments with conventional medicine in Cuba.
Keywords: Traditional Medicine, Complementary Therapies, Health Survey

INTRODUCTION

Globally, the landscape for traditional and complementary medicine (T&CM) has been improving consistently. According to the World Health Organization (WHO), 170 nations have acknowledged their use of T&CM, which represents 88% of the WHO Member States.[1] These non-mainstream health approaches from T&CM are sometimes the primary source of treatment for millions of people. They are also the sole recourse for individuals facing barriers to[2, 3]. However, the use of T&CM has been increasing not only in low- and middle-income nations but also in developed countries for various purposes like disease prevention, health promotion, and improving general wellbeing.[4, 5, 6] It seems that this trend has increased during the COVID-19 pandemic, according to reports from countries like Netherlands and India, as well as from the China's Special Administrative Region of Hong-Kong.[5, 7, 8]

Cuba was one of the countries from the Pan-American Health Region (PAHO) that contributed to the last WHO Global Report on T&CM.[1] NTM is the term used in the country for what is internationally called T&CM.[9] NTM is recognized as a medical specialty, with a holistic and integrative approach to health problems using methods of health promotion, disease prevention, diagnosis, treatment and rehabilitation which come from traditional medical systems and other therapeutic modalities that are integrated with each other and with conventional treatments from modern western medicine. Eleven NTM therapies are regulated in Cuba, including acupuncture, phytotherapy, apitherapy, homeopathy, flower therapy, ozone therapy, traditional exercises, medical hydrology, sun-thalassotherapy, natural nutrition, and more recently Ayurveda.[10] The Cuban National Program for the Development

and Generalization of NTM was established in 1997.[11] For more than two decades a successful integration of NTM within the CNHS has been reported.[12, 13] However, there are no previous surveys evaluating the use of NTM from the perspective of the Cuban population. The statistical report of patients' medical care with NTM modalities is guaranteed by the Health Complementary Statistical Information System[14] but the number of nationals using NTM is unknown as long as these statistics could include patients repeating these consultations and treatments within the NHS and the use of NTM for self-care purposes is not considered on these data.

The objective of the present study was to analyze the prevalence of NTM use in the last 12 months as per according to the report from the NHS, Cuba 2018-2019, of which there is no previous history from national population surveys.

MATERIALS AND METHODS

Study design
This observational, descriptive cross-sectional study was part of the NHS, Cuba 2018-2019. The objective of the NHS was to identify the prevalence of chronic non-communicable diseases, selected health events and their determinants, as well as the perception of health risks, preventive activities, and use of health services, according to socio-demographic variables.

Population
This study included the Cuban population aged 6 years and over living in urban and rural areas. A total of 5472472 individuals were included. 14339 of them, aged 15 years old and over (49.3% males, 50.7% females), were interviewed in their homes. Physical and biochemical evaluations of these volunteers were performed by health professionals at local health institutions, complying with the established ethical procedures. The selection was based on a complex sample design, with initial stratification and by conglomerates in stages, with national representation according to sex, age groups, area (urban, rural) and regions (west, center and east). The sampling frame included the population assisted by the Family Doctors and Nurses in 2017. Weights were used according to the aforementioned design and population adjustment at the end of 2018.

Statistics
The statistical packages SPSS version 21 and SAS version 9.3 were used to analyze absolute numbers, percentages, arithmetic means, 95% confidence intervals, homogeneity test (chi-square) and trend test for ordinal variables (Cochran-Armitage) significance for both when $p < 0.05$.

Measures
Socio-demographic characteristics of the survey respondents were collected (living area, gender, age, skin color, marital status, educational level and occupation). The main variable selected for this study was the prevalence of NTM use by the Cuban population in the previous 12 months, of which there is no previous history in NHS. Ayurveda was not included as long as it was regulated after finishing the survey by the end of 2019.[15]

Ethics
The study was approved by the Ethics Committee of the National Institute of Hygiene, Epidemiology and Microbiology. Written consent to participate in the survey was requested from the respondents, according to the Helsinki Declaration.[16] The data collection instruments did not include personal identification and privacy was guaranteed during the interview.

RESULTS
From the 5472472 respondents to the NHS, Cuba 2018-2019, 1270894 reported to have used any NTM treatment in the previous 12 months (Graphic 1).

Analysis of the socio-demographic characteristics of these respondents showed a higher prevalence for the use of NTM therapies by those living in rural areas (29.8%) over respondents from urban areas (21.5%) (p = 0.005). The general prevalence of NTM use was of 23.2%. Females reported more use of NTM therapies than males (24.4% versus 21.7%). People in the age group of 35-44 years old started to report higher use of NTM (21.0%) with a tendency to increase this use until the age group of 65-74 years old (26.9%)(z = 5.3; p < 0.001). Anyway, the age group over 75 reported less use of NTM compared to the previous group of 65-74 years old (24.9% versus 26.9%). There were no differences in the use of these treatments according to skin color (p = 0.966). Those reporting co-habited tended to use NTM more frequently (25.1%), as well as people with university educational levels (25.7%). Retired respondents reported a higher prevalence of NTM use (26.7) (Table 1).
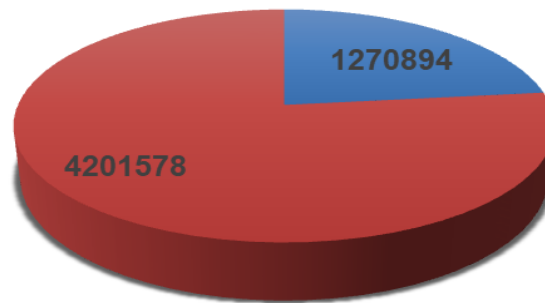
Figure 1. Report of the use/no use of NTM in the previous 12 months by NHS, Cuba 2018-2019 respondents.

Table 1. Socio-demographic characteristics of NHS, Cuba 2018-2019 respondents (n=1270894)

Characteristics	No.	%	IC 95%	X^2 (p)
Living area				
Urban area	929058	21.5	18.3-24.7	59.0 (0.005)
Rural area	341836	29.8	21.7-37.9	
Both areas	1270894	23.2	19.2-27.3	
Gender				
Male	520968	21.7	17.0-26.4	9.2 (0.09)
Female	749926	24.4	20.3-28.5	
Age				
15-24[a]	39247	13.2	5.6-20.8	58.8 (< 0.001)
25-34[a]	102898	18.7	12.1-25.2	5.3 (< 0.001)*
35-44[a]	144950	21.0	16.7-25.3	
45-54[a]	325836	23.7	20.2-27.2	
55-64[a]	275727	25.3	20.8-29.8	
65-74[a]	224023	26.9	21.8-31.9	
Over 75	158213	24.9	20.1-29.7	
Skin color				
White	814964	23.1	18.7-27.5	0.3 (0.966)
Mixed	325532	23.6	17.8-29.4	
Black	130398	23.1	18.3-27.9	
Marital status				
Single	273576	19.9	15.8-24.1	19.9 (0.013)
Married	480173	23.8	19.8-27.8	
Co-habited	296670	25.1	18.9-31.3	
Widowed	110994	24.7	21.3-28.2	
Divorced	109481	24.4	19.1-29.7	
Educational level				
None	34043	24.1	18.6-29.6	15.7 (0.351)
Elementary school	123231	23.0	18.2-27.8	
Junior High School	284659	23.8	18.7-28.8	
Skilled worker	37655	21.7	10.7-32.7	
Technician	191527	20.1	15.2-25.0	
High School	329562	23.2	17.6-28.8	
University	270218	25.7	21.7-29.7	
Occupation				
Government employee	484999	23.2	18.7-27.7	32.0 (0.003)
Mixed Company employee	9093	21.1	12.1-30.2	
Self-employed	130300	20.8	15.3-26.2	
Student	17408	14.1	6.1-22.2	
Housewife	177885	22.6	18.0-27.2	
Retired	346917	26.7	21.8-31.6	
Without/searching labor link	104293	20.6	16.3-24.9	

* Cochran-Armitage trend test for ordinal variable: z (p-value)

Phytotherapy was the NTM therapy most frequently reported (61.6%), followed by "others" (23.8%) and by acupuncture (16.2%). Medical hydrology (0.7%) and sun-thalassotherapy (0.3%) were the less reported therapies (Table 2).

Table 2. The use of NTM therapies reported by respondents to the NHS, Cuba 2018-2019

NTM therapies	n	%	CI$_{95\%}$
Acupuncture	206508	16.2	12.5-20.0
Phytotherapy	782809	61.6	56.6-66.6
Apitherapy	152753	12.0	5.5-18.5
Homeopathy	55082	4.3	2.2-6.4
Flower Therapy	99801	7.9	4.9-10.8
Ozone Therapy	43027	3.4	2.3-4.4
Traditional Therapeutic Exercises	43906	3.5	2.5-4.4
Sun-Thalassotherapy	3669	0.3	0.1-0.5
Medical Hydrology	8578	0.7	0.4-1.0
Natural Nutrition	13979	1.1	0.6-1.6
Others	302015	23.8	18.4-29.2

DISCUSSION

This study focuses on the first inclusion of NTM as a topic in a NHS in Cuba. It represents the first comprehensive examination of the prevalence of NTM use by the Cuban population as per according to the results from a nationally representative health survey.

According to the findings of this study more than one out of five Cubans use NTM therapies, with a higher prevalence of use among people living in rural areas. This prevalence is lower than in Brazil (45%) or Australia (63.1%), also according to national surveys.[6, 17] The tendency to use more these treatments by females and by those with university educational level is also reported in European countries, where the general prevalence of use is of 25.9%.[18]

Some foreign surveys describe a correlation of T&CM use by patients suffering from chronic non-communicable diseases.[17, 19] This could explain the observed increase of the use of these treatments with age, from the 35-44 years old group until the 65-74 years old group, as well as by retired respondents. Anyway, as long as the reasons for using NTM were not explored it could be considered a limitation of this study.

The most common NTM treatment reported was Phytotherapy, which has been practiced in the island since pre-Hispanic times and somehow it is the real Cuban traditional medicine. The use of medicinal plants by Cuban natives was first referred by Columbus on his first trip to America[20] and it is still used daily all over the nation, even during COVID-19 pandemic.[21] "Others" NTM therapies report followed in frequency the use of Phytotherapy. As long as the identification of these treatments was not registered and this information is missing, it is not possible to know what respondents meant by "others". It is a limitation of the study as long as it cannot be determined if there are confusions about NTM therapies or if people were using non-regulated therapies on their own.

Acupuncture was the third most frequent treatment reported and there are different references to its use in Cuba for the treatment of pain, hypertension and other acute conditions, as well as in odontology, sport medicine and for analgesic purposes during surgeries. It is practice in the three levels of the CNHS, with a broader use in primary health care settings.[9] WHO reported in 2019 that Acupuncture was formally acknowledged by Member States as the most common form of T&CM used by their populations (113 Member States), closely followed by Phytotherapy (110 Member States).[1] In Cuba, with a most representative use of Phytotherapy, these two therapies are also the most commonly used because as it was mentioned it cannot be determined what respondents meant by "others".

It is also a limitation of this study the impossibility to know whether NTM therapies were prescribed by a physician or if they were self-administered. The self-reported nature of the survey, which exposes the data to the risk of bias and is related to some of the previous limitations, could be considered as the main weakness of this study. However, its most important impact is that it analyzes for the first time data about the prevalence of NTM use as per as the results from a NHS.

CONCLUSION

This study analyzed for the first time the prevalence of NTM use by the Cuban population. It demonstrated that more than one out of five Cubans used NTM therapies in the previous 12 months to the NHS, Cuba 2018-2019. The data from the CNHS and the information from this particular study would contribute to strengthening the integration of these treatments with conventional medicine in Cuba.

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

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ORIGINAL RESEARCH

Ethnobotany and Phytochemical Composition of *Alchornea cordifolia* in Abia State Nigeria

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Abstract

Objective: This study was carried out to document the medicinal values and therapeutic applications of different parts of *A. cordifolia* in Ekebedi Oboro ancient Kingdom of Abia State, Nigeria, and profile its bioactive constituents.

Material-Method: The ethno-botanical survey involved questionnaires and oral interviews of 81 randomly selected traditional medicine doctors and natives on medicinal values of *A. cordifolia*. GC-MS fingerprinting was adopted to elucidate the bioactive constituents of the methanol leaf extract of the plant.

Results: The survey showed that *A. cordifolia* is prescribed in different treatment regimens for coughs, bronchitis, post-partum bleeding, lacerations, wounds, vaginitis, diarrhea, sickle cell anemia, and venereal diseases, including gonorrhea, syphilis, and prostatitis. Phytochemical fingerprinting revealed the presence of several bioactive compounds as the major active ingredients of the plant. A total of 27 fatty acids and volatile hydrocarbons were detected in the leaf extract of the plant with 9, 12-Octadecadienoic acid (Z, Z)- methyl ester (18.42%), 9-Octadecenoic acid methyl ester (19.93%), Dodecanoic acid 1,2,3-propanetrieyl (15.87%) as most abundant ingredients.

Conclusion: *A. cordifolia* is used to treat various diseases such as sickle cell anemia, diabetes, post-partum bleeding, and HIV infections amongst others. The fatty acids identified in *A. cordifolia* possibly underscored these antimicrobial and therapeutic actions of the plant in traditional medicine.

Keywords: *Alchornea cordifolia*, Medicinal Plant, Phytochemical Composition, Leaf Extract, Chemical Fingerprinting, Therapeutic Action

INTRODUCTION

Alchornea cordifolia (Christmas bush or dove wood) is a straggling evergreen shrub in the family Euphobiaceae which is distributed throughout West Africa.[1] It grows up to 8 meters tall. The leaves are simple, triangular, and margin-toothed. The flowers are unisexual, and the fruit is a 2-lobed capsule whereas the fruits are ovoid, elliptical, smooth, and bright red in color when ripe. In some Nigerian cultures, the young leaves are eaten as appetizers or added to soups as a substitute for the potherb known as, *Ptericarpus osun* also called *"Uha"*. On the other hand, the older leaves are utilized for packaging kola nuts.[2-4] Also, the leaves are eaten to help prevent bleeding on wounds. Moreover, the infusion derived from the plant is utilized in the treatment of various ailments including sore throat, cough, ulcers, bronchitis, dysentery, worms, venereal diseases, and female sterility. Furthermore, it is known to possess sedative and antimicrobial properties.[5-9]

The root and leaf decoctions are used as a mouthwash against mouth ulcers, toothache and decay, stopping post-partum bleeding, bleeding gums, hemorrhage, and treatment of vaginitis. A poultice of leaves and stem bark is used to cure yaw, chancre and dried tissue powders are used to facilitate the healing of fractures.[10-12] A variety of bioactive compounds, including ellagic acid, hyperin, and eugenol have been isolated from the plant.[2,7,13-15] Also, strong anti-inflammatory compounds including 3, 5, 7, 3'-tetrahydoxyflavone-3-0-α-L-rhamnoside, lupenol (lup-20(29)-en-3c-ol), and methyl gallate has also been isolated from different parts of the plant.[16-18]

Some workers reported the presence of bioactive fatty acids such as dodecanoic (lauric) acid, n-hexadecenoic (palmitic) acid, 9, 12-octadecadienoic acid (alpha-linolenic acid), pentadecanoic acid, nonacosane, 9-octadecenoic (oleic) acid, octadecanal, and terpinolene etc. in extracts of *Alchornea* species which contributed to the plants' bio-efficacy.[19-21]

Antimicrobial, antiretroviral, antioxidant and antitoxin activities of *A. cordifolia* have been documented.[22-24] Organic extracts and isolates from the plant demonstrated antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Plasmodium berghei in vitro* and mice, respectively.[9,25-26] The root and bark extracts of *A. cordifolia* have shown strong antiviral activity against strains of HIV I, out-performing AZT in some trials.[2,27] The anti-HIV activity of this plant may be due to its content of hexadecenoic acid reported to bind directly to CD4 receptors and actively blocking HIV-1 entry and infection in humans.[28] Methanol extracts of *A. cordifolia* similarly inhibited *Botrydiplodia theobromae in vitro*. This fungus has been implicated in invasive

fungemenia in humans.[29-30] The presence of dodecanoic acid in extracts of *Alchornea* known to exhibit antibacterial, antifungal and anti-inflammatory properties in both fungi and humans may account for these activities.[31-32]

Several workers have reported higher plants as major sources of potential drugs and healthcare materials in tropical localities.[22,33-35] This paper presents the ethno-botanical potentials of A. cordifolia in Abia state, Nigeria, and its phytochemical fingerprints.

MATERIALS AND METHODS
Study area
Ekebedi Oboro ancient Kingdom is located in Ikwuano Local Government Area (LGA) of Abia State, in the rainforest belt of Southeast Nigeria, on latitude 5° 24' 22"N and longitude 7° 34' 5"E (Fig. 1). The Kingdom has 200 households with a population numbering about 6,000 people. Most of the people of the Kingdom practice mixed farming as predominant occupation, with fishing, traditional medicinal practices and bone setting as minor occupations.
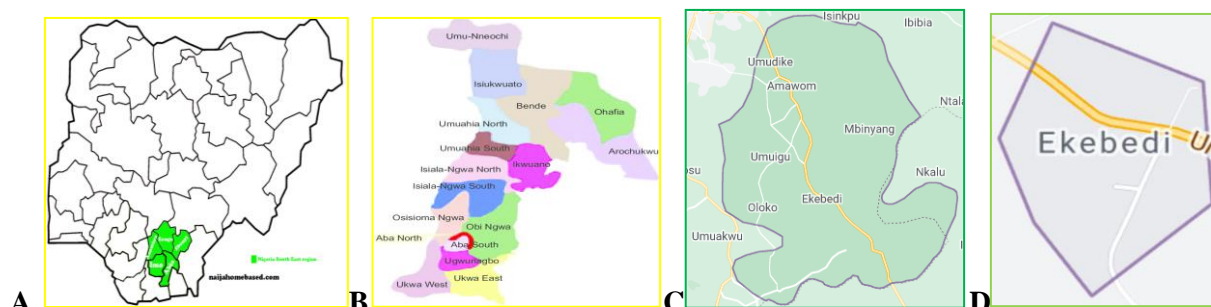
Figure 1. Study area. **A:** Map of Nigeria, Southeastern region in green. **B:** Abia State Map and 17 Local Government Areas (L.G.As.). **C:** Ikwuano L.G.A. of Abia State. **D:** Map of Ekebedi Kingdom.

The ancient Kingdom is known to be endemic to parasitological, pathological and metabolic diseases due to high temperatures and humidity for most of the year. Herbal recipes are frequently employed by indigenes of the area in the management of these diseases.[36-37]

Plant materials
Fresh aerial portions of *Alchornea cordifolia* (twigs, leaves, flowers and fruits) were collected from bushes in the ancient Kingdom. The plant sample was authenticated by Prof. M. C. Dike of the Department of Forestry, College of Natural Resources and Environmental Management (CNREM) of the Michael Okpara University of Agriculture, Umudike, Abia State; and the plant

specimen (Voucher number: AC/7344) was deposited at the Herbarium of the Department.

Preparation of *Alchornea cordifolia* leaf extract
The leaves of the plant were washed in tap water and rinsed in 2 changes of sterile distilled water. They were dried on the laboratory bench for 21 days, enveloped and oven-dried at $40^{o}C$ for 30 minutes, and then milled into powder using a Thomas Wiley machine (ED-5, USA). Then 100g of the milled powder was packed separately into a 2-liter soxhlet apparatus and extracted exhaustively with 250 ml of analytical grade methanol for 24 h. The methanol leaf extract was concentrated using a rotary evaporator at $45^{o}C$ and left on the laboratory bench for two days to afford residue.[1,14]

Survey and data collection

This study sought to document and validate the medicinal uses of *A. cordifolia* by people of Ekebedi ancient Kingdom, in Abia State, Nigeria. For this purpose hence, a random survey to collect data on ethno-botanical and therapeutic significances of *A. cordifolia* was conducted in the Kingdom, using simple questionnaires and, oral interviews methods as adopted by some workers.[36-38] The survey involved a house to house strategy which lasted over a period of 8 weeks (June 04, 2022 – August 07, 2022). Four herbalists (traditional medicine practitioners), 21 herbal vendors and 56 indigenes/natives were randomly selected and interviewed on medicinal uses of *A. cordifolia* in the ancient Kingdom.

Gas chromatography-mass spectrometry (GC-MS) analysis of plant extract

This was conducted following the method of previous workers.[39-41] One ml of leaf extract of *A. cordifolia* residue reconstituted with analytical grade methanol was injected into GC-MS equipment (Model: QP 2010 Plus Schmadzu, Tokyo, Japan) comprising of an AOC-20i auto-sampler and gas chromatograph inter-phased to a mass spectrometer (GC-MS) instrument equipped with a VF 5 MS fixed silica capillary column of 30m length, 0.25 mm diameter and 0.25μm film thickness. The carrier gas was helium (99.99%), flow rate of 1.58 ml/min, injector and mass transfer line temperatures (250 and 200°C respectively), and an injection volume of 1 μl was employed with Split ratio set at 10:1. The oven temperature was programmed from 80°C (Isothermal from 2 min), with an increase of 9°C to 200°C for 4 min, then 10°C/min to 280°C, and end with isothermal at 5°C. The MS operating parameters were as follows: ionization energy 70ev, ion source temperature 200°C, solvent cut time 2.5min, relative detector gain mode, scan speed n/sec, scan range 40-80u, while the interface temperature was 250°C. The total running time of the GC-MS was 1 h.

The percentage of the extract was expressed as percentage with peak and normalization. The relative percentage amount of each phyto-component was calculated by comparing its average peak area to the total areas. The detection process employed was that of the National Institute of Standards and Technology (NIST) (Version 2.0, 2005). The compound prediction was based on the Phytochemical and Ethno-botanical Databases of USDA.[42] The interpretation of GC-MS was conducted using the National Institute of Standard and Technology (NIST) database, which has more than 62,000 structural patterns. The spectra of the unknown phyto-components were compared with that of known components stored in the NIST library. The name, molecular weight and chemical structures of the extracts-derived compounds were ascertained from the National Institute of Standard and Technology (NIST).[43]

Data analysis

Data generated from this study were analyzed using simple descriptive statistical tools such as range, means, and percentages.

RESULTS

Medicinal Importance of *Alchornea cordifolia* in Ekebedi Oboro Kingdom

The results of the survey on uses of *A. cordifolia* as crude medicine in the study area are presented in Table 1. The survey results showed that the plant is used to treat many important pathological and parasitological diseases in the area. All the respondents in the study agreed to use one part or the other of the plant either singly or in combination with other parts of the same or other herbs for treating cases of fevers especially malaria, venereal diseases, toothache, fracture, mouth ulcers etc. Similarly, about 90% of the respondents use different preparations of leaves and twigs of the plant for treating gonorrhea, post-partum bleeding, lacerations, piles etc. About 50% of the respondents on the other hand, prescribe or use the stem bark of *A. cordifolia* for diverse purposes, while 70% others employ the root and root bark of the plant in treating cases of bronchitis, coughs, dysentery (blood in faeces), venereal diseases, toothache, rheumatoid diseases and post-partum bleeding etc. Whereas all the respondents accented to using one or more parts of the plant in treatment regimes, the lowest number of respondents (14%) use the seeds for the treatment of skin diseases (Table 1).

Several solvents are used in preparing these local crude drugs. These include water, palm wine, local gin (popularly called *kai kai*) and supernatant from ground maize kernels (*Mmiri akamu*) (Fig. 2). In this study also, preparations of the test plant as medicine takes the forms of macerations, poultices, decoctions, infusions, tinctures or alcoholic elixirs (*Huma*) using *kai kai* (Fig. 3). *Mmiri akamu* is prescribed for decocting medicines for those who are sensitive to alcohol contents in palm wine or *kai kai*.

Table 1. Medicinal Importance of *Alchornea cordifolia* (Schumach and Thonn.) Arg. Mull. in Ekebedi kingdom

Common/ Local Name of the plant	Number of medicine men interviewed	Respondents Interviewed (%)	Parts Used for Treatment	Uses and significance of charismas bush in folk-Medicare system
Christmas bush	81	90	Leaf / twigs	Malaria fever, typhoid fever, dysmenorrhea, gonorrhea, venereal diseases, syphilis, HIV, diarrhea, toothache, lacerations, sore throat, bronchitis, prostatitis, piles, post-partum bleeding, sickle cell anemia etc.
Ubube in Igbo	81	50	Stem bark	Fever, toothache, mouth sores, rheumatism, prostatitis, HIV, sickle cell anaemia,
	81	70	Root / root bark	Bronchitis, coughs, blood in faeces, prostatitis, venereal diseases, toothache, rheumatism, post-partum bleeding, syphilis, HIV infections.
	81	14	Ripe and unripe seeds	Skin diseases, dyes
	81	100	All parts	Prostatitis, genital discharge, gonorrhea, malaria, toothache, coughs, fevers, venereal diseases, syphilis, toothache, fractures, dysmenorrhea,, rheumatisms
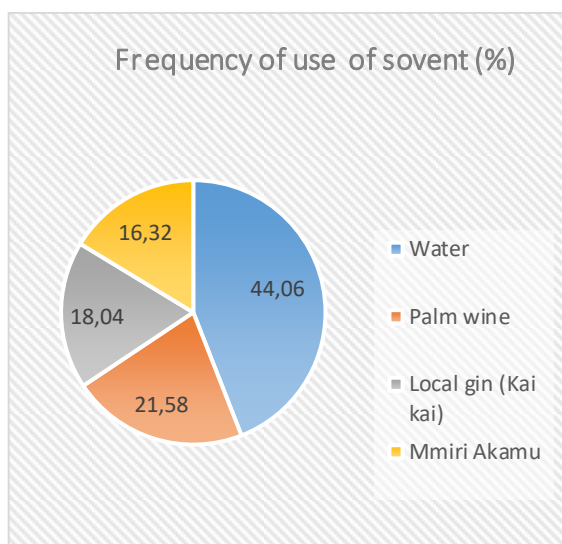
Figure 2. Solvents used in preparing *A. cordifolia* recipes recipes
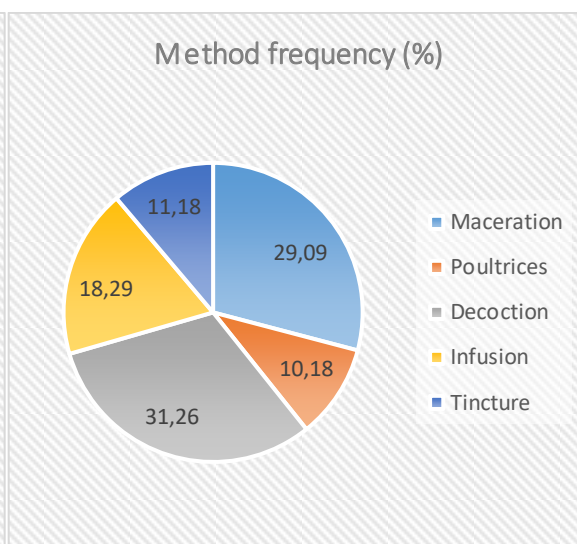
Figure 3. Methods used in preparing *A. cordifolia*

GC-MS analysis of methanol leaf extract of *Alchornea cordifolia*

Mass spectral data of *Alchornea cordifolia*

The gas chromatogram of the GC-MS analysis of the volatile components of 2µl of the methanol leaf residues from *A. cordifolia* are presented in Fig. 4. The mass spectral data shows the presence of bioactive volatile compounds in *A. cordifolia* leaf. A total of 27 volatile compounds (Table 2) were identified which were represented by the various peaks in the chromatogram and some of the peaks and predominant compounds include: peak **7** (9,12-Octadecadienoic acid (Z,Z)- methyl ester (18.42%), **8** (9-Octadecanoic acid methyl ester (19.93%), **11** (Oleic acid, 7.33%); **24** (Dodecanoic acid 1,2,3-propanetriyl (7.79%), **25** (9-. Octadecenoic acid (Z)-2-hydroxy-1- (6.75%), **26** (Dodecanoic acid 1,2,3-

propanetrieyl (15.87%); and **27** (2-Methyltetracosane, 8.78%). Amongst the bioactive constituents which were detected in lower quantities (area %) in the extract as shown in Table 2 include: 9-Octadecenal (3.64%), Hexadecanoic acid (3.09%), Methyl stearate (1.33%), 9-Octadecanoic acid (Z)-2-hydroxy-1- (1.06%), nonanal, trans-1-Nitro-1-propene, Z-10-pentadecen-1-ol and Z,Z-8,12-Hexadecadien-ol-1. Several possible isomers of the same compound were also captured in the chromatogram. Peaks 8, 23 and 25 are possible isomers of 9-octadecenoic acid; peaks 24 and 26 possible isomers of lauric acid while Peaks **13** and **17** are possible isomers of the aldehyde nonanal. The structures of some of the bioactive compounds isolated from *A. cordifolia* are shown in Figure 4.

Table 2. Bioactive volatile compounds identified from *Alchornea codifolia*

Peak#	R.Time	Area	Area %	Height	Height%	A/H	Name of compound
1	4.981	83277	0.09	20218	0.13	4.12	Cyclopropene
2	26.344	521594	0.54	165190	1.05	3.16	Diethyl Phthalate
3	26.582	32036	0.03	17612	0.11	1.82	Phthalic acid, ethyl pentyl ester
4	30.852	63466	0.07	24109	0.15	2.63	trans-1-Nitro-1-propene
5	36.756	2974763	3.09	936288	5.97	3.18	Hexadecanoic acid, methyl ester
6	37.880	421676	0.44	91709	0.59	4.60	n-Decanoic acid
7	41.298	17721159	18.42	4686154	29.90	3.78	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	41.479	19166079	19.93	5216986	33.29	3.67	9-Octadecenoic acid, methyl ester, (E)-
9	41.594	538512	0.56	140724	0.90	3.83	11-Octadecenoic acid, methyl ester
10	42.133	1281690	1.33	403295	2.57	3.18	Methyl stearate
11	42.721	7051263	7.33	536147	3.42	13.15	Oleic Acid
12	44.980	8070	0.01	8864	0.06	0.91	9-Octadecenal
13	46.268	360617	0.37	61350	0.39	5.88	Nonanal
14	47.617	44051	0.05	20557	0.13	2.14	2-Nonen-1-ol, (E)-
15	49.166	247831	0.26	70979	0.45	3.49	1-Hexadecyne
16	49.297	345184	0.36	88350	0.56	3.91	Z-10-Pentadecen-1-ol
17	50.012	96266	0.10	26665	0.17	3.61	Nonanal
18	50.256	1322796	1.38	308143	1.97	4.29	Z,Z-8,10-Hexadecadien-1-ol
19	50.371	3499521	3.64	502875	3.21	6.96	9-Octadecenal, (Z)-
20	51.618	173846	0.18	46096	0.29	3.77	Cyclopropanepentanoic acid, 2-undecyl-, methyl
21	54.279	56671	0.06	19704	0.13	2.88	Oxalic acid, allyl pentadecyl ester
22	54.899	597632	0.62	163300	1.04	3.66	Z,Z-8,10-Hexadecadien-1-ol
23	54.983	1883864	1.96	327059	2.09	5.76	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)- E,E
24	56.850	7490583	7.79	495679	3.16	15.11	Dodecanoic acid, 1,2,3-propanetriyl ester
25	56.967	6493004	6.75	482196	3.08	13.47	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)- E,E
26	57.720	15259931	15.87	558205	3.56	27.34	Dodecanoic acid, 1,2,3-propanetriyl ester
27	59.065	8446482	8.78	252854	1.61	33.40	2-methyltetracosane
		96181864	100.00	15671308	100.00		
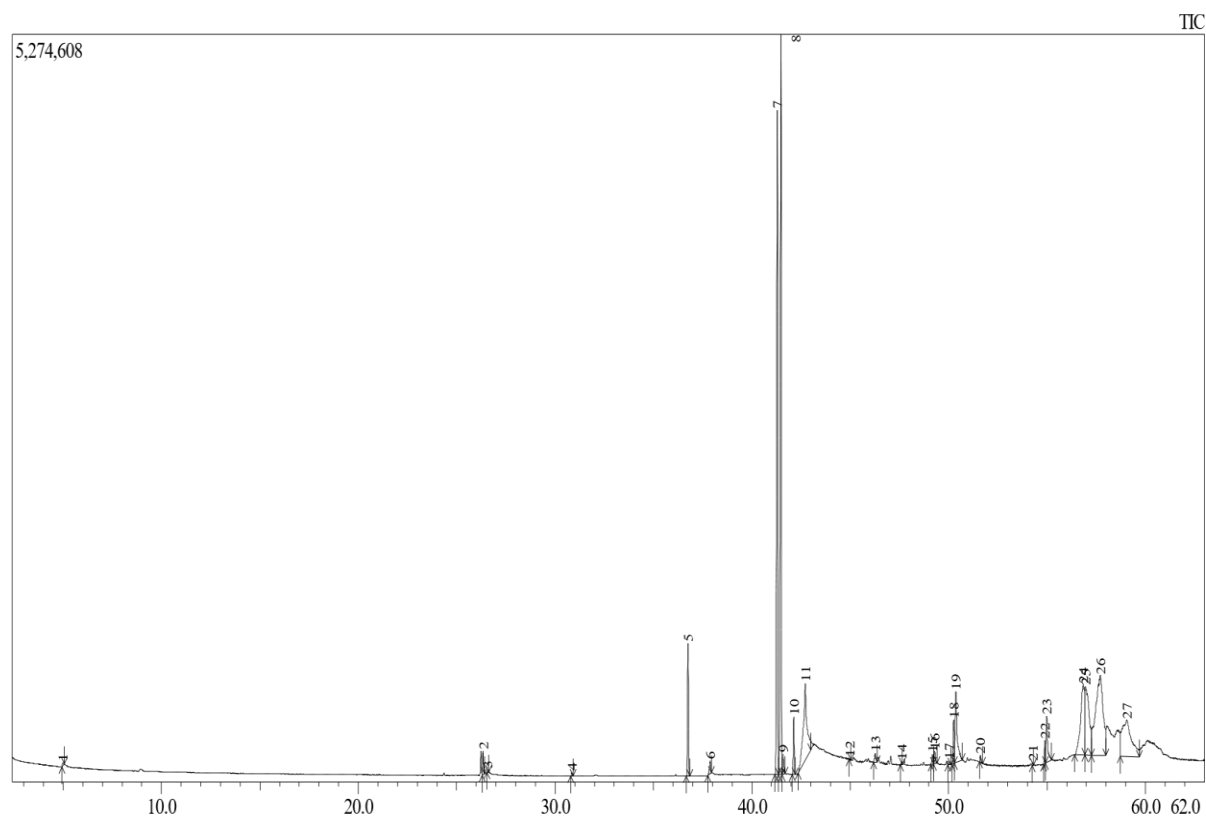
Figure 4. Chromatogram of methanol leaf extract of *Alchornea cordifolia*.
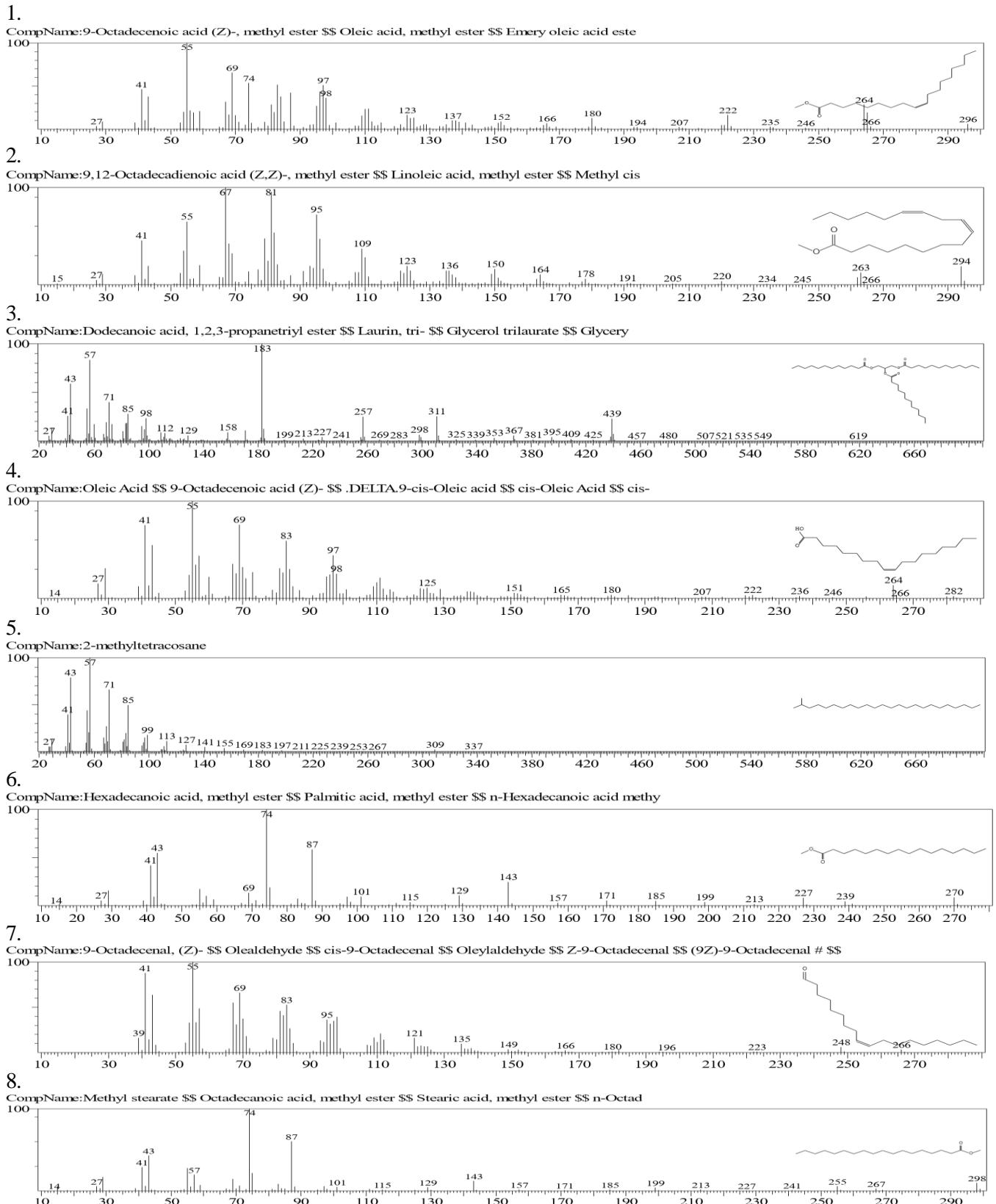
Figure 5. Structures of some compounds detected in methanol leaf extract of *Alchornea cordifolia*. **1.** 9-Octadecenoic acid ME; **2.** 9,12-Octadecadienoic acid ME; **3.** Dodecanoic acid ME; **4.** Oleic acid; 5. 2-Methyl tetracosane; **6.** Hexadecanoic acid; **7.** 9-octadecenal; **8.** Methyl Stearate)

DISCUSSION

Results presented in Table 1 indicated that *A. cordifolia* is medicinally relevant in the traditional healthcare system of the people of the study area. Our findings lend support to the fact that the people of the Kingdom use various parts of the plant (leaves, stem, roots and barks) for treating human diseases. Coughs, bronchitis, gonorrhea, toothache, mouth ulcers, piles, lacerations, post-partum bleeding, sore throats, sickle cell anaemia, boils and venereal diseases amongst other malaises are challenged with preparations from sole or combinations of different parts of *A. cordifolia* in this study. The submissions of previous workers that leaves, stems and roots of *A. floribunda* and *A. cordifolia* are used in various traditional medical practices of Africans for the management of pathogenic diseases, inflammatory, ulcerogenic, respiratory, urino-genital, HIV infections and gastrointestinal conditions or wound infections actively corroborates our findings in this regard.[44-47] Findings in this survey showed that natives of the study area use root bark, stem and leaf extracts of *A. cordifolia* in health matters related to sickle cell anemia, venereal diseases, diabetes, arthritis, rheumatoid challenges, ulcers, HIV infections and its opportunistic microbial overloads etc. This corroborated the report and views of several other workers where extracts of the plant has been employed to treat diabetes, sickle cell disease, arthritis amongst other human disease.[48] It is also consistent with the submission of other investigators where alcoholic root extracts of the plant proved more effective than AZT in inhibiting advancement of HIV.[2,27] Hexadecanoic (palmitic) acid found in moderate amount in the test plant extract has been linked with blocking HIV-1 entry and infection in humans.[28] Mechanism of action of extracts of this plant against microbial and mesobiotic agents appear to hinge on disruption of mitosis and cell proliferation through the arrest of microtubules and spindle formations[20-21,49-52] and/or inhibition of certain enzymes such as HIV integrase and topoisomerase I involved in relaxing DNA supercoils during viral replication and translation.[50-51,53] Our results also indicated that natives of Ekebedi kingdom use *A. cordifolia* to manage conditions of rheumatoid pains, prostatitis, bacterial infections-induced infertility and diarrhea, a view also held by some workers where various preparations of the plant is used for tackling pains, piles, rheumatism and toothaches in humans etc.[11-12,]

[48,54] The anti-arthritic and anti-stress properties of *A. cordifolia* as seen in its use against rheumatoid pains in this study is supported by similar and parallel reports of other workers whereby decoctions and topical applications of *A. cordifolia* effectively ameliorated pains in assayed indigenous phyto-medicine systems.[6,11-12,48,55] Similarly, oral administration of hydro-ethanolic extracts of the plant decreased duration of immobility and paw edema in mice and artificially-induced arthritic rats.[44,56-57] These workers suggested that the mechanism of anti-arthritic activity of this plant may have involved antioxidant action due to suppression of elastase and superoxide anions and increased enzymes (SOD, CAT and GSH) levels observed in both animal and human systems. With respect to prostate conditions, our data showed that *A. cordifolia* is used for treating prostate conditions in the study area. This finding is consistent with submissions from other investigators who reported that extracts and powder formulations of *A. cordifolia* in combination with *Seteria sp., Plastoma sp., Boerhavia* sp. are effectively used to cure sickle cell disease and prostate conditions in South-south, Nigeria.[58-59] Inhibition of certain enzymes involved in cancer cell proliferation is suggested as one of the possible mechanisms for this reported activity.[60] Plants have a long history of use in many traditional Medicare systems of different rural and sub-urban cultures of Africa where over 80% of the population have been reported to use them to prevent or treat malaises.[34,36-37] Presence of active principles such as alkaloids, flavonoids, tannins, anthocyanins and anthraquinones etc. are reported to exert potency, and lend scientific support for the use of many herbs including *A. cordifolia* in traditional medicines.[46-47] In this study, *A. cordifolia* is used effectively for treating coughs and bronchitis in the surveyed Kingdom. In tropical locations, fevers and upper respiratory tract infections (URTI) such as common colds, sore throats, sinusitis, influenza, pneumonia and bronchitis are common diseases of viral origin in humans. These conditions characterized by violent sneezes, headaches, aches, body pains and shortness of breath or syncope in some cases underscore many diseases induced by rhinovirus, adenovirus, influenza virus and coronavirus. In most developing countries, URTIs so far accounts for 40% disability in infants and children, and up to 80-90% deaths of elderly people[19]. The anti-bronchitis activity of *A. cordifolia* in this study is

supported by similar reports in which administration of ethanol root extract of *A. cordifolia* to histamine-induced broncho-constriction in guinea pigs effectively delayed onset of the disease symptoms in the test animal.[22] So far, multiple scientific evaluations, in which 4000 mg/kg body weight of plant extracts were administered to laboratory animals, have upheld that plant extract is non-toxic to mammals.[44,47] Similarly, bio-safety tests on mice (1g/kg body weight) in the tropics affirmed that aqueous extracts of *A. cordifolia* leaves did not adversely affect the anxiolytic and depressant activities of CNS of the animals.[61]

Phytochemicals demonstrate high pathogen-specificity, and pathogen resistance to them are unlikely; therefore they could be useful compounds to complement or potentiate existing azoles against antifungal resistant species.[53] Recent studies showed that traditional medicine has been recognized as a veritable complement to conventional forms of healthcare in developing countries.[22] This was warranted by increasing cases of antimicrobial resistance (AMR) to orthodox drugs and its consequences in terms of loss of human lives, man hours, and economic returns to many nations, and reinforced resort to medicinal plants for health and vitality.[62] In many cultures, medicinal plant leaves, twigs, roots, barks, flowers, fruits and stems find extensive applications in different forms of preparations of crude drugs and constitute principal sources of potential antimicrobial candidates than any other.[22,26-27,33,54] Findings in this study (Table 1) is congruent with the reports of several previous workers in which preparations from *A. cordifolia* were reported to be used effectively for the treatment of various ailments including coughs, bronchitis, dysentery, sickle cell anemia and venereal diseases, etc.[3,26-27]

Though all parts of the plant were used in preparing medicines (Table 1), the leaves and twigs found more applications as crude drugs than the stem bark and roots, being used against diverse ailments by about 90% of the respondents in this study, and this is consistent with the views held by other workers.[3,22,63-64]

Data obtained from this survey indicated that water followed by palm wine were the commonest solvents used for preparing *A. cordifolia*-based medicines while *mmiri akamu* was the least. This is in line with the observation of some scholars.[63] These methods used in preparing medicines from *A. cordifolia* are consistent with those reported for

other herbs by other workers.[24,30] Decoction and maceration ranked foremost as popular ways of preparing *A. cordifolia*, and this strongly agrees with the observations from similar studies.[34,63] The routes of administration are mainly oral as decoctions, infusions and elixirs (*Huma*), and topically as poultices, powders or tinctures. This finding is also in harmony with the views expressed by other workers.[63]

Different chemical compounds were identified in the chromatogram of methanol leaf extract of *A. cordifolia* in this study (Fig. 5, Table 2). The results of GC-MS chemical profiling in this study showed that the most abundant compounds detected in *A. cordifolia* leaf extracts were Dodecanoic acid 1,2,3-propanetriyl (lauric acid), 9-Octadecenoic acid methyl ester (commonly called oleic acid) and 9,12-Octadecadienoic acid (Z,Z)- methyl ester (commonly referred to as linoleic acid). Similar studies indicated that 9-octadecenoic acid and 9,12-Octadecadienoic acid (Z,Z)- methyl ester were among the highest occurring fatty acid constituents of different solvent extracts of *Onosma gmelinii*, *Cnidoscolus aconitifolius* and *Entandrophragma angolense* (Mahogany).[40,65-66] Dodecanoic acid 1,2,3-propanetriyl ester made up 12.8% of oil fraction of *Alstonia boodei*.[67] These results are in agreement with findings in this study where these fatty acid compounds constitute the most abundant constituents in *A. cordifolia* (Table 2). Similar investigations also detected octadecane, oleic acid and cyclopentene in *A. flourubunda*.[68-69] These compounds or their closely related hydrocarbons were also identified in varying concentrations in this study.

Strong antimicrobial and antifungal activities have been ascribed to the predominant and common chemical ingredients in *A. cordifolia* (9, 12-Octadecadienoic acid (Z,Z)- methyl ester, 9-Octadecanoic acid methyl ester, and Dodecanoic acid 1,2,3-propanetriyl.[40,70] Methanol extract and fractions of *A. cordifolia* significantly inhibited growth and replication of the human HSV II *in vitro* in a study.[71] Some scholars attributed the strong anti-HIV I and II, HSV I and II, anti-measles virus and COVID-19 virus activities to dodecanoic acid which is abundantly present in this plant, and this may explain its therapeutic use in HIV cases in the study area.[72,73] The compound in parallel studies has also been implicated for antibacterial and antifungal activities against the growth and reproduction of *Streptococcus* sp., *S. aureus* and *Candida albican* in

a manner which was favorably comparable to ciproflaxacin.[74-75]

Methanol extract of *Alchornea coelophylla* inhibited *in vitro Fusarium spp., Escherichia coli* and *Pseudomonas aureginosa* causal agents of recalcitrant fungenemia and bacteremia in humans.[76] Fatty acids occur widely in plant and animal tissues where they play antimicrobial roles in their cuticles.[77] Dodecanoic, hexadecanoic acid and tetradecanoic acid exert efficient toxicity against phytonotic moulds including *Beauveria bassianai.*[78] Also, the antifungal activity of leaf extracts of *Sesuvium portulacastrum* against various phyto-pathogens was due to its content of some of these fatty acid methyl esters, their derivatives or related compounds while octanoic (caprylic) acid has been reported to potentiate fungitoxicity of some synthetic azoles *in vivo*.[51,79-82,83] The degree of potency of fatty acids reportedly correlated with their chain length and degree of unsaturation.[84] Mechanisms for the antifungal activity of fatty acids against susceptible microbial agents have been suggested to include penetration and disruption of cell membranes, and inhibition of protein synthesis or enzymatic function.[53,83] According to these workers, fatty acids penetrate into cell membrane bilayers of target organisms and upset its integrity, leading to increased fluidity and loss of cellular radicals. These mechanisms may underpin inhibition of fungal agents causing human diseases and their therapeutic action.

Many biological activities such as anti-arthritic, antihistaminic, anti-coronary, anti-androgenic, anti-acne, anti-inflammatory, hepatoprotective, antimicrobial and antioxidant activities have been ascribed to 9,12-octadecadienoic acid (Z,Z)- and related methyl ester.[85] The compound, 9, 12-octadecadienoic acid (Z, Z)- methyl ester was associated with impeding cell proliferation and antimicrobial activity of extracts of *Albisia adiathofolia*.[86] The polyunsaturated fatty acids (PUFA) – 9, 12-octadecadienoic acid and 9, 12, 15-octadecatrienoic acid are known precursors of prostaglandins. Lack of PUFA predisposes humans to inflammations, cancers, viral infections and various kinds of neuro- or cardiovascular diseases.[66] Besides, octadecenoic acids are analogues of hexadecanoic acid and these compounds exhibit significant biological activities including antidiabetic, antioxidant, antimicrobial and anticancer activities.[87] Hexadecanoic and octadecanoic acids were reported to underscore

antibacterial, anticancer, and antimicrobial efficacies in methanol extracts of *Spermacoce articularis* and neem extract against the human pathogen *Salmonella* species respectively. Tetracosane though found in small concentration in the studied extract is known to demonstrate antioxidant and strong effectiveness against some cancer cell lines and forms one of the principles underscoring the efficacy of *Dendrobium crepidatum* used in treating fevers, diabetes, cataracts and cancers in Chinese medicine.[86-88] It was also reported as one of the principal compounds detected in *Acacia pennata* a herb used in Thailand; that is associated with inhibiting β-amyloid aggregation in brain tissues of persons suffering from Alzheimer's disease.[89] 2-Methyl tetracosane may exhibit similar chemical properties. Some studies indicated that oleic acid detected in moderate quantity in this present study demonstrated anticancer, anti-inflammatory, hypocholesterolomic, anti-androgenic, antifungal and food preservative activities.[90-91] It is reported as one of the major constituents of *Sida cordata* used in Asia and Nigeria for treating microbial infections, stomach upset, bronchial asthma, wheezing and edema.[92-93] These compounds may underscore and possibly explain the medicinal or therapeutic significance of *A. cordifolia* in the ethnobotany of the people of Abia State, Nigeria.

The people of the study area practice slash and burn agriculture characterized by land rotation. The survey coincided with the cropping season which made accessing interviewees difficult and erratic. Another limitation encountered during this study was in terms of method of isolation and analysis of the active principle of the test plant adopted in the study which did not allow for isolation and identification of nonvolatile bioactive compounds other than fatty acids from the test plant.

Finally, this study confirms that *A. cordifolia* is used to treat various diseases such as fevers, sickle cell anaemia, prostatitis, lacerations, wounds, venereal diseases etc. in the ancient Ekebedi Kingdom. The natives prepare the crude drug mainly as alcoholic macerations, decoction or infusion using water, local gin (*kai kai*) or palm wine. Phytochemical fingerprinting of the methanol leaf extract of the plant revealed the presence of 27 Fatty acid compounds with linoleic, linolenic and lauric acids being the most abundant active ingredients present in the plant, and these compounds possibly underscored its therapeutic activities in traditional

medicine.
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ORIGINAL RESEARCH

Knowledge, Attitudes, and Behaviors of Medical Residents towards Traditional and Complementary Medicine: A Cross-sectional Study

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Abstract

Objective: This study aimed to evaluate the knowledge, attitudes, and behaviors of medical residents working at a faculty of medicine hospital about traditional and complementary medicine (TCM).

Material-Method: This is a cross-sectional study. The study was conducted with medical residents working at the faculty of medicine between 15 July and 15 December 2021. A survey containing questions to determine the clinical and demographic characteristics of the participants and their knowledge, behavior, and attitudes toward TCM methods was applied to the participants.

Results: A total of 261 medical residents participated. The average age was 29.4±2.6 years, and 51.7% (n=135) were male. 82% of the participants were studying internal medicine sciences. The average time as a physician was 4.4 years, and the average time as a physician in the total branch was 2.6 years. Cupping therapy was the most known method, with 55.6%. The most frequently used method (19.2%) was phytotherapy; the most commonly recommended (16.5%) was phytotherapy; the most formally trained (4.2%) was cupping therapy; the most desired formal training (41%) was acupuncture; the most frequently declared method (11.9%) as another TCM method was diet. 36.4% of the participants found TCM useful. 82% thought that these methods should only be applied by trained physicians.

Conclusion: It was determined that the level of TCM knowledge among medical residents was low. Although the majority of participants had a positive attitude toward TCM, they had low rates of application, implementation, and recommendation.

Keywords: Medical Residency, Physicians, Complementary Medicine, Integrative Medicine, Traditional Medicine

INTRODUCTION

Traditional, complementary, alternative, and integrative medicine have different meanings in this field. The World Health Organization (WHO) defines traditional medicine as "the set of knowledge, skills, and practices that can or cannot be explained based on theories, beliefs, and experiences specific to different cultures".[1] Complementary medicine, on the other hand, refers to health practices that are different from traditional medicine or are not integrated into the current health system. While the terms alternative medicine and complementary medicine are often confused, they describe separate concepts, according to the National Center for Complementary and Alternative Medicine (NCCAM). When used together with traditional medicine, it is called "complementary medicine"; when used instead of traditional medicine, it is called "alternative medicine". On the other hand, integrative medicine adopts a holistic, patient-centered approach to health and well-being, using a combination of traditional and complementary methods.[2] The terms in this area seem unclear, and WHO states that there may be treatment alternatives.[1]

As the concept of health has developed throughout human history, all societies have tried different methods to prevent diseases. Traditional treatment methods have developed, and many effective methods have survived to the present day. These traditions vary according to the region, historical period, and religious culture.[3]

With the development of science, preventive medicine has undergone significant changes and created modern health services known as evidence-based medicine, but in recent years, there has been an increase in interest in traditional and complementary medicine (TCM) in our country and

the world.[4]

The fact that it is less invasive, that modern medicine does not adequately meet the expectations, that it is believed to have fewer side effects, that holistic medicine is more demanded, that it includes factors such as more beliefs and ethnic cultures about the practice may be a factor in its spread. Although TCM is not included in insurance coverage, its widespread use in many countries shows that it is widely accepted and sought after.[5]

Herbal products used in treatments are not considered drugs, but after the preclinical/clinical stage, it is important to evaluate whether the molecule is a drug, how it is licensed, its effectiveness, safety, side effects, and interactions. The uncontrolled use of natural products by people who have the illusion that they are harmless, the lack of information about their use, and the lack of training of doctors on this issue cause traditional treatments to fail or pose a life-threatening threat in today's conditions.[6]

Physicians continue to debate the efficacy, safety, and indications of TCM. Some claim that these methods have therapeutic uses, while others claim that they delay healing; however, the evidence is still considered incomplete.[5]

It cannot be ruled out that people resort to treatments that differ from modern ones, on the contrary, the causes that lead to this condition should be considered in all aspects. While modern medicine has significantly advanced in treating infectious diseases, it has not made any noticeable progress in treating cancer and chronic diseases. Therapy, where examination has become a luxury, has become defensive because it cannot adequately educate patients in the diagnosis and treatment process. It has become a laboratory medicine that depresses the patient with almost useless examinations and images. Clinical attention to the corporate pharmaceutical industry, which sometimes targets private practices, political actions, and rhetoric that pits patients against doctors and discredits the medical profession, has diminished trust in medicine and modern doctors.[6]

The fact that patients with many diseases in different branches have to apply to the relevant departments separately and the process is time-consuming and expensive shakes patients' confidence in the treatments they receive. These conditions strengthen the tendency toward holistic medicine.[7]

Scientific studies on TCM applications have gradually increased.[8-13] This study aimed to evaluate medical residents' knowledge, attitudes, and behaviors toward TCM at a medical school hospital.

MATERIALS AND METHODS
Type and location of research
This research was designed in a descriptive, cross-sectional type. This study was conducted at Ondokuz Mayıs University Faculty of Medicine.

Criteria for Inclusion and exclusion
Criteria for inclusion of participants in the study: 1)To be a medical resident in clinical sciences at Ondokuz Mayıs University Faculty of Medicine, 2)Voluntary participation in the study, 3)Not having a severe physical or mental problem that will prevent communication.

Criteria for exclusion of participants from the study: 1)Becoming an assistant in basic medical sciences, 2)Not being a medical school graduate

Population and sample
The study population consisted of medical residents working in clinical sciences at Ondokuz Mayıs University Faculty of Medicine. During the data collection process, it was unimportant for this study that the physicians were medical residents for major or minor specialties. The size of the study sample was calculated using the Openepi program. According to the sample calculation, at least 241 medical residents should have been reached with an acceptable error of 5% and a confidence level of 95%. An attempt was made to reach the entire universe. All clinical departments were visited.

Data collection material and method
The survey questions were prepared by reviewing previous theses and articles on this subject.[4,8,11,14-17] The form was created in three parts. In the first part, questions were asked to learn sociodemographic data such as age, gender, residence in an internal or surgical clinical sciences department, total duration of medicine, and TCM information sources. It was recorded as 0 (zero) years for participants who have not yet completed one year. In the second part, the subjective knowledge evaluation status of 15 TCM methods separately, the personal knowledge score level between 0-10 (0 = I do not know, 10 = I know very well) about the 15 methods, the use and recommendation status, the official TCM methods, if any, the desire to receive formal training on the relevant techniques, if any, and other TCM methods allowed by the Ministry of Health of the Republic of

Türkiye were questioned. In the third part, 12 Likert-type questions with five options (strongly disagree-disagree-undecided-agree-strongly agree) were asked to evaluate the assistants' attitudes toward TCM. Cronbach's α value of the 12-question attitude scale toward TCM was 0.765. In order to get the highest score when calculating the attitude scale, except for items 1 and 7, it was calculated as 1 point to strongly disagree, 2 points to disagree, 3 points to be undecided, 4 points to agree, and 5 points to strongly agree. Items 1 and 7 were calculated as 1 point, agree 2 points, undecided 3 points, disagree 4 points, strongly disagree 5 points. Thus, the lowest score is 12 points; The highest was arranged in such a way that 60 points could be obtained.

Those who declared that they knew any TCM method were asked to give themselves a score between 0 (I don't know at all) and 10 (I know very well) for each method to measure their subjective level of knowledge.

All the methods allowed by the Ministry of Health of the Republic of Türkiye were taken into account, and the 15 methods we selected individually were added to the questionnaire. The questionnaire was updated and finalized by verbally consulting 15 resident physicians. The questionnaire form was applied to male and female clinical sciences residents over the age of 18 who were studying at Ondokuz Mayıs University Faculty of Medicine and agreed to participate in the study. The study was conducted between 15.07.2021 and 15.12.2021. It was stated to the participant that participation is voluntary and that he can stop participating at any time without giving any reason. No credentials were requested. The survey took an average of four minutes to complete.

Internal and surgical branches were included in the study because they were actively involved in the treatment process. Basic medical sciences were not included in the study because they did not actively treat, and the majority of them were not medical school graduates; in addition, research assistants in the departments of medical pharmacology and medical genetics who are not medical school graduates were not included.

Statistical analyses
Statistical analyses were performed with IBM SPSS Statistics version 25.0. Means, standard deviations, percentages, and median values were used to present descriptive analyses. The agreement between the normally distributed variables was confirmed using the Kolmogorov-Smirnov test. Mann-Whitney U test was used to evaluate nonparametric groups. $p<0.05$ was considered statistically significant.

Ethics
Ethics committee approval for the research was obtained from the Clinical Research Ethics Committee (OMUKAEK-2021/353

RESULTS
A total of 261 physicians participated in the study. Of the participating physicians, 51.7% (n=135) were male, and 81.9% were from internal medicine sciences. The mean age of the participants was 29.4±2.6 (min=24, max=40) years. The total range of working time in the profession is 0-15 years; the average duration is 4.4 years. The entire duration of the branch medicine range was between 0 and 11 years, and the average time was 2.6 years. 55.6% of the participants stated that they knew cupping therapy, 52.1% acupuncture, and 43.7% hirudotherapy. The least known methods were prolotherapy (7.3%) and osteopathy (8.4%) (Table 1). Osteopathy and prolotherapy had the highest knowledge score among the TCM methods declared to be known. The lowest knowledge score was found to belong to hirudotherapy and reflexology (Table 1).

The most frequently used TCM methods were phytotherapy, with 19.2%, and massage therapy with 14.6%. The least used were apitherapy and homeopathy. It was declared that larval therapy and prolotherapy were not used at all (Table 2). Among the TCM methods recommended by the participants to their patients/clients, phytotherapy, with 16.5%, and massage therapy, with 11.9%, were the most common. Larval therapy and homeopathy were recommended at least (Table 2). The most formal training methods of the participants were cupping therapy, with 4.2%, and mesotherapy, with 2.7%. The method with the least formal training was music therapy, with 0.4%. It was found that there was no formal training in hirudotherapy, larval therapy, apitherapy, homeopathy, chiropractic, and reflexology (Table 2). The methods in which the participants declared that they wanted to receive formal training were acupuncture with 41% and hypnosis and mesotherapy with 32.2%. The methods that required the least formal training were larval therapy, with 7.3%, and apitherapy, with 8.4% (Table 2).

Table 1. The TCM methods that the participants declared that they had knowledge of and the level of knowledge they perceived about these methods

TCM methods	No n (%)	Yes n (%)	Level of knowledge Median (q1-q3)
Acupuncture therapy	125 (47.9)	136 (52.1)	4 (1-5)
Apitherapy (bee therapy)	223 (85.4)	38 (14.6)	4 (2-6)
Phytotherapy (plant therapy)	150 (57.5)	111 (42.5)	4 (1-6)
Hypnosis therapy	180 (69.0)	81 (31.0)	4 (3-5)
Hirudotherapy	147 (56.3)	114 (43.7)	3 (1-5)
Homeopathy therapy	225 (86.2)	36 (13.8)	3 (1-6)
Chiropractic therapy	216 (82.8)	45 (17.2)	4 (2-5)
Cupping therapy	116 (44.4)	145 (55.6)	4 (2-6)
Larval therapy	228 (87.4)	33 (12.6)	4 (2-6)
Massage therapy	176 (67.4)	85 (32.6)	4 (1-6)
Mesotherapy	163 (62.5)	98 (37.5)	4 (2-5)
Music therapy	205 (78.5)	56 (21.5)	3 (2-6)
Osteopathic therapy	239 (91.6)	22 (8.4)	5 (3-6)
Prolotherapy	242 (92.7)	19 (7.3)	4 (3-7)
Reflexology	224 (85.8)	37 (14.2)	3 (1-5)

Table 2. TCM methods that participants declare that they use, recommend, have received formal training, and want to receive

TCM method	Declared to be used n (%)	Recommended to Patients/Clients n (%)	Formal Education Received n (%)	Formal Education Desired n (%)
Acupuncture therapy	10 (3.8)	29 (11.1)	5 (1.9)	107 (41.0)
Apitherapy (bee therapy)	1 (0.4)	4 (1.5)	0 (0.0)	22 (8.40)
Phytotherapy (plant therapy)	50 (19.2)	43 (16.5)	6 (2.3)	77 (29.5)
Hypnosis therapy	5 (1.9)	6 (2.3)	4 (1.5)	84 (32.2)
Hirudotherapy	4 (1.5)	12 (4.6)	0 (0.0)	29 (11.1)
Homeopathy therapy	1 (0.4)	1 (0.4)	0 (0.0)	31 (11.9)
Chiropractic therapy	3 (1.1)	6 (2.3)	0 (0.0)	57 (21.8)
Cupping therapy	27 (10.3)	27 (10.3)	11 (4.2)	70 (26.8)
Larval therapy	0 (0.0)	1 (0.4)	0 (0.0)	19 (7.30)
Massage therapy	38 (14.6)	31 (11.9)	2 (0.8)	59 (22.6)
Mesotherapy	8 (3.1)	14 (5.4)	7 (2.7)	84 (32.2)
Music therapy	18 (6.9)	14 (5.4)	1 (0.4)	56 (21.5)
Osteopathic therapy	2 (0.8)	7 (2.7)	3 (1.1)	40 (15.3)
Prolotherapy	0 (0.0)	4 (1.5)	4 (1.5)	41 (15.7)
Reflexology	4 (1.5)	3 (1.1)	0 (0.0)	46 (17.6)

Diet (n=31, 11.9%) and hot springs (n=23, 8.8%) were the most frequently declared among those declared to be allowed by the Ministry of Health of the Republic of Türkiye and known as another TCM method. Access bar, meditation, HRV biofeedback, bioresonance, reiki, tai-chi, ozone therapy, Botox, sound frequency therapy, and neural therapy were the least known methods (each; n=1, 0.4%) (Table 3).

Table 3. Other methods that they declare, known as other TCM methods

TCM method	n (%)
Diet	31 (11.9)
Spa	23 (8.8)
Yoga	22 (8.4)
Aromatherapy	9 (3.4)
Functional Medicine	6 (2.3)
Pilates	3 (1.1)
Access Bar	1 (0.4)
Meditation	1 (0.4)
Hrv Biofeedback	1 (0.4)
Bioresonance Therapy	1 (0.4)
Reiki	1 (0.4)
Tai-Chi	1 (0.4)
Ozone Therapy	1 (0.4)
Botox	1 (0.4)
Sound Frequency Therapy	1 (0.4)
Neural Therapy	1 (0.4)

The statement "TCM methods can replace evidence-based medicine" with 70.5% and "TCM methods should also be applied by people other than health personnel (physicians, dentists, pharmacists, etc.) who are trained in the relevant subject" with 65.6% were the attitudes that medical residents disagreed with the most. "Patients/clients should inform the physicians they have examined/consulted about the TCM methods they use," with 89.6% and 82% with the statement "Only physicians trained in the relevant subject should be able to apply TCM methods." were the attitudes that physicians agreed with the most. "There is no harm in patients/clients using

the TCM method they want simultaneously in addition to the treatment you recommend." was the attitude that physicians were most undecided with 39.5% (Table 4).

Table 4. Evaluation of attitude statements towards TCM

Attitudes statements towards TCM	Strongly disagree n (%)	I disagree n (%)	I'm undecided n (%)	Agree n (%)	Strongly agree n (%)
TCM methods are risky methods that need to be more scientific.	12 (4.6)	83 (31.8)	80 (30.7)	64 (24.5)	22 (8.4)
TCM methods can replace evidence-based medicine.	83 (31.8)	101 (38.7)	55 (21.1)	18 (6.9)	4 (1.5)
Patients/clients should inform the relevant physicians about the TCM methods they use.	5 (1.9)	2 (0.8)	20 (7.70)	82 (31.4)	152 (58.2)
Only physicians who are trained in the relevant subject should be able to apply TCM methods.	5 (1.9)	13 (5.0)	29 (11.1)	84 (32.2)	130 (49.8)
Other health professionals (nurses, pharmacists, etc.) who have been trained in the relevant subject should also be able to apply TCM methods.	63 (24.1)	62 (23.8)	66 (25.3)	59 (22.6)	11 (4.2)
Persons other than health personnel (physicians, dentists, pharmacists, etc.) who have been trained in the relevant subject should also be able to apply TCM methods.	109 (41.8)	62 (23.8)	37 (14.2)	38 (14.6)	15 (5.7)
The reason for the popularity of TCM methods is religious and cultural factors.	5 (1.9)	29 (11.1)	75 (28.7)	120 (46.0)	32 (12.3)
There is no harm for patients/clients to use the TCM method they want simultaneously in addition to the treatment you recommend.	28 (10.7)	44 (16.9)	103 (39.5)	71 (27.2)	15 (5.7)
TCM methods should also be included in the reimbursement of state insurance.	34 (13.0)	49 (18.8)	64 (24.5)	67 (25.7)	47 (18.0)
TCM departments should also be opened in medical faculty hospitals, and resident training should be started.	26 (10.0)	42 (16.1)	74 (28.4)	80 (30.7)	39 (14.9)
TCM courses should also be given within the medical school education.	22 (8.4)	28 (10.7)	70 (26.8)	100 (38.3)	41 (15.7)
Optional TCM training should also be given within the residency training.	25 (9.6)	16 (6.1)	42 (16.1)	109 (41.8)	69 (26.4)

Attitude scale scoring was determined as a minimum of 13 points and a maximum of 53 points. The mean score of the attitude scale was found to be 37.5±7.0. It was determined that there was no statistically significant difference between TCM attitude scores according to the status of declaring that they know TCM methods (p>0.05) (Table 5).

Table 5. Comparison of the knowledge of the TCM method declared by the participants according to the TCM attitude score

TCM method	Response	Attitude toward TCM Mean±SD*	p-value**
Cupping therapy	No	37.1±7.2	0.660
	Yes	37.8±6.8	
Hirudotherapy	No	37.8±6.8	0.554
	Yes	37.1±7.1	
Massage therapy	No	37.6±6.9	0.607
	Yes	37.3±7.1	
Hypnosis therapy	No	37.1±7.0	0.279
	Yes	38.3±6.9	
Acupuncture therapy	No	37.5±6.8	0.691
	Yes	37.5±7.2	
Larval therapy	No	37.4±7.0	0.633
	Yes	38.0±7.0	
Apitherapy (bee therapy)	No	37.6±6.8	0.579
	Yes	37.0±7.8	
Homeopathy therapy	No	37.3±6.9	0.421
	Yes	38.9±7.0	
Phytotherapy (plant therapy)	No	37.0±6.9	0.345
	Yes	38.1±7.1	
Chiropractic therapy	No	37.3±7.1	0.546
	Yes	38.3±6.4	
Osteopathic therapy	No	37.3±6.9	0.212
	Yes	39.5±7.4	
Music therapy	No	37.4±6.8	0.728
	Yes	37.8±7.7	
Reflexology	No	37.4±6.9	0.964
	Yes	38.2±7.5	
Prolotherapy	No	37.4±7.0	0.588
	Yes	39.0±5.7	
Mesotherapy	No	37.8±6.8	0.094
	Yes	36.9±7.2	

*SD: Standart Deviation, ** Mann Whitney U test

DISCUSSION

In his study, Yüksel et al. stated that most of the physicians had little or no knowledge about TCM methods in their own opinions. Doctors have stated that they have heard of acupuncture, hirudotherapy, and hypnosis the most.[4] In another study conducted by Özçakır et al. with general practitioners and examining physicians' knowledge, attitudes, and behaviors about TCM, most of the physicians stated that they had a low level of knowledge about TCM methods according to their opinions.[18] In the study conducted by Kırsoy, the participating medical faculty students stated that the methods they had the most knowledge about were massage and acupuncture, respectively.[19] In Ağrak's study on specialty and medical faculty students, it was determined as diet and prayer.[20] In a study conducted by Bjersa et al. among doctors, nurses, and physiotherapists, almost all of the participants reported having little or no knowledge about TCM.[10] According to the study conducted by Mistik et al., the most well-known methods were acupuncture and phytotherapy, contrary to this study.[21] In a study of primary care physicians and pediatricians by Orhan et al., more than half of the physicians reported a low level of knowledge about TCM.[22] In another study conducted by Şenol et al., spa and massage were the most popular TCM methods. The least known method was chiropractic.[17] In another study conducted by Altan et al. with preclinical students, the most common methods of the students were massage, herbal therapy, yoga meditation, hypnosis, and spirituality prayer.[23] In a study conducted by Doğanay et al., the most prominent TCM practitioners were religious practice/prayer, massage, cupping, and hydrotherapy/spa practice.[24] Mak et al. In a study with doctors in Australia, participants reported being most familiar with acupuncture, yoga, and Tai Chi.[25] A study conducted by Verhoef et al. on general practitioners in Canada found that doctors were most knowledgeable about chiropractic, acupuncture, and hypnosis.[26] In this study, cupping therapy, acupuncture, and hirudotherapy were the most well-known methods, and prolotherapy and osteopathy were the least known. The reason for this; can be explained by the fact that cupping, acupuncture, and hirudotherapy are more common in the information sources in the medical community in Türkiye, while prolotherapy and osteopathy are less frequently mentioned methods. This study determined that most medical residents working in the clinic had a low rate of knowledge of TCM methods, and those who declared that they knew had low subjective knowledge levels. This can be explained by the fact that TCM training is not given in medical education, and few willing people do partial or detailed studies on this subject.

In a study conducted in Zonguldak, acupuncture, leeches, and cupping were doctors' most commonly used methods, in contrast to the current study.[4] A study in India reported that most doctors had previous experience with TCM methods. The most commonly used methods were homeopathy, Ayurveda, and yoga.[27] In this study, homeopathy was one of the least used methods by the participants. A study conducted with resident physicians in a university hospital reported that physicians utilized cupping therapy and phytotherapy most frequently, respectively.[28] In a study conducted with medical students, it was reported that they utilized phytotherapy most frequently, a finding that is consistent with the current study.[19] In a study of 423 osteopathic primary care physicians who were members of the Michigan Osteopathic Association, physicians reported that they preferred vitamins and massage for themselves or their families.[29] In this study, massage therapy was the second most frequently used method. In a study of medical students in Düzce, the most frequently used TCM methods were phytotherapy and cupping.[30] Similarly, phytotherapy was the most frequently used method in this study. In a study conducted in Elazığ, it was reported that the most frequently used TCM methods by medical students were massage, diet, and phytotherapy. It was reported that students never used the homeopathic, osteopathic, and Reiki methods of TCM.[9] In a study conducted in a hospital in Istanbul, massage, hydrotherapy, acupuncture, and herbal treatment were the most commonly used methods.[12] In a study conducted with physicians and nurses in a hospital in Ankara, medicinal plants, massage, and hydrotherapy were the most commonly used methods.[31] In a study involving primary care physicians in Bursa, physicians reported using herbal therapies and vitamins.[18] In this study, phytotherapy was the most frequently used method among the participants, followed by massage therapy. Apitherapy and homeopathy were the least frequently used. Larval therapy and prolotherapy were not used at all. The

high rates of use of phytotherapy and massage therapy by our participants can be explained by the fact that these methods are known and accepted by society. The low use of apitherapy and homeopathy can be explained by the fact that these methods are less known and accepted by society. The lack of use of larval therapy and prolotherapy may be attributed to the fact that the conditions for which these therapies are indicated are not commonly encountered in the medical resident population.

In a joint study by Salomonsen et al., acupuncture was the most recommended method for patients.[32] In a study conducted by Elbi et al. with a group of general practitioners, similar to this study, it was determined that they recommended herbal medicines and vitamins to the patients the most, followed by massage, cryotherapy, reflexology, and relaxation techniques.[8] In contrast, in this study, the recommendation rates of phytotherapy and massage therapy were lower. According to a study by Yüksel et al., doctors recommend acupuncture to their patients the most.[4] In Ağrak's study, the methods that the participants recommended to their patients the most were diet, vitamin supplementation, and phytotherapy, respectively.[20] According to Giannelli et al.'s study on the knowledge and practice levels of general practitioners in Italy about TCM practices, patients were most often recommended acupuncture and less frequently manipulative treatments, homeopathy, and herbal medicines. Being young (<54) and female increased the likelihood of recommending TCM applications.[33] The study conducted with resident physicians in Zonguldak revealed that physicians recommended TCM methods to their patients based on their needs. However, in contrast to this study, acupuncture was the most frequently recommended method.[28] Among the methods the participants stated they recommended to their patients/clients, phytotherapy and massage therapy were the most frequently recommended. The least recommended were larva therapy and homeopathy. The reason for phytotherapy and massage therapy being recommended more may be explained by the fact that the physicians are more knowledgeable, experienced, and experienced in this regard. The reason for larval therapy being the least recommended may be explained by the lack or inadequacy of knowledge and experience in this regard and the fact that such a method is not applied in our hospital. The reason for homeopathy being among the least recommended may be explained by

the fact that it is a rarely known method and has a low level of evidence.

In a study conducted by Verhoef et al., 20% of 200 doctors in Canada reported that they received TCM education, primarily in medical education or seminars on hypnosis and acupuncture.[26] In a study conducted by Özkaptan et al. with Turkish doctors and nurses, the majority of nurses and doctors reported that they had never received any TCM education.[34] In a study conducted by Yüksel et al., almost all of the doctors did not participate in any training program related to TCM, and about half of the doctors wanted to attend a TCM training course.[4] In a study conducted by Koçdaş et al., it was determined that almost all of the doctors did not see the use of TCM, and about half of them received training and wanted to use it in their patients.[12] In a study conducted by Özçakır et al., it was reported that almost all of the doctors did not attend any training or course related to TCM, and about three-quarters of them wanted to receive training.[35] The methods that the participants stated that they received the most formal education were cupping therapy and mesotherapy. The method that received the least formal education was music therapy. It was determined that no formal education was received on hirudotherapy, larva therapy, apitherapy, homeopathy, chiropractic, and reflexology. In this study, similar to other studies conducted in Türkiye, it was determined that the majority of medical residents did not receive any TCM education; however, the rate of people who received education was higher than in other studies. Mobbing, heavy workload, and the decrease in the purchasing power of the physician's salary lead to physician resignations.[36] Together with the increase in the public's demand for TCM methods, it may contribute to the shift of the interest of medical residents to this sector, which has become a profitable sector. The fact that the most education was received in cupping therapy and mesotherapy can be explained by the fact that there is a lot of demand for these two methods.

In Ağrak's study, hypnosis, phytotherapy, and acupuncture were the methods that the participants wanted to receive the most training.[20] In this study, the methods that the participants declared that they wanted to receive formal training were acupuncture, hypnosis, and mesotherapy, and the methods that required the least formal training were larval therapy and apitherapy. The reason for this may be that popular and more demanded methods affect

medical residents.

Limitations

The fact that the study population is limited to clinical sciences medical residents working in the faculty hospital of a province will limit the generalizability of the results to the country. The fact that the information in this study is based on personal statements and carries the risk of differing from the facts may adversely affect the results. Although the entire universe is tried to be reached, the fact that the majority of medical residents do not participate in the study is a limitation. Since no valid and reliable questionnaire measures physicians' knowledge about TCM and legislation in Türkiye, the use of a questionnaire created by literature review can be considered as a limitation. Since the study was carried out during the pandemic period, conducting it as an online survey instead of face-to-face to reduce transmission can be considered as a limitation because it reduces the number of questionnaire fillings.

CONCLUSION

Medical residents have been assessed on their knowledge of TCM methods, with results indicating that their knowledge needs to be improved. It is found that medical residents predominantly rely on and recommend phytotherapy to their patients, although they have yet to receive formal training on the subject. This suggests that phytotherapy is being used and recommended based on informal knowledge. Acupuncture is identified as the area where medical residents desire the most training. Although medical residents have a positive attitude towards TCM, they believe that it should only be practiced under physician control and as part of medical education. The limited knowledge and lack of training in TCM contribute to the lack of TCM practice among medical residents. It would be useful for the Ministry of Health to establish an official platform for all physicians to obtain at least basic information on the subject through a centralized online training channel (such as ministry training on smoking cessation). Specifying participation in the training platform on these methods as a prerequisite for the application of these methods or participation in the certification process will ensure a comprehensive awareness of all TCM methods.

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ORIGINAL RESEARCH

Antibacterial, Phytochemical and Toxicological Activities of *Garcinia kola* Extracts against Multidrug Resistant Clinical Bacteria

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Abstract

Objective: The rise in multiple antibiotic-resistant microorganisms has led to a decline in the efficacy of many antibiotics, prompting the investigation of alternative solutions such as medicinal plants. To tackle this concerning issue, this study assessed the phytochemical composition, antibacterial activity, and toxicological characteristics of *Garcinia kola* extracts against multidrug-resistant bacteria commonly found in clinical settings.

Material-Method: The cold maceration technique was employed to extract the root and leaf of *Garcinia kola* using water and methanol. The extracts were then subjected to phytochemical screening. Extracts were evaluated for the ability to inhibit the growth of five multi-drug resistant isolates used in this study. An agar well diffusion assay was used to determine the zones of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth macro dilution technique. Additionally, brine shrimp lethality assay was conducted to determine the 50% lethal concentration (LC50) of the extracts. Synergistic effects of combination of the extracts for each solvent (root and leaf) were tested against the isolates.

Conclusion: The results showed that the root extract in methanol had the highest yield at 25.58%. All four extracts contained ten out of the fourteen tested phytochemicals. The methanol leaf and root extracts exhibited the highest and lowest inhibition zones of 20 mm and 9 mm against *Staphylococcus aureus*, respectively. The MIC values ranged from 250 to 600 mg/mL indicating broad-spectrum antibacterial activity. The cytotoxicity test showed a range of 33.03 to 126.3 µg/mL for the aqueous and methanol extracts. Although Garcinia kola shows potential as a source of antibacterial compounds, caution should be exercised due to its toxic effects.

Keywords: *Garcinia kola*, Phytochemicals, Minimum Inhibitory Concentration (MIC), Inhibition Zones, Cytotoxicity

INTRODUCTION

Researchers continue to monitor the utilization of botanical substances for addressing various conditions, and traditional herbal remedies have become increasingly significant as alternative treatments for a diverse array of illnesses [1, 2]. The majority of individuals in economically disadvantaged regions perceive plant-based remedies as more cost-effective and safer alternatives [3]. The use of these plant products has expanded in industrialized, emerging, and impoverished nations due to the introduction of new diseases and the development of microorganism resistance [4,5].

Numerous pharmacologically active compounds present in medicinal plants can improve health through various mechanisms, whether individually, in combination, or both simultaneously [6]. The identification of phytochemicals within plants,

which could potentially serve as significant medications in modern medicine, has fueled ongoing interest in evaluating natural compounds derived from plants as potential chemotherapeutic agents [7]. Apart from generating potentially harmful bioactive molecules, plants also develop defense mechanisms against predators [8]. The increasing popularity of medicinal plants has underscored the need for comprehensive scientific investigations to assess both their potential toxicity and effectiveness [9]. While the general public often perceives herbal medicines as safe and devoid of potential toxicity, the risk of toxicity remains a significant barrier that limits their widespread use [10]. Common toxicities associated with herbal medicines include hepatotoxicity, nephrotoxicity, neurotoxicity, cardiac toxicity, pulmonary toxicity, adult respiratory distress syndrome, convulsions, and

acute eosinophilic pneumonia [11]. The exploration of medicinal plant toxicity can be advantageous for both the development of novel therapeutic compounds and the progress of conventional medicine [9].

Many African nations rely on traditional medicine to address their healthcare needs [7]. Furthermore, in the folk medicine of the Ilaje people of Ondo State, Nigeria, appendicitis is treated using the root of the botanical plant *Garcinia kola*. This practice stems from the abundance of secondary metabolites, or phytochemicals, found in plants, which may possess pharmacological properties effective against various ailments [13]. Because of its bitter, astringent taste and stimulating qualities, the seeds are greatly valued as an oral masticatory aid. They, along with other plant components, are utilized as an aphrodisiac and for the treatment of various conditions including diarrhoea, bronchial issues, throat infections, fever, colds, and malaria [14]. In customary, cultural, and social rituals, people traditionally chew the seeds for their aphrodisiac properties [15]. Around 80% of the population in many West African countries resort to medicinal herbs for therapy, primarily due to the limited affordability of new medications [16].

The multifunctional *Garcinia kola* Heckel (Clusiaceae) tree is commonly encountered in tropical and subtropical wet lowland forests across sub-Saharan Africa, including countries like Nigeria and Cameroon. Virtually every part of this tree has been harnessed in traditional medicine for centuries to address a diverse array of ailments. Consequently, it is referred to by various names such as bitter kola, false kola, and occasionally dubbed the "wonder plant" [14]. In recent years, *Garcinia kola* has garnered significant research interest [14, 15, 17, 18] primarily due to its unique biflavonoid complex called kolaviron, which seems to be exclusive to this plant species. However, it is worth noting that *Garcinia kola* contains various other chemicals alongside kolaviron, including garcinianin, kolanone, gakolanone, garcinoic acid, garcinal, garcifuran A and B, and garcipyran [19]. Additionally, these compounds appear to be highly specific to *Garcinia kola*, with no other botanical sources confirming their presence. The objective of this study was to investigate the cytotoxic properties, antimicrobial properties, and phytochemical activities of extracts derived from *Garcinia kola*.

MATERIALS AND METHODS

Plant materials
The plant materials were obtained from a nearby plantation in Shagamu, Ogun State. To confirm the plant's identity, it was examined and authenticated at the herbarium, Department of Botany, University of Ibadan. A voucher specimen labeled UIH-23235 was submitted for future reference. The leaves and roots of the plant were air-dried, pulverized, and soaked in clean distilled water and cold methanol separately. The resulting extracts were concentrated using a vacuum and stored at a temperature of 4°C for later use.

Test organisms and media
The bacterial species [*Staphylococcus aureus, Escherichia coli, Acinetobacter baumannii, Enterobacter cloacae, and Enterococcus faecalis*] used in this study were obtained from the Department of Microbiology, University of Ibadan and University College Hospital, Ibadan, Nigeria. These bacteria were cultured on nutrient agar [No. 2] and nutrient broth [pH 7.4] from Oxoid, England. Prior to testing, all bacteria were kept on agar slopes at 4°C. Brine shrimp eggs [*Artemia salina* Sander®] were purchased from the Great Salt Lake Company in the USA.

Phytochemical screening
Preliminary phytochemical screening was carried out according to Evans [20] and Edeoga *et al.* [21]. An analysis was conducted to determine if the leaves and roots contained certain secondary metabolites. These included alkaloids, tannins, cardiac glycosides, flavonoids, saponins, steroids, phenols, coumarins, terpenoids, diterpenes, flavonoids, anthocyanins, charcones, and proteins.

Cytotoxicity [Brine-Shrimp Lethality] assay
Cytotoxicity test was carried out using the methods of Meyer *et al* [22]. Artemia salina eggs were incubated in seawater for a period ranging from 48 to 72 hours. The nauplii were divided into tubes with different concentrations of extracts and each of the tube contained 10 nauplii. The concentrations used were 1000, 500, 250, 125, 62.5, and 31.25 in µg/mL. After 24 hours, the number of surviving nauplii was recorded to estimate the number of dead nauplii. The mortality percentage for Brine Shrimp nauplii was determined for each concentration using the following formula;

$$\% \text{ Mortality} = N1/N0 \times 100$$

Where,

$N1$ = The total count of deceased nauplii after 24 hours of being kept at room temperature.

$N0$ = Total number of nauplii

The Probit analysis was conducted using the conventional probit table. Using this information, we determined the median Lethal Concentration [LC_{50}] following the methodology outlined by Finney [23].

Toxicity testing criteria

Herbal extract toxicity is often evaluated using either Meyer's or Clarkson's toxicity index. In line with Meyer's index, extracts are considered toxic if the LC_{50} value is below 1000 µg/mL, and non-toxic if the LC_{50} value is above 1000 µg/mL[22]. On the other hand, Clarkson's toxicity criterion categorizes plant extracts as follows: non-toxic for LC_{50} values above 1000 µg/mL; low toxic for LC_{50} values between 500 and 1000 µg/ml; medium toxic for LC_{50} values between 100 and 500 µg/mL, and highly toxic for LC_{50} values between 0 and 100 µg/mL[24].

Determination of antibacterial activity

Antibacterial activity of the extracts was determined by agar well diffusion method of Perez et al. [25], with slight modifications Idowu et al. [26]. The samples were dissolved in sterile distilled water and methanol to achieve concentrations ranging from 125-750 mg/mL. Each test organism was sub-cultured in Nutrient broth and incubated for 24 hours and adjusted using the 0.5 McFarland standard. A sterile cotton swab was placed into the prepared liquid and rotated a few times by applying pressure to the inside of the tube. This action was done to remove any extra liquid from the swab. Next, the swab was inoculated onto the Mueller Hinton Agar (MHA) plate by moving it back and forth across the whole surface. The plate was also rotated about 60° after each streak to ensure the inoculum was evenly spread. Afterward, the MHA plates remained uncovered for three to five minutes to enable the absorption of any additional moisture present on their surfaces (CLSI) [27]. An 8.0 mm cork borer was utilized to create holes in agar, and then 100µL of extracts were added to each well. Controls were established using a concentration of 15µg/mL of Erythromycin for the bacteria, while 40% methanol served as the negative control. The size of the inhibition zones was employed as an indicator of the antibacterial effectiveness.

Determination of minimum inhibitory concentration [MIC]

Minimum Inhibitory Concentration [MIC] was determined on test bacteria by broth macro dilution using the method of Andrews [28]. The agar was combined with the extracts to create a range of dilutions in each test tube, including concentrations of 600, 500, 400, 300, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 mg/mL. All test tubes contained 2 mL of nutrient broth, and then 0.1 mL of each extract at the desired concentration was added to the broth. Next, 0.1 mL of standardized inoculums of the specific test pathogens were introduced into the test tubes that contained both the nutrient broth and the extract suspensions. All the test tubes were incubated at 37°C for 24 hours. Following the incubation period, each test tube was examined to determine whether visible signs of bacterial growth were present or absent. The minimum concentration of the substance being tested, at which no observable bacterial growth was detected, was defined as the MIC [Minimum Inhibitory Concentration].

Determination of minimum bactericidal concentration [MBC]

To determine the minimum bactericidal concentration [MBC] of an extract, a loop full from each broth culture that did not show any growth in the minimum inhibitory concentration [MIC] tubes was inoculated onto freshly prepared nutrient agar plates and incubated for 24 hours. At the end of the incubation period, the extract with lowest concentration that prevented any bacterial growth on the solid medium was considered as the MBC for the extract. This observation was associated with the MIC test tube that exhibited no signs of growth within 24 hours of incubation.

RESULTS

Table 1 shows the percentage of plant extract obtained from the different solvents. The root extracted using methanol demonstrated the highest yield of 22.60%, whereas the aqueous root extraction was the lowest with 4.24%.

The composition of phytochemicals in *Garcinia kola* extracts from the leaf and root are presented in Table 2. The results demonstrated that both the root and leaf samples of *Garcinia kola* contain various phytochemicals of interest, including alkaloids, tannins, flavonoids, saponins, phenols, and quinones. The quantitative analysis of phytochemicals indicates that ten out of the fifteen tested bio-active constituents were found in both the methanolic and aqueous extracts of *Garcinia kola*. However, charcones, anthocyanins, and chalcones were not detected. Coumarins were only absent in the aqueous root extract, and cardiac glycosides were only present in the aqueous root extract.

Table 1. Percentage yield of *Garcinia kola* extracts

Plant parts	Solvent Type	Weight of sample [g]	Weight of extracts [g]	Yield [%]
Leaves	Aqueous	1000	66.30	6.63
Leaves	Methanol	1000	168.00	16.80
Root	Aqueous	1000	42.40	4.24
Root	Methanol	1000	226.00	22.60

Table 2. Qualitative phytochemical Screening of *Garcinia kola* Extract

S/N	Phytochemicals	Extracts			
		Aqueous Root	Methanol Root	Aqueous Leaf	Methanol Leaf
1	Saponins	+	+	+	+
2	Tannins	+	+	+	+
3	Flavonoids	+	+	+	+
4	Steroids	+	+	+	+
5	Quinones	+	+	+	+
6	Terpenoids	+	+	+	+
7	Phenols	+	+	+	+
8	Di-terpenes	+	+	+	+
9	Proteins	+	+	+	+
10	Alkaloids	+	+	+	+
11	Cardiac glycosides	+	-	-	-
12	Coumarins	-	+	+	+
13	Chalcones	-	-	-	-
14	Anthocyanins	-	-	-	-

Table 3 displays the results of our findings on the phytochemical screening of the *Garcinia kola* extracts. The table presents the percentage of active compounds found in the aqueous and methanol extracts. It was observed that the root and leaf extracts from methanol had higher levels of active compounds compared to the aqueous root and leaf extracts. Specifically, the methanol extract of the root exhibited the highest concentration of phenols [83.501±0.053], while the aqueous extract of the root had the lowest concentration of saponins [0.60±0.10%].

Table 3. Quantitative phytochemical screening of *Garcinia kola* extracts

Sample	Saponins [%]	Alkaloids [%]	Flavonoids [mg QE/g]	Phenols [mg GAE/g]	Tannins [mg GAE/g]
Methanol Root	4.28±0.02	12.14±0.06	42.889±0.00	83.501±0.05	10.608±0.05
Methanol Leaf	9.50±0.01	10.40±0.00	29.630±0.13	31.795±0.03	5.474±0.04
Aqueous Root	4.61±0.02	3.91±0.01	10.963±0.78	26.472±0.05	4.982±0.00
Aqueous Leaf	0.60±0.10	1.82±0.02	3.778±0.00	4.899±0.03	3.123±0.04

Values are in mean of duplicate values ± Standard error

Table 4 presents the results of the study on the antibacterial properties of aqueous leaf and root extracts of *Garcinia kola* measuring the zones of inhibition, in millimeters, of the extracts against different bacteria. The results revealed that the extracts displayed varying levels of antibacterial activity against the test bacteria. In general, the root extracts exhibited higher inhibition zones than the leaf extracts, particularly against *Staphylococcus aureus, Escherichia coli,* and *Enterococcus faecalis.* Notably, the root extracts were particularly effective against *Enterococcus faecalis*, with a zone of inhibition of 13.50±0.71 mm. However, the aqueous extracts exhibited no antibacterial activity against *Acinetobacter baumannii* and *Enterobacter cloacae*. Table 5 shows the antibacterial activity of methanol leaf and root extracts of *Garcinia kola* depicted by zone inhibition of the isolates in millimeters. Results also showed variations in the antibacterial activities of the extracts against the test bacteria. The zones of inhibitions of the methanol extracts were generally higher than those of the aqueous extracts. The extracts were very effective against three of the test isolates [*S. aureus, E. coli, E. faecalis*], although more effective on *Staphylococcus aureus* [20.00±0.00mm] than the other two isolates. The methanol extracts showed no antibacterial activity on *A. baumannii* and *E. cloacae*.

Table 4. Antibacterial activity of Aqueous Leaf and Root Extracts of *Garcinia kola*

Extracts	Conc. In mg/mL	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*
AR	750	13.00±2.83	0.00±0.00	12.00±1.41	13.50±0.71	0.00±0.00
	500	11.00±1.41	0.00±0.00	12.00±0.00	11.00±0.00	0.00±0.00
	250	9.00±1.41	0.00±0.00	11.50±0.71	11.00±0.00	0.00±0.00
	125	9.00±0.00	0.00±0.00	9.00±0.00	9.50±0.71	0.00±0.00
AL	750	10.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	500	11.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	250	9.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	125	9.50±0.71	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ARAL	750	13.50±0.71	0.00±0.00	14.50±0.71	12.50±0.71	0.00±0.00
	500	12.00±0.00	0.00±0.00	14.00±0.00	10.50±0.71	0.00±0.00
	250	11.00±0.00	0.00±0.00	13.50±0.71	10.50±0.71	0.00±0.00
	125	11.00±0.00	0.00±0.00	12.50±0.71	9.00±0.00	0.00±0.00
Control	Erythromycin [15µg]	0.00±0.00	8.00±0.00	0.00±0.00	10.00±0.00	7.00±0.00
	Methanol [40% v/v]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

AR: Aqueous Root   AL: Aqueous Leaf   ARAL: Aqueous Root and Aqueous Leaf

Table 5. Antibacterial activity of Methanol Leaf and Root Extracts of *Garcinia kola*

Extracts	Conc. in mg/mL	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*
MR	750	12.50±0.71	0.00±0.00	14.50±0.71	12.00±0.00	0.00±0.00
	500	13.00±0.00	0.00±0.00	13.00±1.41	14.00±0.00	0.00±0.00
	250	12.50±0.71	0.00±0.00	12.50±2.12	13.00±1.41	0.00±0.00
	125	11.00±0.00	0.00±0.00	11.00±0.00	15.00±0.00	0.00±0.00
ML	750	12.50±0.71	0.00±0.00	19.00±0.00	12.00±1.41	0.00±0.00
	500	13.00±0.00	0.00±0.00	17.50±0.71	12.00±1.41	0.00±0.00
	250	12.50±0.71	0.00±0.00	17.50±0.71	18.50±0.71	0.00±0.00
	125	11.00±0.00	0.00±0.00	15.00±0.00	17.00±0.00	0.00±0.00
MLMR	750	12.00±0.00	0.00±0.00	15.00±0.00	15.00±0.00	0.00±0.00
	500	9.50±0.71	0.00±0.00	20.00±0.00	14.50±0.71	0.00±0.00
	250	11.00±0.00	0.00±0.00	17.00±0.00	9.00±0.00	0.00±0.00
	125	12.00±0.00	0.00±0.00	13.00±2.82	14.50±0.71	0.00±0.00
Control	Erythromycin [15µg]	0.00±0.00	8.00±0.00	0.00±0.00	10.00±0.00	7.00±0.00
	Methanol [40% v/v]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

MR: Methanol Root   ML: Methanol Leaf   MLMR: Methanol Root and Methanol Leaf

Tables 6 and 7 show the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous and methanol extracts [Root and Leaf] of *Garcinia kola*. Results showed the MIC values to be within the range of 125 – 600 mg/mL.

Table 8 showed the LC_{50} as calculated by Graph Pad Prism 2021 (Computer software programme) to be within the range of 33.03-126.3 µg/mL. Table 9 shows Finney's table for transformation of percentage of mortality to probit values.

Table 6. Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal Concentration of the aqueous extract [Root and Leaf] of *Garcinia kola*

EXTRACT	MG/ML	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*	Sterility
AR	MIC	300	>600	300	300	>600	----
	MBC	600	>600	>600	500	>600	NG
AL	MIC	300	>600	250	250	>600	----
	MBC	>600	>600	>600	>600	>600	NG
ALAR	MIC	600	>600	125	400	>600	----
	MBC	600	>600	400	>600	>600	NG
Control	EI	NG	+	NG	+	+	NA
	MI	+	+	+	+	+	NA
	BI	+++	+++	+++	+++	+++	NA

AR: Aqueous Root   ---: No turbidity   ARAL: Aqueous Root and Aqueous Leaf   +++: Massive Growth
AL: Aqueous Leaf   NG: No growth   NA: Not applicable   +: Growth
EI: Erythromycin + Inoculum   MI: Methanol + Inoculum   BI: Mueller Hinton Broth + Inoculum

Table 7. Minimum Inhibitory Concentration [MIC] and Minimum Bactericidal Concentration of Methanol extract [Root and Leaf] of *Garcinia kola*

EXTRACT	MG/ML	*Escherichia coli*	*Acinetobacter baumannii*	*Staphylococcus aureus*	*Enterococcus faecalis*	*Enterobacter cloacae*	Sterility
MR	MIC	300	>600	250	250	>600	----
	MBC	>600	>600	400	400	>600	NG
ML	MIC	400	>600	300	300	>600	----
	MBC	>600	>600	500	>600	>600	NG
MLMR	MIC	400	>600	300	400	>600	----
	MBC	600	>600	500	>600	>600	NG
Control	EI	NG	+	NG	+	+	NA
	MI	+	+	+	+	+	NA
	BI	+++	+++	+++	+++	+++	NA

MR: Methanol Root ---: No turbidity   ML: Methanol Leaf NG: No growth   MLMR : Methanol Root and Methanol Leaf
NA: Not applicable   +++: Massive Growth   +: Growth
EI: Erythromycin + Inoculum   MI: Methanol + Inoculum   BI: Mueller Hinton Broth + Inoculum

Table 8. Toxicity of Aqueous and Methanol Extracts of *Garcinia kola* [Root and Leaf] using Brine Shrimp Lethality Assay

Extract	Concentration [µg/mL]	No. of 1st tube	Survived 2nd tube	nauplii 3rd tube	No. of dead nauplii	% Mortality	Probit	LC$_{50}$
AR	1000	0	0	0	30	100.00	8.09	33.03
	500	0	0	0	30	100.00	8.09	
	250	0	1	2	27	90.00	6.28	
	125	1	2	2	25	83.33	5.95	
	62.5	3	3	4	20	66.66	5.41	
	31.25	4	5	6	15	50.00	5.00	
AL	1000	0	0	0	0	100.00	8.09	76.06
	500	0	0	0	0	100.00	8.09	
	250	0	0	0	0	100.00	8.09	
	125	4	2	3	4	70.00	5.52	
	62.5	5	5	6	5	46.66	4.90	
	31.25	8	9	8	8	16.66	4.01	
MR	1000	0	0	0	30	100.00	8.09	84.29
	500	0	0	0	30	100.00	8.09	
	250	2	2	3	23	76.67	5.71	
	125	3	4	4	19	63.33	5.33	
	62.5	4	6	5	15	50.00	5.00	
	31.25	7	8	8	7	23.33	4.26	
ML	1000	0	0	0	30	100.00	8.09	126.30
	500	0	0	0	30	100.00	8.09	
	250	0	0	0	30	100.00	5.71	
	125	4	6	6	14	46.67	5.33	
	62.5	8	7	8	7	23.33	5.00	
	31.25	10	9	10	1	3.33	4.26	
CYCLOPHOSPHAMIDE	1000	2	2	2	24	80	5.84	61.82
	500	3	3	4	20	66.67	6.41	
	250	4	3	4	19	63.33	5.33	
	125	5	4	6	15	50	5.00	
	62.5	7	7	6	10	33.33	4.56	
	31.25	7	9	9	5	16.67	4.01	

AR= Aqueous Root; AL= Aqueous Leaf; MR= Methanol Root; ML= Methanol Leaf

Table 9. Finney's table for transformation of percentage of mortality to probit values

%	0	1	2	3	4	5	6	7	8	9
0	__	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.25	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
__	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

(Finney, 1952).

DISCUSSION

In response to the increasing problem of drug-resistant bacteria, there is an ongoing need to research new treatments to tackle the global issue of antimicrobial resistance. The World Health Organization firmly advocates for the exploration of traditional medicines as potential sources of safe and effective remedies, not only for microbial infections but also for non-microbial illnesses (WHO) [29]. This research explored the potential medicinal properties of *Garcinia kola* extract, an indigenous plant, and its ability to effectively treat drug-resistant bacteria responsible for wound and urinary tract infections often encountered in healthcare settings. It equally replicated the use of traditional folk medicine practices that have been proven effective in treating infections over time. In ancient times, the use of medicinal plants provided significant benefits in managing infectious diseases. Therefore, it is important to follow a similar approach as closely as possible to the traditional methods used in those historical periods. In this study, a cold maceration method was used, which is in line with traditional practices, as it enables a gentle extraction process. This method was observed to effectively preserve most of the phytoconstituents found in the plant materials. The differences observed in the percentage yield, as illustrated in Table 1, can be ascribed to the utilization of various plant components and solvents as opined by Heinrich *et al.* [30]. Both methanol root and methanol leaf had the highest percentage of yield coming in with 22.6% and 16.80%, respectively, and this might be due to the high volatile property of methanol as a solvent which is able to extract both polar and non-polar compounds. Qualitative phytochemical screening of *Garcinia kola* extracts revealed the presence of tannins, saponins, flavonoids, alkaloids, steroids, quinones, terpenes, di-terpenes, phenolic compounds in all test extracts, and this is in accord with the work of Ukaoma *et al.* [31] who equally detected four of these compounds (Flavonoids, tannins, alkaloids and saponins) in their study. In the study of Emmanuel *et al.* [32] on *G. kola* roots, bioactive compounds such as saponins, tannins, flavonoids, cardiac glycosides, and alkaloids were also identified; however, it is noteworthy that the researchers did not utilize methanol as a solvent in their study. This indicates that these key bioactive components are naturally found in the *G. kola* plant, regardless of the choice of solvent for extraction. Flavonoids are well-known for their capacity to defend the body against damaging molecules like hydroxyl and superoxide anion radicals, which ultimately contribute to promoting overall health [32]. Flavonoids possess various beneficial properties, such as anti-inflammatory, anti-allergic, analgesic, and antioxidant effects; this backs the traditional usage of *Garcinia kola* in folk medicine to treat various infections [33]. Tannins have been identified as a phytochemical compound in *Garcinia kola* extract; these compounds have antimicrobial properties and can inhibit the growth and multiplication of microorganisms by binding to iron, forming hydrogen bonds, and interacting with important proteins in these organisms [34]. Presence of tannins makes *Garcinia kola* a suitable medicinal plant for treating microbial infections, especially gastrointestinal ailments like diarrhoea and dysentery [35]. Additionally, tannins have shown promise in preventing and acting as anticancer agents, making them valuable compounds in cancer research [36]. Various factors, both prior to and during the extraction process, can impact the extraction of phytochemicals. These factors encompass the specific part of the plant utilized, the locality and particle size, the drying technique employed, fluctuations in daily and seasonal conditions, and the degree of processing, among others. Additionally, factors associated with the extraction process itself, such as the chosen extraction technique, solvent type, ratio of solvent to sample, solvent pH and temperature, and extraction duration, can also influence the extraction process. In summary, these diverse factors can shape the results and render different variations in the extraction process feasible [37].

The results of the quantitative phytochemical analysis in Table 3 indicate that both solvents (methanol and water) were effective in extracting active compounds from the plant. Methanol was more effective in extracting certain active compounds, particularly flavonoids [42.889±0.000] and phenols [83.501±0.053]. The high efficiency observed in methanol solvent may be due to its high volatile nature. Methanol-extracted root had the highest content in four out of five of the tested phytochemicals, which can be attributed to high polyphenol content commonly found in *G. kola* root. This is consistent with the presence of bioflavonoids in the seeds, stem bark, and roots of *Garcinia kola* [38, 39].

In the antibacterial activity, extracts from the root [Table 4] largely had higher inhibition zones on three of the test isolates [*S. aureus, E. coli, E. faecalis*], although it was more effective on *Enterococcus faecalis* [13.50±0.71 mm] than other isolates. The aqueous extracts showed no antibacterial activity on *A. baumannii* and *E. cloacae*. These findings showed that *G. kola* can be used comprehensively in the treatment of bacteria diseases, mainly the root extract [40].

The combination of the aqueous root and aqueous leaf extracts (ALAR) (1:1), as shown in Table 4, showed a higher antibacterial activity across all four concentrations against the test bacteria than when it was tested singly, and the highest antibacterial activity was against *S. aureus* with a zone of inhibition of 14.50mm at 750 mg/mL. These results indicate that there may be a synergistic effect when these extracts are combined, resulting in enhanced effectiveness. This could be because the compounds in the extracts collaborate to target various aspects of bacterial physiology, resulting in a more significant overall impact compared to using the compounds individually. Furthermore, both the methanolic and aqueous extracts of *Garcinia kola* demonstrated inhibition of the growth of both Gram-positive and Gram-negative bacteria. The use of methanol in the extraction process resulted in a stronger inhibitory effect compared to the aqueous extract, possibly due to the higher concentration of active compounds in the methanol extract. The results of this study indicate that there is a range of antibacterial activities among the different extracts, which is likely attributed to variations in the quantity of compounds present in each plant extract. Phenols [83.501±0.053 mg GAE/g] from methanol extraction were the highest observed and this can be attributed to the high volatility of methanol, which led to more active ingredients than in water. The lethal effect, as observed in the results, was higher in *Staphylococcus aureus* [20.00 mm]. No antibacterial activity for both *A. baumannii* and *E. cloacae* clearly shows resistance of both isolates to the *Garcinia kola* root and leaf extracts, and this could be due to several reasons. Both bacteria are opportunistic pathogens and are often associated with nosocomial infections and can be difficult to treat due to their high antibacterial resistance. The bacteria being resistant to the extracts even at high concentrations suggests that the active compounds in the extracts are ineffective against the strains. It is

possible that these bacteria have developed resistance mechanisms that allows them to resist the action of the antimicrobial compounds present in the extracts. Reports on the antimicrobial activities of *G. kola* vary considerably as there are studies with different views and ranges of antibacterial action [41, 42, 43]. However, it is noteworthy to mention that different parts of the plant were used.

In this study, it was noticed that the isolates resistant to multiple antibiotics displayed higher minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, which varied among the different bacteria strains. When the extracts were used at concentrations ranging from 1.95-62.5mg/mL, it was observed that they were not effective in inhibiting bacterial growth. However, at higher concentrations (125-600mg/mL), we observed varying inhibitory and lethal concentrations for different bacteria. Interestingly, both *A. baumannii* and *E. cloacae* showed no MIC or MBC values, indicating that none of the extracts was able to inhibit or kill these bacteria even at the highest concentration tested (600mg/mL). This lack of activity may be attributed to the factors mentioned earlier in the study. It is worth noting that unlike the agar well diffusion assay, there was no evidence of synergy in the MIC assay. In the study of Osungunna [44], it was suggested that the extracts may have shown a combined effect in the agar well diffusion assay, but not in the MIC test, possibly because of variations in the experimental settings. The agar well diffusion assay is a qualitative technique that can detect even trace amounts of extracts, whereas the MIC test is a quantitative approach that determines the lowest concentration needed to hinder microbial growth. Aqil and Ahmad [45] suggested that the differences in the results of the two tests could be attributed to various factors. One possibility is that the microorganisms in the two tests may have had different susceptibility patterns, which could impact the outcomes. Additionally, variations in the preparation and dilution of the substances being tested, as well as the incubation conditions, could also contribute to disparities in the results between the two tests. The significant variations in the values of MIC and MBC observed in this study may be due to the bacteria's ability to develop resistance to multiple antibiotics. Furthermore, the diverse types of plant components and their combined effects, along with the inherent resistance of each multi-drug resistant bacterium, may also play a role in

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these differences, as different bacteria may react differently to the test samples. Thormar [46] suggested that researchers should prioritize antimicrobial agents with MIC values lower than 1% vol/vol (equivalent to 10,000 ppm) in laboratory settings. This means these agents can effectively hinder or eliminate bacteria at low concentrations. It is essential to keep in mind that findings from laboratory experiments may not accurately represent real-life situations within the body. *In vivo* conditions involve varying concentrations of antibacterial agents and bacteria throughout different bodily regions, and these values are not considered fixed constants [44].

In contemporary times, the brine shrimp (*Artemia salina*) lethality assay has become a frequently employed method to assess the potential harmful impact of bioactive substances. This assay serves as an initial step in evaluating the toxicity of plant extracts for screening purposes. [47,48,49]; pesticides [50] and nanostructures [51]. The initial proposal for this assay was made by Michael *et al.* [50], and it was further developed by other researchers. This lethality assay has been used as a guide for evaluating the cytotoxic and antitumor properties of active agents, as demonstrated by Meyer *et al.* [22] in 1982. In general, it is commonly believed that extracts obtained using alcohol or organic solvents tend to be more toxic than those obtained using water. However, our study yielded contrasting results, as we discovered that the aqueous extracts were actually more toxic than the methanol extracts. Our research findings indicate that the aqueous root, aqueous leaf, and methanol root extracts of *Garcinia kola* are not safe and exhibit toxicity towards the nauplii, with LC_{50} values of 33.03, 78.06, and 84.09 µg/ml, respectively. Additionally, the methanol leaf extract displayed medium toxicity towards the nauplii, with an LC_{50} value of 126.3 µg/ml, in accordance with Clarkson et al. [24] findings. This report corroborates the study of Onajobi et al [52] where they also found that the aqueous extract of *Garcinia kola* was more toxic than the methanol extract. The authors suggested that the higher toxicity of the aqueous extract could be due to the higher solubility of some toxic compounds in water compared to methanol.

CONCLUSION

The findings of this study emphasize the promising potential of *Garcinia kola* as a valuable treatment option for infectious diseases caused by strains that are resistant to multiple antibiotics. The *Garcinia kola* plant showed effective activity against bacteria used in this study and could be further studied for use in management of infections when compared to conventional antibiotics. These findings offer a scientific foundation to consider utilizing the plant for medicinal purposes in treating various infectious diseases such as skin infections, wound infections, and urinary tract infections. Although the results have demonstrated the antibacterial properties of this medicinal plant, further research and development are necessary before the plant can gain broader recognition among the general population. However, it is important to note that further extensive and thorough toxicological assessments are required to establish the safety of these extracts before they can be confidently used.

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ORIGINAL RESEARCH

Investigation of Attitudes Toward Complementary and Alternative Medicine among Young Adults

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Abstract

Objective: This study was designed to investigate the attitudes of individuals aged 18-24 towards complementary and alternative medicine.

Material-Method: The sample of the cross-sectional study, an analytical type of research, consisted of 178 people aged 18-24, with at least primary education, technological devices, and the ability to use internet networks. The data were collected using the Socio-Demographic Information Form and the Complementary, Alternative, and Conventional Medicine Attitudes Scale (CACMAS). Descriptive statistics, independent groups t-test, ANOVA, Mann-Whitney U, Kruskal-Wallis, Tukey tests, and regression analysis were used to analyze the data. The significance level was taken as 0.05 in the interpretation of the results.

Results: Several factors were found to positively impact mean scale scores ($p < 0.05$), including having a large family, perceiving complementary or alternative medicine practices as beneficial, recommending these practices to close relatives or friends. Additionally, it has been found that believing complementary medicine practices to be more beneficial than treatments provided by healthcare institutions also positively influenced scale score averages ($p < 0.05$).

Conclusion: The study found that around half of participants aged 18-24 used complementary and alternative medicine practices, and that some personal attitudes influenced attitudes towards complementary medicine. Hence, there exists a necessity to disseminate accurate information regarding the appropriate utilization of complementary medicine practices, ensuring their application by qualified individuals, and to engage the younger people in such initiatives.

Keywords: Alternative Treatment, Knowledge, Attitude, Complementary Medicine, Alternative Medicine

INTRODUCTION

Medicine is generally defined as the branch of science and art concerned with the sustenance and advancement of health and the elimination, control or prevention of disease. Complementary and Alternative Medicine (CAM) includes all methods used either alongside or instead of traditional and evidence-based medicine.[1] The World Health Organization (WHO) defines CAM applications as comprising culturally diverse value-based information, skills, beliefs, and practices aimed at preserving health, preventing, enhancing, or remedying physical and emotional problems.[2] The global prevalence of CAM usage is on the rise, attributed to several factors such as the widespread availability of information via the internet, a growing interest in individual health management, and heightened awareness among patients regarding independent treatment choices.[3] Incorporating a wide array of modalities like herbal medicines, vitamins, probiotics, psychotherapy, acupuncture, and cupping, the spectrum of CAM encompasses over 1800 distinct types.[4]

Examining the historical utilization of CAM practices reveals that Hippocrates, the esteemed ancient Greek physician hailed as the father of medicine, stated in his writings how the body could be healed through cupping and leeches.[5] In addition, these teachings influenced most medical practices in medieval Europe and served as the cornerstone for much of the educational curriculum in universities of that era.[5] In India, renowned for its utilization of alternative medicine practices, various medical systems are founded on the principle that diseases can be effectively treated with special herbal medicines as well as practices such as yoga and meditation. These traditions, which have been in existence for over 5000 years, form integral components of India's healthcare landscape.[6]

Traditional Chinese medicine relies on techniques such as acupuncture, moxibustion, and herbal remedies to restore balance within the body, with a history spanning over 3000 years.[7] In our country, the roots of CAM practice can be traced back to antiquity, with the first legal framework established by the "Acupuncture Treatment Regulation" aimed at grounding acupuncture treatments in scientific principles.[8] Following this, certain universities commenced acupuncture training programs with authorization from the Ministry of Health. The most recent advancement in this domain occurred with the issuance of the "Regulation on Traditional and Complementary Medicine Practices" in 2014. This comprehensive regulation delineates various aspects including the methodologies of traditional and complementary treatment practices, the requisite training and certification procedures for practitioners, as well as the operational standards for healthcare facilities offering these modalities.[9] Covered under this regulation are a range of treatments such as acupuncture, apitherapy, phytotherapy, hypnotherapy, leech therapy, homeopathy, chiropractic, cupping, larva therapy, mesotherapy, prolotherapy, osteopathy, ozone therapy, reflexology, and music therapy.[10]

Upon reviewing studies on CAM applications, Çekiç et al. (2021) found a notable prevalence of CAM usage among children with allergic diseases, with a higher inclination observed among younger parents. Güveli et al. (2021) reported that 46.8% of the participants in their study involving oncology patients endorsed CAM methods, incorporating plant-based remedies, herbal medicines, and religious and spiritual practices. Şahin et al. (2019) observed positive and moderate attitudes towards CAM among nursing students. Liu et al. (2014) conducted a systematic review and meta-analysis revealing the beneficial effects of St. John's wort oil on psychological problems during menopause. Aboufaras et al. (2023) noted that 37.5% of cancer patients perceive CAM as potentially beneficial, albeit hindered by socioeconomic and scientific barriers.[11-15] Despite the valuable insights provided by these studies, research remains limited to specific patient demographics. Hence, there is a pressing need for further investigation, particularly among healthy young adults. This study aims to evaluate attitudes towards complementary alternative medicine among individuals aged 18-24.

Research questions
- To what extent do the socio-demographic characteristics of 18-24-year-old young adults influence their attitudes towards complementary and alternative medicine?
- What are the prevailing attitudes of individuals aged 18-24 towards complementary medicine?

MATERIALS AND METHODS
Research design
The present research, conducted in Karatay district of Konya province between January and August 2023, adopts a cross-sectional study design within an analytical framework.

Population and sample
The study included people aged 18-24 living in Konya. For the sample calculation of the study, the G*Power 3.1 package was used. Based on the "Attitudes and Behaviors of the Students of the Faculty of Health Sciences towards Complementary, Alternative Treatment and Modern Medicine" score in the study titled "Complementary, Alternative and Conventional Medicine Attitudes Scale" by Ağan (2019), it was determined that the minimum sample size should be 176 with an effect size of 0.5, 95% confidence interval and 5% margin of error.[16] The research was carried out on a voluntary basis and a total of 178 participants who were willing to participate in the study formed the sample group for this research.

Inclusion and exclusion criteria
Individuals aged 18-24, with an education level of primary school and above, who have at least one technological device and can use internet networks were included in the study. People who struggled to communicate or worked in a healthcare setting were excluded from the study. Data from participants who provided incomplete responses to the questionnaires were excluded from the study subsequent to data analysis.

Data collection tools
The Socio-Demographic Information Form and the Complementary, Alternative and Conventional Medicine Attitudes Scale (CACMAS) were used to collect data.

Socio-demographic information form
This form, devised by the researchers following a thorough review of the literature, comprises 20 questions aimed at eliciting socio-demographic characteristics and attitudes toward complementary alternative medicine.[13,17,18]

Complementary, alternative, and conventional medicine attitudes scale (CACMAS)
The scale was developed by McFadden et al. in

2010.[19] The validity and reliability study of the scale was conducted by Ağan.[16] The seven-point Likert-type scale (strongly disagree=1, disagree=2, slightly disagree=3, undecided=4, slightly agree=5, agree=6, strongly agree=7) consists of 25 items. The scale has three sub-dimensions; "Philosophical harmony (items 1 to 13)", " Being displeased with Modern Medicine (items 18 to 24)" and "Holistic Balance (items 14 to 17)". Items 5, 12, 18, 19, and 20 of the scale are reverse-coded during analysis as they include negative expressions. High scores indicate that individuals have a positive view towards complementary alternative medicine and a negative view towards modern medicine. In Ağan's study, the Cronbach's alpha coefficient of reliability was calculated between .80-.82 for the subdimensions of the scale.[16] In this study, the Cronbach's alpha coefficient of reliability was calculated between .75-.80 for the sub-dimensions.

Application of data collection tools
To reduce the carbon footprint, the data collection tools have been converted into a link that can be accessed online via the Google Forms page. This link, created by the researchers, includes the consent form for voluntary participation, the questions in the Socio-Demographic Information Form and the items of the Complementary, Alternative and Conventional Medicine Attitudes Scale. This survey link was disseminated to individuals aged 18-24 through social platforms. Social network platforms

created by schools were used to reach these age groups. It took approximately 5-10 minutes for the participants to complete the survey questions.

Statistical analysis
The data obtained from the study were analyzed using SPSS 25.0 (Statistical Package for Social Science) software. Descriptive statistics, t test in independent groups, Mann Whitney U test, one-way analysis of variance (ANOVA) in independent groups and regression analysis were used to analyze the data. The level of statistical significance was set at $p < 0.05$ level.

Ethical considerations
Ethical approval was obtained from the ethics committee of KTO Karatay University prior to the commencement of the study (Decision Number: 2023/019, Decision Date: 22.12.2023). Participants were informed about the purpose of the study, and their consent was obtained verbally and via the online consent system.

RESULTS
The results of the analyses of data from 178 people aged 18-24 who participated in the study were examined.

The total score of the CACMAS was determined to be 107.23±13.10, with scores ranging from 57 to 145 points. Table 1 presents the average scores of the participants based on their socio-demographic characteristics.

Table 1. Distribution of Participants' Attitudes towards Complementary, Alternative and Modern Medicine according to Socio-demographic Characteristics (n=178)

	Variables	n (%)	Mean±SD/ Mean Rank	Test Value	p
Gender	Female	130 (73)	108.37±12.46/ 93.80	U=2561.50	0.067
	Male	48 (27)	104.14±14.40/ 77.86		
Education level	High school graduate	20 (11.2)	108.47±13.52	t=-0.429	0.668
	University graduate	158 (88.8)	107.10±13.52		
Marital Status	Single	173 (97.2)	106.95±13.12	t=1.698	0.091
	Married	5 (2.8)	117.00±8.57		
Family Type	Nuclear family	149 (83.7)	106.17±13.31	F=3.489	**0.033***
	Extended Family	26 (14.6)	113.42±10.93		**0.025***
	Family Integrity Disrupted	3 (1.7)	105.66±2.08		
Family Income Status	Income Equal to Expenditure	15 (8.4)	102.06±13.81	t=-1.603	0.111
	Income Exceeds Expenditure	163 (91.6)	107.71±12.98		

*$p < 0.05$, SD: Standard deviation t= Independent sample t-test, U=Mann Whitney U test, F= One-way analysis of variance in independent groups (Advanced analysis: Tukey HSD)

The analysis revealed that 73% of the participants were female, 88.8% were university graduates, 97.2% were single, 83.7% had a nuclear family, and

91.6% had an income exceeding their expenses. Upon examination of the data, no statistically significant differences ($p > 0.05$) were found between

gender, educational status, marital status, family income status, and the mean total score of the CACMAS among the socio-demographic characteristics. However, a significant difference was observed between family type and mean total CACMAS scores (p<0.05). The results of Tukey multiple comparison analysis, conducted to investigate the source of the difference, showed that the participants from extended families exhibited higher attitudes towards complementary, alternative and modern medicine compared to those from nuclear families, with a mean score of 113.42±10.93 (p=0.025) (Table 1).

Table 2 presents the CAM applications used by the participants and the reasons behind their usage. The data revealed that cupping was the most prevalent practice among participants, with 56.2% reporting its use, followed by leech therapy at 14%, hypnosis at 12.4%, acupuncture at 9.6%, phytotherapy at 8.4%, and bloodletting at 3.9%. Furthermore, the primary reasons cited for CAM usage were muscle and joint pain, accounting for 62.9% of the participants, followed by headache/migraine at 15.7%, stress at 10.7%, weight management at 6.2%, and chronic conditions at 4.5% (Table 2).

Table 2. Preferred Applications and Reasons for the Participants to Use CAM (n=86)

		n	%
Applied CAM practices	Cupping	100	56.2
	Leech	25	14
	Hypnosis	22	12.4
	Acupuncture	17	9.6
	Phytotherapy	15	8.4
	Osteopathy	9	5.1
	Mesotherapy	7	3.9
	Bloodletting	7	3.9
	Ozone therapy	5	2.8
Reasons for using CAM applications	Muscle and joint pain	112	62.9
	Headache-Migraine	28	15.7
	Stress	19	10.7
	Weight	11	6.2
	Chronic conditions	8	4.5

*CAM: Complementary Alternative Medicine

Table 3 presents the comparison of the variables between the individual characteristics of the participants in the study on complementary medicine and the mean scores of the CACMAS. It was found that there was no significant difference between the individual characteristics concerning CAM applications, previous application status, number of applications, perceived benefits of CAM applications, and the overall mean score of the CACMAS (p>0.05).

However, the participants who thought that perceived CAM practices as beneficial exhibited the highest mean score, with a mean of 107.93±13.06. Similarly, those who recommended CAM practices to their close circles or friends had a mean score of 108.72±12.95. Notably, individuals who believed in the superior efficacy of CAM practices over treatments offered in healthcare institutions had the highest mean score of 112.14±12.69 (p<0.05) (Table 3).

A simple linear regression analysis was conducted to investigate the impact of family type, among other socio-demographic characteristics, on participants' attitudes towards complementary medicine. The analysis also explored the influence of beliefs regarding the usefulness of CAM practices, recommendations of CAM practices to close relatives or friends, and the perception of CAM practices providing greater benefits than conventional treatments, as well as the intention to apply CAM practices, on the total mean level of CACMAS. The results revealed that individuals from larger family types exhibited a significant positive effect of 6.426±2.369 points on the total mean level of the CACMAS. Similarly, those who believed that CAM practices provided more benefit than treatments administered in healthcare institutions demonstrated a significant positive effect of 6.787±1.937 points on the total mean level of CACMAS (Table 4).

Table 3. Comparison of Participants' Attitudes Towards Complementary, Alternative and Modern Medicine and Individual Characteristics Towards Complementary Medicine (n= 178)

	n (%)	Mean±SD	t/F	p
Thinking that CAM applications are useful				
Yes	152 (85.4)	107.93±13.06	**t=-4.340**	**0.000****
No	26 (14.6)	99.42±16.83		
Previous use of CAM practices				
Yes	86 (48.3)	108.29±13.44	t=1.038	0.301
No	92 (51.7)	106.25±12.78		
Number of CAM applications				
Never used	86 (48.3)	105.58±15.09	F=0.156	1.881
Once or twice	52 (29.2)	105.55±10.44		
Three times and more	40 (22.5)	110.40±14.67		
Benefit from CAM applications				
Yes	90 (50.6)	108.41±13.30	t=1.629	0.301
No	88 (49.4)	104.93±14.36		
Recommending CAM practices to close relatives or friends				
Yes	125 (70.2)	108.72±12.95	**t=3.011**	**0.003****
No	53 (29.8)	102.52±14.98		
Thinking that CAM practices provide more benefits than the treatments applied in health institutions				
Yes	70 (39.3)	112.14±12.69	**t= 4.533**	**0.000****
No	108 (60.7)	103.40±13.58		

*p<0,05, **p<0,01, CAM: Complementary Alternative Medicine, SD: Standard deviation, t= Independent sample t test, F= One-way analysis of variance in independent groups

Table 4. Regression Analysis on the Contribution of Socio-demographic and Complementary Medicine Characteristics to Attitudes towards Complementary, Alternative and Modern Medicine

	Variables	Unstandardized	Standardized		
		B±SE	Beta	t	p
	Constant	83.990±4.583	-	**18.328**	**0.000****
	Family type	6.426±2.369	0.188	2.712	0.007
CACMAS	Thinking that CAM applications are useful	3.684±2.758	0.100	1.336	0.183
	Recommendation of CAM practices to close relatives or friends	2.504±2.209	0.088	1.133	0.259
	Thinking that CAM practices provide more benefit than the treatments applied in health institutions	6.787±1.937	0.254	3.504	**0.001****

Durbin-Watson=2.011
R^2=0.200
F=8.604   **p= 0.000***

*p<0.05; **p<0.01 [1]: Regression Analysis (F); 2: Standard Error (SE); Coefficient Analysis (t); Summary statistics are given as Regression Coefficient (Standard Error) values.

DISCUSSION

This study aimed to investigate the perspectives of individuals aged 18 to 24 regarding complementary and alternative medicine. Our findings revealed that there were no significant differences in the mean total CACMAS score based on gender, educational status, marital status, or family income status. However, a significant relationship was observed between family type and the mean total CACMAS score. Specifically, participants from extended family backgrounds displayed markedly higher attitudes towards complementary, alternative and modern medicine compared to those from nuclear families. Arslan et al. (2020) reported that individuals from extended families were approximately 8 times more likely to use CAM compared to those from nuclear families, with significantly higher CAM scores observed in the former group.[20] Similarly, Yeşil et al. (2018)

reported that 13.3% of the participants started to use CAM under the influence of family members.[21] Our research findings are consistent with the literature, indicating that elders within extended families may have an impact on other family members' attitudes and behaviors towards CAM. In addition, the higher average age within large families may contribute to factors such as chronic diseases and unmanageable pain, potentially driving increased interest in CAM applications perceived as more affordable and accessible.

In our study, 50.6% of participants reported benefiting from CAM treatments, with 62.9% stating they used CAM treatments specifically to alleviate muscle and joint pain. The rates of benefit from CAM treatments can vary depending on the reason for the application and the specific method employed, as evidenced by previous studies. Dedeli et al. (2011) found that patients' skepticism regarding the efficacy of these treatments influenced the reported benefits. Güveli et al. (2021) observed a benefit rate of 26.1% among oncology patients using CAM, while Çekiç et al. (2021) reported a benefit rate of 70% among allergic patients. Additionally, Arslan et al. (2020) reported that 52.7% of the participants perceived CAM treatments as beneficial in their study.[11,12,20,22] This study identified that the most prevalent CAM practices among the participants, with cupping being the most commonly used at 56.2%, followed by leech therapy at 14%, hypnosis at 12.4%, acupuncture at 9.6% and bloodletting at 3.9%. Ak et al. (2020) reported cupping as the most commonly used CAM application at 39.7%, followed by leech therapy at 17.7% and acupuncture at 16.1%. Boccolini et al. (2022) reported that the most commonly used practices were phytotherapy, acupuncture, homeopathy, meditation and yoga.[23,24] Despite slight variations, our findings align with previous studies in the literature, which have consistently identified cupping, leech therapy, hypnosis and acupuncture as among the most commonly utilized CAM methods, with usage rates ranging from 5% to 55%.[13,23,25,26,27] It is worth noting that cultural characteristics may influence the prevalence of certain CAM practices, such as cupping, in different regions. Therefore, variations in cupping practices between studies conducted in Turkey and those in other countries are expected due to cultural differences.

The study revealed that 39.3% of the participants perceived CAM practices to be more beneficial than treatments provided by healthcare institutions, and individuals holding this belief demonstrated significantly higher mean scores compared to those who did not share this view. In alignment with our findings, Güveli et al. (2021) found that 46.8% of the participants believed in CAM practices, while Çekiç et al. (2021) reported that 27.8% of CAM users resorted to these practices due to dissatisfaction with medical treatments. Similarly, Aboufaras et al. (2023) observed high expectations of benefits from CAM practices among cancer patients, leading to their preference for CAM.[11,12,15] The findings echo those in the literature, indicating that perceptions such as dissatisfaction with medical treatments and concerns about their side effects, coupled with the perceived efficacy of CAM practices, contribute to the growing preference for CAM over conventional treatments provided by healthcare institutions.

In their study, Sharifi et al. (2022) reported that 72.7% of women who utilized CAM did so based on recommendations from family and close friends. Similarly, Başer et al. (2023) found that 50.8% of the participants adopted CAM practices upon hearing about them from their friends, while Liem et al. (2017) (59%) noted that 59% of individuals in Indonesia primarily acquired information about CAM from friends. Moreover, Arslan et al. (2020) indicated that 44.5% of the participants in their study turned to CAM practices through recommendations from relatives or friends. Another study revealed that 99.5% of the participants obtained information about CAM from their close environment such as friends, neighbors and relatives.[20,27,28,29,30] The similarity between our findings and those in the literature underscores the similarity in the ways individuals are informed about CAM methods, predominantly through interpersonal networks such as friends, neighbors, and relatives.

CONCLUSION

Complementary and alternative medicine encompasses a wide range of health practices rooted in traditional medicine or cultural traditions, which may not be fully integrated into conventional healthcare systems. The utilization of CAM is on the rise today. The study revealed that 48.3% of individuals aged 18-24 utilize CAM and have positive attitudes towards complementary medicine. Moreover, attitudes towards CAM were significantly higher among those from larger

families and individuals who believed in the superior benefits of CAM practices compared to treatments provided by healthcare institutions. It is therefore advisable to educate young people about the potential benefits and risks associated with CAM, as well as how they can effectively integrate these practices into their healthcare regimen.

Limitations

This study has several limitations. Firstly, the findings of the research are primarily applicable to the participants who were involved in the research and may only be valid for the specific time period during which the research was conducted, given its cross-sectional design. Additionally, collecting the data online via web-based platforms may introduce bias, as it limits the sample to individuals with internet access, computer or smartphone ownership, and technical literacy. Another limitation stems from the use of self-report questionnaires, which may be subject to response bias or inaccuracies. It is crucial for future studies to address these limitations and consider alternative methodologies to ensure more comprehensive and representative results. Despite these limitations, the study contributes significantly as the first investigation into young adults' attitudes towards complementary and alternative medicine.

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ORIGINAL RESEARCH

Alternative Therapies for Eye Inflammation: Patient Preferences and Patterns Complementary Medicine in Inflammatory Eye Disease

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Abstract

Objective: To evaluate the prevalence and patterns of complementary and alternative medicine (CAM) use among patients with inflammatory eye disease.

Material-Method: This cross-sectional study was conducted over three months through direct interviews with 150 consecutive patients at a tertiary care clinic specializing in inflammatory eye diseases. Sociodemographic data and clinical characteristics were extracted from clinical records for analysis. Descriptive and comparative analyses were performed using IBM SPSS Statistics software.

Results: In this cohort, 110 of the 150 patients (73.3%) reported employing complementary and alternative medicine (CAM) strategies to mitigate their ocular conditions. The CAM interventions included vitamin supplementation (n=53, 35%), herbal remedies (n=41, 27%), acupuncture (n=23, 15%), and leech therapy (n=11, 7%). Notably, a significant portion—roughly one in three patients—engaged in multiple CAM therapies.
Statistical analysis revealed a robust association between CAM applications and several demographic and clinical variables. Females were more likely to use CAM (p=0.04), as were individuals with higher occupational standing (p=0.03) and income levels (p=0.03). Furthermore, patients diagnosed with uveitis exhibited a higher propensity for CAM usage than those with other inflammatory eye diseases (p=0.03). Patients diagnosed with any inflammatory eye disease (≥5 years) exhibited a higher propensity for CAM use (0<0.01). Most patients using CAM therapies were convinced of their therapeutic benefits to eye health, and adverse effects were infrequent. Referrals by healthcare providers constituted 57% of CAM informational resources, underscoring the need for healthcare professionals to be proactive in discussing CAM use with patients.

Conclusion: The employment of CAM modalities is prevalent among patients with inflammatory eye diseases, indicating an essential role for these therapies in this patient population. The influence of CAM on disease trajectory, potential side effects, and interactions with established immunosuppressive treatments necessitates that ophthalmologists and other healthcare professionals maintain a comprehensive understanding of CAM usage to ensure optimized and safe patient care.

Keywords: Alternative Medicine, Complementary Medicine, Inflammatory Eye Disease

INTRODUCTION

Inflammatory eye diseases such as uveitis, scleritis, and episcleritis pose complex therapeutic challenges that significantly affect patients' quality of life and visual functionality. While conventional treatments primarily involve corticosteroids and immunosuppressants, their efficacy is often affected by the risk of adverse effects and the development of resistance, necessitating the exploration of complementary and alternative medicine (CAM) as viable adjunctive or primary treatments. A growing body of evidence supports the patient-driven and clinician-endorsed integration of CAM strategies to mitigate these concerns and enhance patient outcomes [1-3].

CAM's multifaceted nature of CAM encompasses an array of modalities, including, but not limited to, phototherapeutics, acupuncture, nutraceuticals, and mind-body interventions. Rooted in a holistic health perspective, these practices are increasingly recognized for managing inflammation and immune dysregulation inherent to ocular conditions. Notably, the phytochemical components in herbal remedies have shown anti-inflammatory effects that can complement or substitute traditional pharmacotherapy, particularly in patients who do not tolerate or respond to standard treatments [4,5].

Additionally, CAM's holistic ethos of CAM, which emphasizes the interconnectivity of bodily systems, potentially addresses deeper systemic factors contributing to ocular inflammation. Mind-body techniques, such as yoga and meditation, are under investigation for their immunomodulatory and stress-reducing effects, which may play roles in the onset and aggravation of inflammatory eye

disorders. This integrative perspective promises a personalized and comprehensive approach to patient care [4-6].

This study was dedicated to an in-depth examination of the role of Complementary and Alternative Medicine (CAM) in managing inflammatory eye diseases. This study aimed to systematically evaluate the prevalence and patterns of CAM usage among affected patients, delving into the diversity of CAM practices, their frequency of use, and the relationship between CAM adoption and various sociodemographic and clinical factors. This research analyzed the empirical evidence for CAM's potential therapeutic benefits, including patient-perceived efficacy and safety, while considering healthcare providers' influence on patients' CAM-related decisions. The study acknowledges anecdotal and preliminary support for CAM's effectiveness but recognizes the variation in research methodologies and the absence of standardized protocols. Therefore, a crucial goal is to advocate for more stringent clinical trials to validate the findings and confirm the role of CAM in clinical settings for inflammatory eye conditions.

MATERIALS AND METHODS

Study design

This cross-sectional observational study investigated the patterns and prevalence of complementary and alternative medicine (CAM) utilization among patients diagnosed with inflammatory eye diseases. The interviews were conducted per the Declaration of Helsinki and were approved by the Hospital Administration Board of the responsible care center.

Patient selection

Patients were consecutively recruited from an ophthalmology outpatient clinic specializing in inflammatory eye disease. Inclusion criteria mandated that patients were 18 years or older, had a confirmed diagnosis of an inflammatory eye condition, such as uveitis, scleritis, or episcleritis, and had attended the clinic within three months of data collection.

Informed consent

Before participation, all patients were informed of the study's objectives, the nature of their involvement, and the confidentiality of their responses. Written informed consent was obtained from each participant, ensuring voluntary participation and the right to withdraw from the study at any point without any consequences to their treatment.

Interview process and questionnaire design

The questionnaire and interview process were amended to outline the training of interviewers, the development process of the questionnaire, and the domains covered, including demographic, clinical information, and details of CAM use. We also specified how we accounted for potential biases and the pilot testing of the questionnaire for reliability and validity.

Training interviewers conducted face-to-face interviews using a standardized questionnaire developed for this study. The questionnaire was designed to capture a comprehensive range of information on CAM use, including the types of CAM therapies, frequency and duration of use, perceived effectiveness, and sources of CAM information.

The interview questions encompassed the following domains: Demographic information included age, sex, occupation, and income level. Clinical information: specific diagnosis, duration of the eye condition, and previous and current treatments.

- Details of CAM use: types of CAM used, duration of CAM use, reasons for choosing CAM, perceived benefits, adverse effects experienced, and disclosure of CAM use to ophthalmologists.

Patient selection and exclusion criteria

Eligible participants were individuals actively undergoing treatment or follow-up for inflammatory eye diseases at our ophthalmology clinic. The inclusion criteria were refined to ensure a representative sample:

- Age 18 and above with a confirmed diagnosis of an inflammatory eye disease, including uveitis, scleritis, or episcleritis.

- A history of attendance at the clinic for their condition within the last six months to ensure recent and relevant experience with CAM therapies.

Exclusion criteria were extended to improve the study's focus and eliminate potential confounding factors:

- Patients with non-inflammatory eye conditions, such as refractive errors, cataracts, or age-related macular degeneration, were excluded to maintain the study's relevance to inflammatory conditions.

- Individuals unable to provide informed consent, including those with cognitive impairment or language barriers that could compromise understanding of the consent process.

- Those with conditions or treatments that could interfere with CAM therapies' effects, such as immunosuppressive drugs or recent eye surgery, were also excluded.

These criteria were designed to select a cohort that could provide reliable data on the use and effects of

CAM therapies in the context of inflammatory eye diseases.

Data analysis

Descriptive statistics were used to characterize the study population regarding demographic and clinical variables. Comparative analyses were performed to examine the relationships between CAM use and patient characteristics using chi-square tests for categorical variables and t-tests or ANOVA for continuous variables, as appropriate. Logistic regression was used to identify the factors independently associated with CAM use. The level of significance was set at $P < 0.05$. Statistical analyses were conducted using the IBM SPSS Statistics software. The results are presented as means with standard deviations for continuous variables and as frequencies with percentages for categorical variables. Correlation coefficients were calculated to determine the strength of the associations between different variables. All statistical tests were two-tailed, and a p-value of less than 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of patients with confirmed diagnoses of inflammatory eye disease at the ophthalmology outpatient clinic for chronic follow-up were analyzed. One hundred fifty participants were enrolled, including individuals who voluntarily agreed to be interviewed and provided written consent.

Of the total cohort, 110 patients (%73.3) reported employing CAM strategies: 69 (62.7%) women and 41 (37.2%) males. The overall mean age of the patient's using CAM was 52 years, with a range extending from 43 to 77 years; the mean age for the women was 50 (SD ±12) and 54 years of age (SD ±15) for men.

The most frequently adopted CAM method was vitamin supplementation, with 53 patients (48.18% of CAM users) using this approach. Herbal remedies were the second most popular, used by 41 patients (37.27% of CAM users). Acupuncture was employed by 23 patients (20.91% of CAM users), leech therapy was used by 11 patients (10% of CAM users), and manual therapy/yoga was preferred by seven patients (%6,36 of CAM users) engaging in this practice.

Among patients utilizing Complementary and Alternative Medicine (CAM), 34% reported engaging in multiple CAM interventions. Specifically, 34% of these patients had adopted at least three different CAM modalities, while 66% had incorporated at least two modalities into their treatment regimen.

Females were more likely to use CAM treatments than males (p=0.04). Patients with higher occupational standing and greater income levels were significantly inclined towards CAM usage (p=0.03 for both groups). Patients diagnosed with uveitis were notably more inclined to use CAM than those with other conditions (p=0.03).

Time from diagnosis is a critical factor in CAM adoption, with those diagnosed over 10 years ago and between 5-10 years ago being significantly more likely to use CAM (p<0.01 for both groups). **Table 1** presents a detailed comparative analysis of CAM utilization.

Table 1. Comparative Analysis of CAM Utilization by Demographic and Clinical Characteristics in Patients with Inflammatory Eye Diseases

Demographic or Clinical Characteristic	CAM Users	Non-CAM Users	p-value
Gender			
Females	70 (63%)	40 (37%)	**0.04**
Males	40 (37%)	60 (63%)	**0.04**
Occupational Standing			
Higher occupational standing	75 (68%)	35 (32%)	**0.03**
Lower occupational standing	35 (32%)	65 (68%)	**0.03**
Income Level			
Higher income	80 (72%)	30 (28%)	**0.03**
Lower income	30 (28%)	70 (72%)	**0.03**
Inflammatory Eye Disease Diagnosis			
Uveitis	50 (45%)	20 (18%)	**0.03**
Scleritis	20 (18%)	40 (36%)	0.42
Keratitis	20 (18%)	40 (36%)	0.31
Orbital Cellulitis	10 (9%)	50 (45%)	0.51
Orbital Scleritis	10 (9%)	50 (45%)	0.43
Time from diagnosis (years)			
>10	60 (54.5%)	10 (9%)	**<0.01**
5--10	40 (36.4%)	30 (27%)	**<0.01**
<5	10 (9.1%)	60 (54.5%)	0.059

*Chi-square test

Most patients reported receiving information about CAM from healthcare professionals (57%). The proportion of patients who expressed satisfaction with CAM was 73%, and the preference for professional administration of these therapies was 76%. **Table 2** illustrates the distribution of the responses, providing insights into CAM's role in managing inflammatory eye diseases.

Table 2. Sources of Information, Satisfaction, Practitioner Preferences, Frequency, and Budget Allocation for CAM among Patient

Question	N=110	%
Source of CAM Information		
Healthcare professionals	63	57%
Social media	25	23%
Family and friend recommendations	22	20%
Satisfaction and Benefit from CAM		
Yes	80	73%
No	30	27%
CAM Practitioner Preference		
Physician	84	76%
Other healthcare professionals	23	21%
Others	3	3%
Frequency of CAM Usage		
As per physician's advice and regularly	47	43%
Irregular	63	57%
Budget for CAM Treatments		
More than 10% of monthly budget	44	40%
Less than 10% of monthly budget	66	60%

CAM: complementary and alternative medicine.

DISCUSSION

The array of CAM modalities embraced by the patients in this study highlights an emerging paradigm in managing inflammatory eye conditions. The pronounced reliance on CAM reflects active patient engagement in self-care and signals a broader trend towards holistic treatment regimens.

The present study revealed that a significant majority (73.3%) of the patients with inflammatory eye diseases who visited an ophthalmology clinic reported using Complementary and Alternative Medicine (CAM). This finding aligns with the trends observed in the global healthcare, where CAM is increasingly accepted, especially among patients with chronic conditions such as eye diseases. Notably, the predominance of female patients (62.7%) employing CAM in our study reflects a gender-related propensity towards CAM usage, which is supported by literature demonstrating a higher inclination among women to use alternative therapies for health issues, including eye diseases [4,7].

The average age of CAM users was 52 years, suggesting that the middle-aged demographic might be more open or in need of alternative therapeutic options, perhaps because of a higher incidence of chronic conditions or a more established health self-management approach. This is consistent with research indicating that CAM use is often prevalent in older patients with chronic diseases [8,9].

Vitamin supplementation has emerged as the most frequently adopted CAM method in patients. This is attributed to the general perception of vitamins as a safe and essential aspect of health management and increasing awareness of the potential role of nutritional deficiencies in eye diseases [10]. Herbal remedies were the second most preferred CAM strategy, which aligns with findings from Palestine and Central Africa, where herbal therapies were reported by 24.1% of patients with eye diseases [1,11,12]. This preference could be due to historical and cultural trust in plant-based remedies and their potential anti-inflammatory properties. A scholarly review conducted by West et al. noted the extensive use of nutritional supplements and herbal medications in ophthalmology. Ophthalmologists must be aware of this use, mainly when these substances manage eye diseases [13]. This awareness is crucial for providing comprehensive patient education and ensuring patients make informed decisions about using these alternative therapies in conjunction with conventional treatment plans.

Acupuncture was used by 20.91% of CAM users in our study, suggesting a moderate but significant interest in this traditional Chinese medicine practice. This is somewhat mirrored by findings that also reported the use of acupuncture in patients with various eye conditions, indicating its perceived benefit in symptom management [14]. Tong et al.

reported acupuncture as a safe adjunctive therapy to artificial tears (AT) in patients with mild to moderate dry eye, offering additional benefits up to one month after treatment [15]. This therapeutic effect is accompanied by molecular markers indicative of reduced inflammation. Given the availability of qualified practitioners to administer standardized acupuncture treatment, this study supports the recommendation of acupuncture as a complementary treatment to conventional AT therapy for the management of dry eye symptoms. This finding is significant, as it suggests that integrating certain CAM practices, such as acupuncture, can enhance the standard care for ocular conditions by providing symptomatic relief and addressing underlying inflammatory processes. Such integration into clinical practice must be supported by evidence of efficacy and safety, as demonstrated in controlled trials conducted by Tong, Yang, and Na et al. [14-16]. Leech therapy and yoga were less commonly employed, reflecting a lack of awareness, accessibility, or both. However, it is essential to note that despite the lower usage rates, such therapies still represent critical components of a holistic approach to patient care.

Many patients use multiple CAM strategies, reflecting a desire for diverse therapeutic approaches. This multi-pronged strategy might be driven by the multifactorial nature of these conditions and the desire for personalized care. The literature has reported a growing acceptance of CAM across various conditions, which supports our findings regarding the high rate of CAM usage, especially among female patients and those with a higher socioeconomic status [2,4].

The preference for CAM among uveitis patients aligns with broader trends of CAM usage in chronic diseases, where patients seek additional relief from symptoms and the potential side effects of conventional therapies [2,17,18]. The significant correlation between longer diagnosis duration and CAM usage suggests that patients might turn to CAM when traditional treatments do not fully address their needs over time.

Given these insights, healthcare providers should be equipped with knowledge of CAM and engage in open discussions with their patients about these options. An integrative care approach, which includes an understanding of CAM modalities, could potentially enhance patient outcomes by offering a broader spectrum of therapeutic options aligned with patient preferences and clinical evidence.

In the context of CAM use for inflammatory eye diseases, the high reliance on healthcare professionals for information aligns with a study by Wolsco et al. [19], emphasizing the need for medical practitioners to be well-versed in CAM to effectively guide patient decisions. The reported satisfaction (73%) and preference for physician-administered CAM therapies (76%) in our study are echoed in the literature, underscoring patient trust in professional healthcare guidance for CAM integration into treatment plans. Contrasting findings from Dorcas indicate the need for patient education on evidence-based CAM use [12].

The varying frequency of CAM usage, with 43% adhering to physician advice and 57% using CAM irregularly, reflects the diversity in patient adherence and potential for improved educational outreach, as suggested by Keenum et al. [20]. Financial commitment to CAM treatments, with 40% of patients allocating more than 10% of their monthly budget, signifies the perceived value of CAM despite cost concerns, which is a point of discussion in the economic analysis of CAM by Herman and Maxion-Bergemann [21].

These insights necessitate a balanced discussion on incorporating CAM into standard care, acknowledging its benefits while addressing the challenges posed by its integration, as analyzed in recent comprehensive reviews by Almazrou [22]. Analytically, the data point towards a paradigm shift in patient management, where individual preferences informed by professional advice shape a more personalized approach to care in ophthalmology.

The findings of this study underscore the importance of integrating patient education into clinical practice, particularly in the context of CAM therapies for inflammatory eye diseases. Ophthalmologists should be prepared to engage in open discussions with their patients about the use of CAM, providing evidence-based information on the potential benefits and risks. This dialogue can empower patients to make informed decisions about their medical treatment plans. Furthermore, educational materials or programs can be developed to further support patients' understanding of CAM therapies, enhancing their ability to manage their condition proactively and holistically. Such initiatives could lead to more personalized patient care and potentially better clinical outcomes.

Study Limitations

The current study provides valuable insights into the use of CAM therapies for inflammatory eye

diseases, yet it is not without limitations that warrant further investigation. One notable limitation is the cross-sectional design, which captures data at a single point in time and limits the ability to assess long-term outcomes and causality of CAM therapies. Future research should employ longitudinal studies to observe the sustained effects and safety profiles of CAM over time. Additionally, there is a need to standardize the dosage and administration of CAM interventions to evaluate their efficacy rigorously. Controlled trials are recommended to establish a stronger evidence base that could guide clinical practice and patient education on the integration of CAM into conventional treatment paradigms for inflammatory eye conditions.

CONCLUSION

The present study emphasizes the significant engagement of patients with inflammatory eye diseases in CAM, highlighting a shift towards holistic healthcare approaches. The reliance on various CAM modalities demonstrates a patient-driven move towards personalized care, reinforced by the substantial trust in healthcare professionals for CAM-related guidance. With a considerable proportion of patients reporting satisfaction with CAM outcomes, medical practitioners must incorporate an understanding of CAM into their practice, ensuring informed discussions and integrated patient care. This study catalyzes ongoing research and dialogue on the effective integration of CAM into conventional treatment paradigms, advocating for a patient-centered approach in ophthalmology.

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ORIGINAL RESEARCH

Treatment of Burn Wounds with a Chitosan-Based Hydrogel Dressing Containing *Artemisia absinthium* L.: A Comprehensive In Vivo Study

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Abstract

Objective: Wormwood (*Artemisia absinthium* L.) is a valuable medicinal plant that has been used for the wound healing since ancient times. Hydrogel dressings are often preferred for wound care in treatment. In this study, the effects of chitosan-based hydrogel dressings containing wormwood were investigated in rats with a burn wound model for the first time.

Material-Method: Certain phenolic compounds in wormwood extracts were detected by LC-MS/MS and antioxidant activities were calculated using the DPPH. The antibacterial activity of the dressings was tested using the disk diffusion method. Tissues taken from 48 female Sprague-Dawley rats were histopathologically examined at days 3rd and 21st. Skin tissue Il-1α, Il-6, TNF-α and Il-10 levels were measured using ELISA. All data obtained from histopathological examination and cytokine levels were statistically evaluated.

Results: Fifteen phenolic compounds were quantitatively determined in wormwood extracts. The antioxidant activities of high-, medium-, and low-dose wormwood extracts were 91.1% ± 0.054, 89.6% ± 0.012 and 84.1% ± 0.02, respectively. The hydrogel dressings showed no antibacterial activity against *S. aureus* (ATCC 29213) or *P. aeruginosa* (ATCC 27853). Granulation tissue formation, collagen increase, and regular scar appearance were higher in all three wormwood groups. Wound contraction was completed and remodeling phase started at day 21st, especially in the high-dose wormwood treatment group. Tissue cytokine levels were determined in pg/ml in all groups.

Conclusion: It was determined that *A. absinthium* L. can promote wound healing through various mechanisms of action and shows immunomodulatory effects, and is appropriate for use as a wound dressing in the form of a chitosan-based hydrogel.

Keywords: Burn, Chitosan, Hydrogel, Wormwood, Wound Dressing

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INTRODUCTION

Burn injuries are a public health issue that has existed since the beginning of humanity and continues to be a major concern across the world. They originate from varying sources, including friction, cold, heat, radiation, chemical, or electrical[1]. Heat originated thermal burns are the most prevalent form of burn injury that may necessitate treatment in a burn center. They account for approximately 86% of all burn injuries and are caused by fire or flames, hot liquids, or contact with hot objects[2].

Burns are divided into four categories based on the extent of tissue damage. First-degree burns damage only the epidermis, which is the outermost layer of the skin. Second-degree burns are partial-thickness burns that affect the epidermis and underlying dermis to different levels. Third-degree burns are full-thickness injuries that damage the entire dermis. Fourth-degree, deep full-thickness burns are the most severe types of burns that destroy skin, nerves, bones and organs[3,4].

Burn healing is pathophysiologically similar to

cutaneous wound healing in that it involves inflammation, proliferation, and remodeling phases. Although the phases are the same for all wound types, the duration of each phase varies depending on the type of wound[5]. The inflammatory phase comprises homeostasis through clot formation and the initiation of the healing process by immune system activation. The migration of epithelial cells, formation of granulation tissue, angiogenesis, and the beginning of wound contraction occur during the proliferation phase. Lastly, in the remodeling phase, scar formation and tissue strengthening are observed.

Burn care includes multifaceted procedures with several components. The key components of this procedure include wound dressing, infection management, fluid balance, and surgical intervention. Covering the burn area has been practiced since ancient times for a variety of reasons, including prevention of contamination and physical damage, keeping the wound moist and promoting re-epithelialization, avoiding water and heat loss and lowering pain[6,7]. Chitosan, known for its homeostatic characteristics[8], contributes in the wound healing by affecting multiple mechanisms. Various chitosan-based hydrogel dressing formulations have function on different stages of wound healing, encouraging healing or attenuating the impact of negative factors. It has been shown to influence macrophage activity, promote cell proliferation and formation of granulation tissue[9,10].

Artemisia absinthium L. is a bitter-tasting, aromatic perennial bush-like herb that grows 40-120 cm tall and has silver green hairy divided leaves, and small yellow flowers[11]. Because of its unique bitter flavor, it was given the name absinthium in Ancient Greek, which means undrinkable. It is also known as wormwood because it has been used to cure intestinal worms since ancient times[12]. This species has been of great ethnopharmacological interest in several cultures. Its medical usage has been dated back to the Ebers Papyrus (1552 BC) in Egypt[13]. Wormwood is registered in certain pharmacopoeias and monographs, and widely used in folk medicine for different ailments including the gastrointestinal diseases, inflamed wounds[14], diabetes, hypertension, malaria, wounds[15], diarrhea, dysentery, stomachache, bacterial infections, boils, cuts, wound healing, erysipelas[16] and acne vulgaris[17,18].

There are limited studies focusing on the in vivo effects of *A. absinthium* L. on wound healing. Existing investigations on the wound healing activity of *A. absinthium* L. have tended to focus on excisional wound models rather than on experimental burn models. In present paper, we produced a chitosan-based hydrogel dressing containing wormwood extract and studied its effects on an experimental rat burn model. We aimed to develop a practical commercial wound dressing for burn wounds based on the outcomes of this study.

MATERIALS AND METHODS
Plant materials and extraction
Wormwood saplings were purchased from a local medicinal plant garden in Yalova, Turkey. The purchased plant was identified by a taxonomist in the herbarium of Düzce University, Turkey. Fresh leaves were harvested, washed with distilled water, and dried at room temperature. The dried leaves were converted into a fine powder using a mechanical grinder and stored in an airtight bag. Powdered plant materials were extracted in ethanol %70 respectively 20, 50, and 100 g/500 ml using a magnetic stirrer for 12 h at room temperature. All the samples were centrifuged, filtered, and removed solvents using a rotary evaporator at 60°C. The dried extracts were stored at +4°C prior to use.

Quantitative analysis of phenolic compounds by LC-MS/MS
The hydroethanolic extract of wormwood was analyzed for 26 phenolic compounds using liquid chromatography-tandem mass spectrometry (LC-MS/MS; Shimadzu 8030). An Inertsil ODS-4 (2.1x50 mm, 2μm) column was used for chromatographic analysis. The column oven temperature was set at 40°C during the analysis. A binary gradient system was used with mobile phase A (ultrapure water + 0.1% formic acid) and mobile phase B (methanol + 0.1% formic acid), adjusted to 0-95% B (0-4 mins), 95% (4-7 mins), and 5% (7.01-12 mins). The flow rate was set at 0.400 ml/min and the injection volume was 10 μl. Calibration curves were drawn using at least four concentration points in triplicates for each standard. The extract containing 10 mg/100 ml of wormwood was passed through a 0.22 μm single-use filter and analyzed.

Determination of antioxidant activity
The antioxidant activity of wormwood hydroethanolic extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl; $C_{18}H_{12}N_5O_6$) free-radical scavenging method. It is based on mixing 1.8 ml of 0.1 mM DPPH solution prepared in ethanol with 0.2 ml of extract and incubating in the dark for 30 min. After incubation, the

absorbance was read using a spectrophotometer against a blank at 517 nm. Low, medium, and high doses (2%, 5%, and 10%) of wormwood extract were measured three times each. The antioxidant activity was calculated using the equation given below (Equation 1), using the average absorbance values obtained from each dose.

Antioxidant Activity % = $100 \times (A_0 - A_s) / A_0$ (Eq. 1)

(A_0: Blank absorbance, As: Sample absorbance)

Preparation of chitosan-based hydrogels

Hydrogels were synthesized using low molecular weight chitosan (molecular weight 50-190 KDa, Sigma Aldrich). Chitosan with a final concentration of 2% (w/v) was stirred in a 1% acetic acid solution for approximately 4 h at room temperature until completely dissolved. Gelatin (240 bloom) was dissolved in distilled water at 40°C to a final concentration of 1% (w/v). Equal volumes (245 ml) of chitosan and gelatin solutions were added to the dry wormwood extract. Glycerol (5 ml) was used as the plasticizing agent and 2% glutaraldehyde (5 ml) was used for cross-linking. A total of 500 ml of mixture was cross-linked for 6 hours at room temperature by stirring. After cross-linking, prepared hydrogels were transferred to a Petri dish with a thickness of 6-10 mm and matured at +4°C for 12 hours.

Testing antimicrobial activity

The antimicrobial activities of the hydrogels were tested against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) strains using the disk diffusion method. 6 mm diameter wells were opened on Mueller-Hinton agar with an agar driller. Bacterial suspensions fixed to 0.5 McFarland standard were spread on agar Petri dishes with a swab. Hydrogels containing three different doses of wormwood and the vehicle chitosan hydrogel were loaded into the wells. Hydrogels loaded into the wells were tested in liquid form, skipping the maturation step at +4°C. For comparison, commercial antibiotic discs containing vancomycin (30 μg) and meropenem (10 μg) were used for *S. aureus* and *P. aeruginosa*, respectively. The prepared agar disks were incubated at 37°C for 18-24 hours.

Burn model in rats

The animals included in the study were obtained from the Experimental Animals Application and Research Center of Düzce University (Düzce, Turkey), with permission from the local ethics committee. A randomized experimental design was carried out in six groups of eight animals each, with a total of 48 female 20-24 weeks old Sprague Dawley rats. The animals were weighed prior to anesthesia (ketamine 90 mg/kg, xylazine 10 mg/kg intraperitoneal injection), and the back of each rat was shaved once sedated. Each animal was injected with 2 ml of intraperitoneal saline to prevent dehydration. After antisepsis with povidone-iodine, a burn wound model was made with an iron seal measuring 2 cm × 2 cm on the backs of the animals. To create a third-degree full-thickness burn, the iron seal was exposed to open fire for 40 s and applied to the back of the animal for 20 s under mild pressure. The temperature of the iron seal was fixed at 150°C in each application and controlled by an infrared thermometer. The experimental groups were as follows:

1. Positive Control Group: Silverdin cream containing 1% silver sulfadiazine was applied topically to the animals once a day after the burn model.

2. Negative Control Group: The animals were modeled for burns, but no treatment was applied.

3. Vehicle Group: After burn model was created, the chitosan-based hydrogel without wormwood extract was applied topically once a day.

4. Low-Dose Wormwood Group: 2% wormwood extract in a chitosan-based hydrogel was applied topically once a day.

5. Medium-Dose Wormwood Group: 5% wormwood extract in a chitosan-based hydrogel was applied topically once a day.

6. High-Dose Wormwood Group: 10% wormwood extract in a chitosan-based hydrogel was applied topically once a day.

Histopathological examination

On the third day of the trial, 24 animals were sacrificed to assess acute outcomes, and on the 21st day, the remaining 24 animals were sacrificed to study chronic outcomes. Full-thickness tissue samples were removed from the treated burn areas. The collected tissue samples were divided into two equal parts, and the parts to be used for the analysis of cytokines were stored at -80°C. Tissue samples for histopathological examination were fixed in a 10% formaldehyde solution for at least 48 h. Tissue samples were fixed in paraffin blocks, sliced into 5 μm sections and stained with hematoxylin-eosin. Histopathological slides were examined blindly by a pathologist under light microscope at Düzce University Faculty of Medicine. Granulation tissue, giant cells, collagen increase, loss of skin

appendages, epidermal loss, and deterioration in collagen fibers were evaluated as 0 (Absent) or 1 (Present). Acute inflammation, chronic inflammation, fibrosis, epidermal regeneration, and eosinophil parameters were scored as 0 (None), 1 (Low), 2 (Medium), 3 (High).

Analysis of immunomodulator cytokines
The proinflammatory cytokines interleukin 1 alpha (IL-1α), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and the anti-inflammatory cytokine interleukin 10 (IL-10) were determined by ELISA (ELK Biotechnology, China). Tissue samples stored in a -80°C were brought to room temperature and lysed in 1 ml of 1X PBS solution with a homogenizer for 2 min. The supernatants obtained from centrifuged homogenates were separated for analysis. The ELISA was performed in line with the user manual instructions.

Ethical statement
This research was approved by the Düzce University Local Ethics Committe Presidency On Animal Testings with decisions number 2022/06/08.

Statistical analysis
The tissue cytokine levels analysis was performed using JASP 0.17 software. The Shapiro-Wilk test was used to determine the normal distribution of tissue IL-1α, IL-6, IL-10, and TNF-α levels. The homogeneity of variances was evaluated using Levene's test. The variation of dependent variables according to day and group factors was analyzed using Two-Way ANOVA. Tukey's test was applied for post-hoc analyses that included multiple comparisons, with the findings provided as mean and standard deviation. Results with $p<.05$ were considered statistically significant. Histopathological data were analyzed using the IBM SPSS Statistics version 26.0. Histopathological scores between the experimental groups were analyzed using Fisher's Exact Test with Monte Carlo Simulation. The simulation was repeated 10.000 times with a confidence interval of 99%, and the significance level was set at $p<0.05$.

RESULTS
Quantified phenolic compounds
A total of 26 specific phenolic compounds were quantitatively analyzed in the extract using LC/MS-MS. Of the 26 compounds analyzed, 15 were identified; the remaining 11 compounds could not be detected in the extract. Undetectable phenolic compounds are catechin hydrate, vanillic acid, hydroxybenzoic acid, protocatechic acid, salicylic acid, phlorizin hydrate, 2-hydroxy-1,4-naphthoquinone, naringenin, silymarin, kaempferol, alizarin. The retention times and amounts of detected compounds are listed in Table 1.

Table 1. Name, retention time, and amount of detected compounds

Compounds	RT (min)	Amount (µg/l)
Acetohydroxamic acid	0.406	315.55
Syringic acid	3.001	500.69
Thymoquinone	3.337	762.06
Resveratrol	3.366	717.06
Fumaric acid	0.809	3593.71
Gallic acid	1.278	421.76
Caffeic asit	2.836	120.71
4-hydroxycinnamic acid	3.489	37.31
Oleuropein	3.567	79.82
Myricetin	3.644	35.04
Ellagic acid	3.681	130.1
Quercetin	3.891	268.79
Butein	3.935	196.97
Luteolin	4.069	57.07
Curcumin	4.672	15.09

*RT: Retention time

Antioxidant activity
The highest antioxidant activity for the 0.1 mM DPPH solution was found in the high-dose extract containing 100 mg/ml wormwood as %91.1 ± 0.054. The medium-dose extract containing 50 mg/ml wormwood had an antioxidant activity of %89.6 ± 0.012, while the low-dose extract containing 20 mg/ml wormwood had an antioxidant activity of %84.1 ± 0.02.

Antibacterial activity
Hydrogels containing three dosages of wormwood extract and the vehicle chitosan hydrogel exhibited no antibacterial action against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). The antibacterial activity of the hydrogels was tested using the disk diffusion method, as illustrated in figure 1.
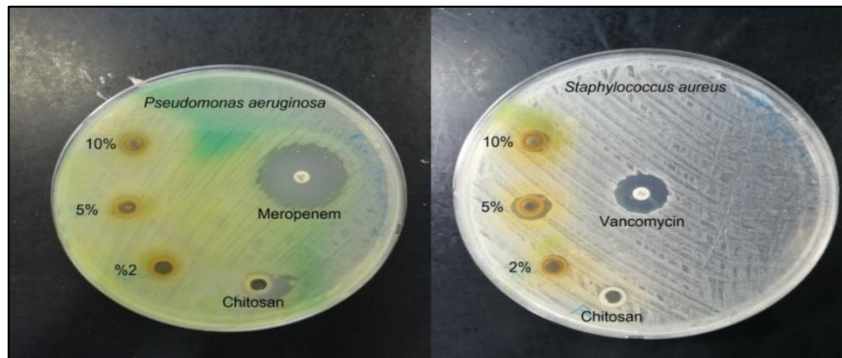
Figure 1. The antibacterial activity of the hydrogels against *S. aureus* and *P. aeurginos*

Histopathological examination

Histopathological changes between the 3rd and 21st days were used to evaluate wound healing.

Statistical evaluation of histopathological data using Fisher's Exact Test with Monte Carlo simulation is shown in Table 2.

Table 2. Statistical distribution of histopathological scores in the experimental groups

Parameter	Day	p-value
Acute Inflammation	3rd	0.257
	21st	0.184
Chronic Inflammation	3rd	0.064
	21st	0.276
Fibrosis	3rd	0.131
	21st	**0.018***
Epidermal Regeneration	3rd	0.429
	21st	0.269
Granulation	21st	0.417
Giant Cell	21st	0.362
Loss of Skin Appendages	21st	1.000
Collagen Increase	3rd	0.626
	21st	0.123
Epidermal Loss	3rd	1.000
	21st	0.265
Deterioration in Collagen Fibers	3rd	0.135
	21st	0.097

*No significant findings were obtained from the parameters of acute and chronic inflammation, epidermal regeneration, granulation, giant cells, loss of skin appendages, collagen increase, epidermal loss, and deterioration of collagen fibers on the 3rd and 21st days. The findings of the fibrosis evaluation on the 3rd day were not significant, however the results obtained on the 21st day were. Granulation and giant cells were not seen in the histopathologically analyzed tissues on the 3rd day, and all samples had a loss of skin appendages. In any of the 48 tissues analyzed, no eosinophils were observed.

Results of cytokine levels

The proinflammatory cytokines IL-1α, IL-6, TNF-α, and the anti-inflammatory cytokine IL-10 were determined at pg/ml levels in tissue samples using

ELISA. The average amounts of tissue cytokine levels in the groups depending on the day are shown in Table 3.

Table 3. The average amounts of tissue cytokine levels

Group	Day	IL-1α	IL-6	TNF-α	IL-10
Vehicle	3rd	227.8±104.9	375.2±234.9	545.8±229.9	543.0±235.4
	21st	588.6±192.4	844.6±465.2	684.9±42.0	589.7±241.1
Positive Control	3rd	520.0±117	232.1±150.7	503.2±130.9	358.4±102.9
	21st	497.7±155.3	632.7±411.4	681.6±18.1	463.0±195.1
Negative Control	3rd	606.8±93.5	312.8±34.5	507.6±156.5	388.3±131.7
	21st	581.3±38.2	487.2±240.4	610.8±42.2	454.7±118.6
Low-Dose	3rd	295.3±143.4	305.2±152.3	532.4±50.7	273.0±33.6
	21st	531.1±168.0	385.8±263.8	384.1±112.8	462.2±344.9
Medium-Dose	3rd	451.9±308.5	430.8±171.8	547.4±142.4	368.4±53
	21st	447.8±76.3	577.7±256.2	629.9±135.7	385.5±131.7
High-Dose	3rd	341.1±76.1	255.8±46.9	412.4±270.2	476.8±106.8
	21st	376.9±79.0	277.7±81.2	746.6±158.2	721.4±401.1

*Two-way ANOVA was used to analyze changes in cytokine levels based on group and day as the main effects, as well as interactions between them. Statistical significance was set at $p<.05$. The p-values obtained from the Two-Way ANOVA for tissue cytokine levels are shown in Table 4.
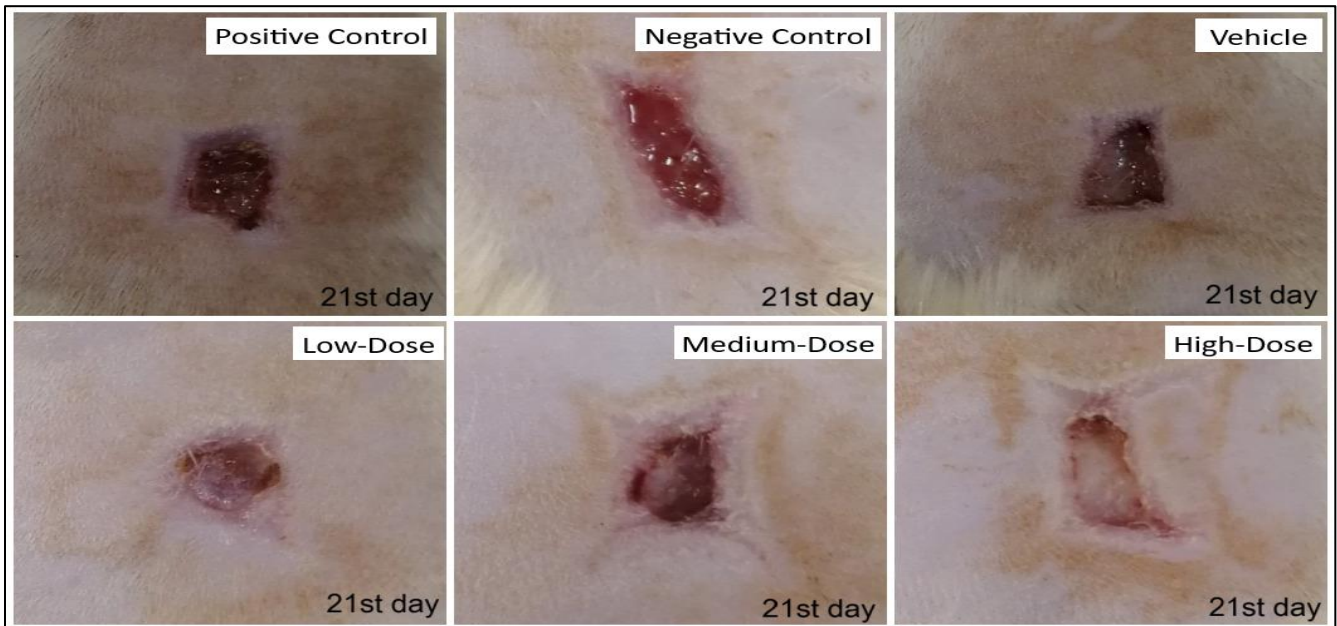
Figure 2. A comparative pictures of scar tissues between experimental groups

*Granulation tissue formation persists in the positive control, negative control, and vehicle groups. Wound beds continue to exude, are soft and edematous, and scar tissue has not fully developed. Granulation, on the other hand, was completed in all wormwood treatment groups. Scar tissue was developed in varying degrees, particularly in the high-dose wormwood group. Scar tissue has lessened in appearance and turned pale pink, indicating that the remodeling phase has begun.
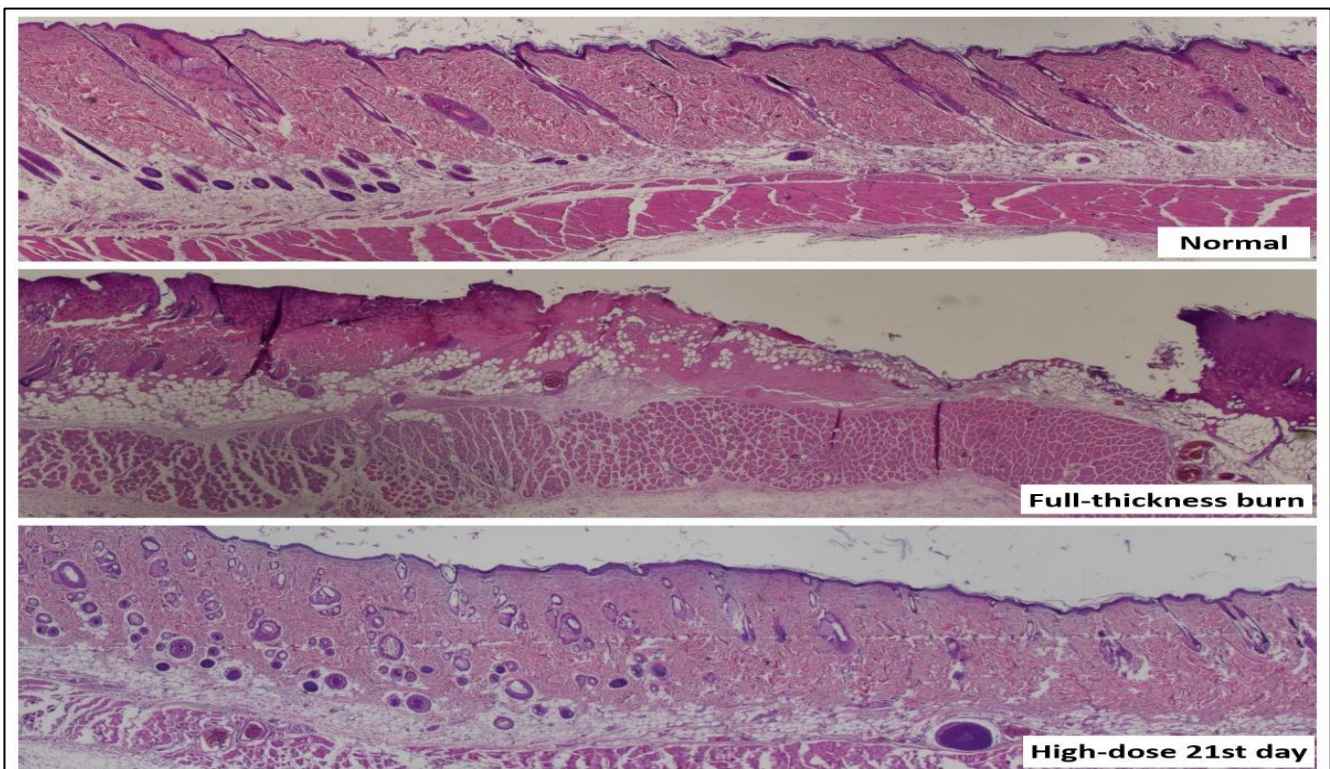
Figure 3. Normal, full-thickness burn and high-dose 21st day rat skin histopathological comparison

*The epidermis and its outermost section the stratum corneum, dermis, hypodermis, adipose tissue, and muscle tissue are all present in normal rat skin. The appendages of the skin were whole, the pale pink collagen fibers were regular and filamentous, and the vascular systems were intact. The epidermis and skin appendages are destroyed in full-thickness burn. Vascular structures and muscle tissue were discovered to be severely ruptured. The integrity of the dermis, particularly collagen fibers, has deteriorated significantly. There was no acute or chronic inflammation observed in the high-dose wormwood group. Granulation and epidermal regeneration were increased and no epidermal loss was seen. There is also an increase in collagen fibers and blood vessels.

Table 4. The statistical results of tissue cytokine levels from the Two-Way ANOVA

	IL-1α	IL-6	TNF-α	IL-10
Group	0.033*	0.113	0.357	0.120
Day	0.025*	0.004*	0.024*	0.068
Group × Day	0.044*	0.392	0.068	0.866

*The main effects and the interaction of tissue IL-1α levels were statistically significant. While the day's main effects on tissue IL-6 and TNF-α levels were statistically significant, the interactions were not. As the main effect or the interaction, changing in tissue IL-10 levels were not statistically significant.
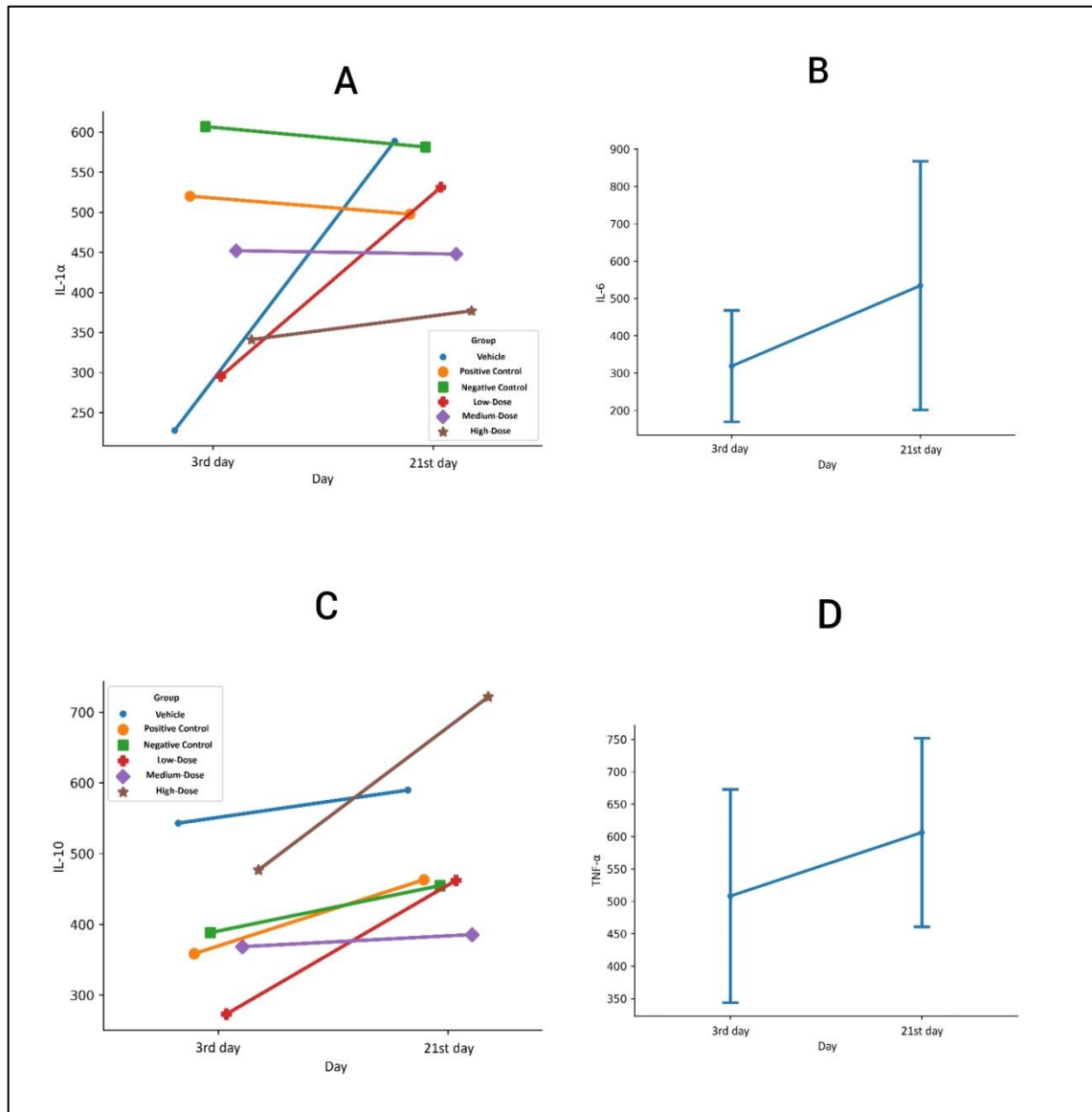

Figure 4. Time-dependent changes in tissue cytokine levels in the groups
*A. When tissue IL-1α levels in the groups were evaluated, the changes on the 3rd and 21st days in the vehicle (p=0.001) and low-dose treatment groups (p=0.026) were statistically significant. Although not statistically significant, IL-1α levels decreased slightly over time in the positive control, negative control, and medium-dose treatment groups while increasing in the high-dose treatment group. **B.** The day-independent variable had a statistically significant effect on IL-6 levels. Multiple comparisons revealed that tissue IL-6 levels on the 3rd day were 318.702 ± 149.2 pg/ml, according to the post-hoc Tukey's test findings. Tissue IL-6 levels were determined to be 534.3 ± 332.9 pg/ml on the 21st day. **C.** Although not statistically significant, tissue IL-10 levels increased in all groups with various scales. **D.** According to the post-hoc Tukey's test findings of the day variable, tissue TNF-α levels were found to be 606.3 ± 145.3 pg/ml on the 3rd day and 508.1 ± 164.4 pg/ml on the 21st day.

DISCUSSION

Wound healing consists of several repair processes, including inflammation, epithelial regeneration, granulation, neovascularization, and wound contraction. Several new treatment techniques targeting various stages of wound repair have been tested in vitro and in vivo. The usage of plants containing bioactive phytochemicals from diverse chemical families is one of these techniques. Phytochemicals are known to affect repair processes through various mechanisms. These mechanisms include reducing oxidative damage due to their antioxidant and antibacterial effects, regulation of the immune system, and acceleration of tissue repair. Considering all of these, we conducted the first study on the effects of chitosan-based hydrogel containing wormwood on burn wound treatment.

Artemisia absinthium L. has been found in the current study to have strong antioxidant properties and a high phenolic component concentration. It was determined that chitosan-based hydrogels containing the extract of the plant can be synthesized and applied as a functional wound dressing on burn treatment. On the 21st day of treatment, considerable changes in wound appearance were observed in individuals with the similar histopathological evaluations. In the positive control, negative control, and vehicle groups, the wound sites remained edematous and exuded with no scab development. On the other hand granulation and scar tissues developed in the wormwood treatment groups. The color of the scar tissue paled and began to disappear, particularly in the high-dose wormwood group.

Moacã et al. (2019) reported that they detected chlorogenic acid, caffeic acid, p-coumaric acid, isoquercitrin, rutin, quercitrin, luteolin and apigenin in their study in which they analyzed ethanolic wormwood extract by LC-MS[19]. Lee et al. (2013) analyzed the methanolic extract by HPLC and found myricetin, quercetin and kemferol among flavanols; caffeic acid, p-, o-, m-coumaric acid and ferulic acid among hydroxycinnamic acid derivatives; gallic acid, protocatechic acid, β-resorcylic acid, vanillic acid and salicylic acid from hydroxybenzoic acid derivatives; rutin, vanillin, hesperetin, resveratrol, naringenin from other phenolic compounds[20]. Koyuncu (2018) reported that he detected coumarin, p-coumaric acid, caffeic acid, vanillic acid, salicylic acid, quinic acid, ferulic acid, chlorogenic acid, rosmarinic acid, protocatechuic acid, cinnamic acid, sinapinic acid, vanillin, malic acid, syringic acid, hesperetin, naringenin, rutin, quercitrin, apigenin, isoquercitrin, rhoifolin in wormwood aqueous methanol extract by LC-MS/MS[21].

In our study, wormwood extract was prepared by maceration in 70% ethanol and its effect on wound healing was investigated quantitatively and phytochemically using LC/MS-MS. Fifteen of the 26 phenolic compounds investigated in the extract were detected at μg/L level. Detected compounds caffeic acid (120.71 μg/l), 4-hydroxycinnamic acid (37.31 μg/l), syringic acid (500.69 μg/l), acetohydroxamic acid (315.55 μg/l), thymoquinone (762.06 μg/l), resveratrol (717.06 μg/l), curcumin (15.09 μg/l), myricetin (35.04 μg/l), gallic acid (421.76 μg/l), quercetin (268.79 μg/l), oleuropein (79.82 μg/l), butein (196.97 μg/l), fumaric acid (3593.71 μg/l), ellagic acid (130.1 μg/L) and luteolin (57.07 μg/l). When compared with the chromatographic analysis results given above, it was observed that caffeic acid, cinnamic acid, syringic acid, myricetin, gallic acid, quercetin, and luteolin compounds were similar. The phytochemicals detected may vary qualitatively and quantitatively owing to the different solvents, extraction techniques, and devices used in the studies.

Many studies have shown that *A. absinthium* L. has high antioxidant activity because of the polyphenols it contains, and as a result, it is an effective free radical scavenger. It has been suggested that this high antioxidant activity and free radical scavenging ability accelerates wound healing processes[22]. Minda et al. (2022) reported that aqueous ethanolic wormwood extract at a dose of 1 mg/ml showed approximately 87% antioxidant activity for 0.1 mM DPPH[23]. In another study where the extract obtained by a similar method was investigated; Moacã et al. (2019) calculated the amount of wormwood required for the IC_{50} value, which means scavenging half of the free radicals contained in 1 mM DPPH solution, as approximately 0.5 mg/ml[19].

In the study we conducted, high-dose extract containing 100 mg/ml wormwood versus 0.1 mM DPPH solution was 91.1 ± 0.054%; medium-dose extract containing 50 mg/ml wormwood 89.6% ± 0.012; low-dose extract containing 20 mg/ml wormwood showed 84.1 ± 0.02% antioxidant activity. Because of the different amounts of wormwood used in these studies, it is thought that there are different antioxidant activity values for the same amount of DPPH. Additionally, owing to its limited antioxidant capacity, it was concluded that

there is no linear relationship between the amount of wormwood and its antioxidant activity.

Several studies have shown that wormwood essential oils have strong antimicrobial and antifungal effects. However, different results have been reported for extracts prepared using various solvents and extraction methods. Erel et al. (2010) investigated the antimicrobial effects of wormwood methanolic Soxhlet extracts using the disk diffusion method. They reported that the extract did not show activity against *Staphylococcus aureus* (ATCC 6538/P), but showed antimicrobial activity (9 mm inhibition) against *Pseudomonas aeruginosa* (ATCC 27853)[24]. Erdogrul (2002) investigated the antimicrobial effects of wormwood extracts obtained by Soxhlet extraction using ethyl acetate, methanol, acetone, and chloroform on the *Staphylococcus aureus* (ATCC 25923) strain by the disk diffusion method. According to the study results, while no effect was observed in methanol, acetone, and chloroform extracts, antimicrobial activity (14 mm inhibition) was observed in the ethylacetate extract[25]. Hrytsyk et al. (2020) investigated the antimicrobial effects of wormwood, which they extracted with 70% ethanol with the help of fractional maceration, using the disk diffusion method. The study concluded that it showed antimicrobial activity on *Staphylococcus aureus* MS (9.37 ± 0.85 mm inhibition) and *Pseudomonas aeruginosa* (5.33 ± 0.54 mm inhibition)[26].

In our research, hydrogels containing low, medium, and high doses of wormwood extract prepared by simple maceration with 70% ethanol did not show antibacterial effects against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) strains. This may be due to the differences in the strains, tested dose amounts as well as the extraction method. Another plausible explanation is that chitosan-based hydrogels cannot disperse adequately due to their viscous nature.

Proinflammatory cytokines mediate critical cellular responses through biochemical increases and decreases. Sultan et al. (2020) investigated the effects of wormwood methanolic Soxhlet extract in a wound healing study on Wistar rats. They concluded that changes in serum IL-6 and TNF-α levels contributed to the wound healing process as immunomodulators[27].

In our study, tissue IL-1α, IL-6, IL-10, and TNF-α levels were determined at the pg/ml level by ELISA. There is no previous research on the determination of cytokine levels in skin tissue. Tissue IL-1α levels showed a statistically significant increase from day 3 to day 21 in the vehicle and low-dose groups. Although not statistically significant, there was a certain level of increase in the high-dose treatment group. There was a slight decrease in the medium-dose treatment, positive control, and negative control groups, although the difference was not statistically significant. In contrast, although not statistically significant between the groups, tissue IL-6 and IL-10 levels tended to increase over time in all experimental groups. Tissue TNF-α levels decreased over time in the low-dose treatment group but increased significantly in the remaining groups. We also concluded that the highest increase in tissue TNF-α level was observed in the high-dose treatment group. When these results are evaluated, it is thought that wormwood aqueous ethanol extract shows immunomodulatory effects on tissue cytokine levels and contributes positively to the wound healing process.

There are in vitro and in vivo wound healing studies on wormwood used for wound healing purposes in traditional medicine. Minda et al. (2022) investigated the effects of aqueous ethanolic wormwood macerate on the human keratinocyte cell line (HaCaT). According to the results of this study, wormwood stimulates more than 97% of keratinocyte migration and substantially contributes to wound closure[23]. In a study examining the effects of essential oil in the excisional wound model created on Wistar rats; they reported that the group treated with 10% wormwood essential oil improved better than the negative and positive control groups[28]. Another excisional wound study on Wistar rats concluded that 10% Soxhlet extract of wormwood strongly affects granulation and epithelialization formation and accelerates tissue repair[29].

The effects of wormwood on the in vivo burn wound model were investigated for the first time in this study. It was established that wormwood at different doses stimulates wound healing and is suitable for use as a wound dressing in the form of a chitosan-based hydrogel for burn. Wormwood treatment groups, especially high-dose, formed a more consistent scar tissue than the other treatment groups. On the 21ˢᵗ day, the wound had completely contracted, and the scar color began to disappear.

Although macroscopic and histopathological appearance of the treatment groups were superior, the negative control group also healed over time. Larger-scale studies or human trials might provide additional information regarding the efficacy and adverse effects of topical treatments containing

wormwood extract.

CONCLUSION

Burn wounds continue to be a significant public health issue, affecting millions annually with varying degrees of severity. Bioactive molecules contained in medicinal plants contribute to cutaneous wound healing by affecting various mechanisms such as inflammation, re-epithelialization, granulation tissue formation, angiogenesis, and fibrosis. They can help treat persistent burn wounds with secondary infections or when other topical therapies are ineffective or have negative side effects.

Artemisia absinthium L. is quite abundant in phenolic compounds and has a high antioxidant potential. The obtained phytochemicals affect repair processes through various mechanisms. Antioxidant potentials, reduction of oxidative damage, antimicrobial effects, regulation of the immune system, and acceleration of tissue repair can be considered as these mechanisms. The current study can lead detailed research of the biochemical and metabolic pathways accompanying wound healing processes in future studies. The effects of novel bioactive compounds belonging wormwood such as absinthin, anabsinthin, artabsin, and artemisinin on wound healing should be investigated in future studies. According to the results of further research, it will be obtain an important cost-effective wound care product.

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ORIGINAL RESEARCH

Associations and Predictors of Psychological Distress among Students Following Disruption of Academic Activities by University Workers Strikes

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Abstract
Objective: Psychological distress is heightened among students during health distress and strikes. This study sought to determine the associating and predicting factors of anxiety and depression among university students following the disruption of academic activities by strikes.
Material-Method: This cross-sectional face-to-face/online study conveniently recruited a total of 317 students aged 15-51 years. The student's socio-demographic, socio-economic, physical characteristics, and psychological distress were assessed using a structured questionnaire, Patient Health Questionnaire-2, and General Anxiety Disorder–7. Data analysis includes descriptive statistics, Pearson's Chi-square test, and Logistic regression, at a significant level of $p < 0.05$.
Results: Student category $\chi^2 = 7.928$, $p = 0.005$ Vs $\chi^2 = 27.282$, $p = 0.000$, steady family income $\chi^2 = 11.846$, $p = 0.001$ Vs $\chi^2 = 14.049$, $p = 0.000$, and lost interest $\chi^2 = 7.676$, $p = 0.006$ Vs $\chi^2 = 16.500$, $p = 0.000$ were significantly associated with depression and anxiety. Steady family income was a predictor for both anxiety (OR = 0.518, $p = 0.031$) and depression (OR =0.501, $p = 0.013$). Student category (OR =3.545, $p = 0.017$), leaving home (OR = 2.182, $p = 0.011$), lost interest in my studies (OR = 2.407, $p = 0.007$), and frequency of exercise (OR = 0.419, $p = 0.036$) also predict anxiety.
Conclusion: Strike causes adverse effects on the psychological states of students. Lack of steady family income, low frequency of exercise, loss of interest, contemplating dropping out of school, and leaving home were outstanding predicting and associating factors of depression and anxiety.
Keywords: Psychological Distress, Anxiety, Post-COVID, Strike, Depression, Physical Activity

INTRODUCTION

The fulfillment of every student who gains entry into the university is to acquire the necessary knowledge and skill and then at the stipulated time be conferred a degree having fulfilled all requirements in character and learning.[1] These graduates are soon expected to take over the nation's workforce and continue the cycle of national development.[2] When this process is hampered by a series of conflicts between the federal government, which is the primary investor in public tertiary institutions and the members of the Academic Staff Union of Universities (ASUU) who are the chief imparters of knowledge and professional skills then the continuum of development is bound to become chaotic.

Students of public tertiary institutions who unfortunately happen to be at the receiving end of the ASUU federal government conflict tend to suffer a myriad of setbacks as a result of a series of unprecedented ASUU strikes.[2-3] ASUU is a trade union formed in 1978 preceding the Nigerian Association of Teachers (NAT). The union is saddled with the responsibility of organizing all academic staff who are qualified for membership; regulating the relationship between academic staff members and employers; establishing and

maintaining a high standard of academic performance and professional practice; establishing and maintaining a just and proper condition of service for its members; and protecting and advancing the socio-economic and cultural interests of the nation (ASUU constitution, 1978 as amended in 1984).[3]

During the ASUU strike, students are made to stay at home away from learned course content for an alarming duration and then in a bid to rush the academic session, they are expected to sit for examination upon resumption from the ASUU strike. Lengthy stay at home also gives room for indulgence in diverse social vices including alcoholism, gangster activities, substance abuse, rape, armed robbery, and internet fraud amongst others.[2-4] Aside from the idle students being a nuisance to society, other socioeconomic implications of months of stay at home include extra expenses on parents who are obligated to provide for students' needs while at home and further extends parental sponsorship until eventual graduation from the university.[5] Parents also have to settle landlords to retain occupancy of students' school accommodations even through the months of the ASUU strike. The pathetic state and uncertainties of education in public universities compel parents to resolve to send their wards to private and foreign institutions despite the outrageous financial implications.[2-3]

According to Ajayi the ASUU strike affected 71.2% of students at Ekiti State University Nigeria and had a negative influence on the academic performance of 51.6% of the university students.[2] More than 50% of the students of the same institution believed that a favorable response from the federal government would truncate this predicament. Alabi also reported that the ASUU strike had a highly dysfunctional impact on university development by disrupting the academic calendar and extending students' study duration, hence tarnishing the reputation of the university system.[3] Students from public institutions in Nigeria are believed to be under-skilled and no match for their foreign counterparts.[5] These and many other factors are a source of physical and psychological distress among students, causing prospective students and parents to lose confidence in the public education system.

Psychological distress including anxiety, depression, and stress[6] are top among the common mental disorders (CMD) which are in turn regarded as the major cause of disabilities worldwide.[7] Individuals with psychological distress stand at a higher risk of mortality such as death by suicide, morbidity, and substance abuse.[8] Additionally, it has been observed that individuals who engage in regular physical activity (PA) presented with decreased levels of psychological distress.[9] Regular participation in PA is also associated with other health-enhancing benefits, like mood elevation, stabilization of emotion, risk reduction, and prevention of diseases like diabetes, heart attack, and hypertension.[10-11] Many individuals experiencing health distress such as seen during the recent past lockdown of COVID-19 could not meet up with the recommendation that would qualify them as engaging in regular PA resulting in reports of poor health.[12] We therefore hypothesize that the student's low level of participation in PA during the strike may harm their psychological state and probing to explore its impact may offer a possible way to avert it.

The prevalence of psychological distress among students has been recorded in the literature. For example, in Nigeria, it was found that 25% out of 10, 421 students had psychological distress[13]; in Brazil, it was recorded as 33.7%[14], ranging from 14.5-15.04% in India[15-16], and in Ethiopia, ranges from 21.6%-40.5%[17-18]. Psychological distress was heightened during pandemics and epidemics. For example, in the recent past COVID-19 pandemic, there was an increased rate of anxiety, fear, poor sleep, depression[19-20], and suicide.[21-22] Accordingly, during the recent COVID-19 lockdown, the odds of having anxiety were 1.61 times higher among students with a low family income while inactive students were 0.59 times less likely to be anxious. However, the odds of having depression were 0.65 times less among males than female students of Malaysia University.[23] The prevalence of anxiety and depression among university students during COVID-19 was as high as 81.7% and 82.4%, respectively.[24]

Studies have been carried out to communicate the detrimental effect of the ASUU strike on academic staff and university development[3,25] with a paucity of data showing the distress students encounter with each industrial action. This study aims to determine the physical, socio-economic psychological impact of the ASUU strike on students and determine the associating and predicting factors of anxiety and depression among university students following the disruption of academic activities embarked on by university workers in public universities in Nigeria. Additionally, this study may provide possible

suggestions for protecting the mental health of university students.

MATERIALS AND METHODS

This study used a cross-sectional survey design and a non-probability sampling technique to sample students aged 15-51 years from public universities in Nigeria. The recruited subjects were both undergraduate and postgraduate students admitted and studying in both state and federal universities. Additionally, only subjects who are conversant with the use of social media platforms like Facebook, Linked-in, WhatsApp, Messenger, or Telegram, can comprehend spoken and written English and were residing in the country during the period survey were included in the study. Students using medication that can impair their memory or who were seriously sick or studying in universities not affected by the strike were advised not to participate.

Outcome assessment tools

The outcome assessment tools for data collection as detailed in the protocol of the study[26-27] include; 1) a structured questionnaire used to collect information including age, gender, location of residence, marital status, student category, type of university, family income, frequency of leaving home per week, afraid of the future of my education because of this strike, lost interest in my study because of this strike, frequency, duration, type, and place of exercise, hours spent watching television, living condition, do you see anything positive about this strike, do you know any student that died during the strike, and suggestion on way to curb such strike in the future. This questionnaire was modified from a previously used questionnaire called the Physical and Socio-economic Psychological Distress Questionnaire (PASDQ) [28] for workers participating in industrial action. The PASDQ was reported to have a Cronbach α ranging from 0.67 to 0.93 and an ICC ranging from 0.26 to 0.88. 2) Patient Health Questionnaire (PHQ-2) was used to determine the level of depression. It is a self-administered 2-item questionnaire[29] with items scored from 0-3, "0" = not at all, and "3" = nearly every day. The resultant score falls within 0 – 6 and 3 is regarded as the optimal cut-point for screening for depression, hence scores ≥ 3 indicate a like hood of major depression which would warrant further assessment with PHQ-9.[29] The sensitivity = 12.3-90.6; specificity = 65.4-99.8, and positive predictive value = 36.9-92.9 were acceptable. 3) Generalized

anxiety disorder (GAD-7), was used to evaluate the level of anxiety, it investigates the frequency at which a person had suffered from anxiety in the last 2-weeks.[30] It comprises 7 items measured on a 4-point Likert scale (0-3), 3 = almost every day and 0 = not at all with a resultant score ranging from 0 – 21.[30] It is reported to have excellent internal consistency with Cronbach's α = 0.911, hence a validated instrument for anxiety screening.

Procedure

The Health Research Ethic Committee [UNIMED HREC] University of Medical Sciences, Ondo State, Nigeria granted ethical approval for the commencement of this study. We also explained the purpose of the study to each subject and obtained their concern to participate. The social media platforms such as Facebook, Linked-in Messenger, Telegram, and WhatsApp were used to disseminate the survey. To participate in the study prospective subjects were sent a link to the survey and were required to click the link and read the preliminary instructions which were mend to enable them to decide to participate. In the opening instruction, we invited the subjects to participate and informed them that participation was voluntary and that no name was required. In addition, subjects were notified that only a single response is required per individual and collected information would be treated in cumulative only, and no identity would be traced to individual response. They were advised to complete the survey if they chose to participate by clicking "yes" after reading the preliminary instructions. However, they were also informed that they could choose not to participate by clicking "no" without any penalty. However, in the hard copy survey, the subjects gave written consent by signature or thumbprint. No pledge, reward, or gift was offered to the subjects to stimulate participation. However, the subjects were promised that they would be granted access to the findings of the study if they indicated interest.

Statistical analysis

The online survey responses were extracted using Microsoft Office Excel 2007. The extracted data underwent cleansing and coding to enable them to be readable by statistical software such as statistical package for social scientists (SPSS) version 23 which was employed for data analysis. The subject demographics, physical, and socio-economic characteristics were summarized using the descriptive statistics of frequency, percent, mean, and standard deviation. The normality of the

measured and manipulated data/variable was determined by the Kolmogorov–Smirnov test and non-normal data were log-transformed to base 10. Pearson's Chi-square test was used to determine the factors associated with anxiety and depression. Gender variance in frequency, duration, and intensity of exercise, depression, and anxiety was assessed using the Man-Whitney U-test. Logistic regression was used to assess the predictor of anxiety and depression among the students during the strike. The level of significance was set at $p<0.05$.

RESULTS
Socio-demographic and physical characteristics of the subjects
The subject's socio-demographic and physical characteristics are presented in Table 1.

Table 1. Demographic of the participants

Demographic	Frequency	%
Gender		
Male	141	44.5
Female	176	55.5
Age		
15 -25	249	78.5
26-51	68	21.5
Student category		
Undergraduate	273	86.1
Postgraduate	44	13.9
Type of University		
State University	59	18.6
Federal university	258	81.4
Marital Status		
married	24	7.6
single	293	92.4
Residence		
Southern Nigerian	285	89.9
Northern Nigerian	32	10.1
Steady Family income		
Yes	122	38.5
No	195	61.5
Leaving Home		
≤5 times	218	68.8
> 5 times	99	31.2
Afraid of my future		
Agree	298	94.0
Degree	19	6.0
Lost interest		
agree	175	55.2
Disagree	142	44.8
Place of exercise		
At home	168	53.0
Out of home	149	47.0
Hours spent watching television		
≤ 3 hours daily	251	79.2
>3 hours daily	66	20.8
Living condition		
Live alone	73	23.0
Living with family members	244	77.0
Do you know any student that died during the strike		
Yes	128	40.4
No	189	59.6
Suggestions on ways to curb strikes in future		
Government should honor agreements	191	60.3
Salary increments for academics	27	8.5
Revitalization of universities	23	7.3
increase the budget for education	52	16.4
use of a university-friendly platform to pay lecturers	24	7.6

The majority of the subjects were in the age range of 15 -25 years old (78.5%). The majority of the respondents in this study were of the female gender (55.5%) compared to the male (44.5%). Notably, most of the subjects were residents of southern Nigeria (89.9%) whereas (10.1%) of the study subjects resided in northern Nigeria. Major of the subjects were singles (92.4%) than married (7.6%)

who were living with their family member (77.0%) than living alone (23.0%). The majority of the subject were also of the undergraduate student category (86.1%) than postgraduate (13.9%) studying in Federal government-owned universities (81.4%) than state universities (18.6%). The majority of these study subjects also agree (94.0%) that they were afraid of the future of their education and that they had lost interest in their studies because of the strike (55.2%) than those who disagree. The majority of the subjects also do not have a steady family income (61.5%) compared to those who have a steady family income (38.5%). The majority of the subjects exercised at home (53.0%) than outside the home (47.0%), spent ≤ 3 hours daily (79.2%) watching television than >3 hours daily (20.8%). The majority of the subjects suggested that to curb such strikes soon, Government should honor existing agreements (60.3%) reached with the striking lecturer whereas others also suggested that government should increase the budget for education (16.4%) and increase the salaries (8.5%) of the striking lecturers.

Determinant of depression and anxiety among students the strike

Table 2 showed case the results of Pearson's Chi-square test used to determine the association between the subject's physical, demographic, and socioeconomic characteristics with depression and anxiety. Student category $\chi2$ (1, n = 110) = 7.928, p = 0.005, marital status $\chi2$ (1, n = 4) = 4.696, p = 0.030, steady family income $\chi2$ (1, n = 31) = 11.846, p = 0.001, lost interest $\chi2$ (1, n = 77) = 7.676, p = 0.006, and contemplating dropping out of school $\chi2$ (1, n = 44) = 6.764, p = 0.009 were significantly associated with depression. Students whose student category was undergraduate (40.3%) showed an increased level of depression than those who were postgraduate (18.2%), while students who were married (16.7%) were less likely to be depressive than those who were single (38.9). Students who answered yes to steady family (25.4%) were less likely to be depressive than those who answered no (44.6%). Students who agreed (44.0%) that they have lost interest in their study showed increased levels of depression than those who disagree (28.9%), while students who agree (48.4%) to contemplating dropping out of school were more likely to be depressed than those who disagree (32.7%). Anxiety was significantly associated with age $\chi2$ (1, n = 18 1) = 11.079, p = 0.001, student category $\chi2$ (1, n = 201) = 27.282, p = 0.000, marital

status $\chi2$ (1, n = 10) = 8.382, p = 0.004, steady family income $\chi2$ (1, n = 68) = 14.049, p = 0.000, leaving home $\chi2$ (1, n = 160) = 8.881, p = 0.003, afraid of my future $\chi2$ (1, n = 209) = 9.119, p = 0.003, lost interest $\chi2$ (1, n = 136) = 16.500, p = 0.000, contemplating dropping out of school $\chi2$ (1, n = 74) = 10.213, p = 0.001, do you see anything positive about this strike $\chi2$ (1, n = 67) = 10.100, p = 0.001, and do you know any student that died during the strike $\chi2$ (1, n = 105) = 19.086, p = 0.000. Undergraduate (73.6%) students had a higher level of anxiety than postgraduate (34.1%) students while students who were married (41.7%) were less likely to be anxious than those who were single (70.3%). Students who had a steady family income (55.7%) were less likely to be anxious than those who do not (75.9%) while students who leave home ≤5 times/week (73.4%) had increased level of anxiety than those who leave home >5 times/week (56.6%). Students who agree (70.1%) that they were afraid of the future of their education had a higher level of anxiety than those who disagree (36.8%) while students who agree (77.7%) that they have lost interest in their studies had increased level of anxiety that those who disagree (56.3%). Students who agree that they were contemplating dropping out of school (81.3%) had a higher level of anxiety than those who disagree (62.8%) while students who saw something positive about the strike (57.3%) were less likely to be anxious that those who do not (74.5%). Students who know a student that died during the strike (82.0%) had a higher level of anxiety than those who do not (58.7%).

Predictors of depression and anxiety among students during the strike

Logistic regression was used to determine the predictor of anxiety and depression among students during the strike. For anxiety, the model was significant, $X^2 (20) = 84.73$, $p < 0.001$. The model was able to explain 32.8% (Nagelkereke R^2) of the variance in anxiety. The Hosmer-Lemeshow test showed a non-significant Chi-square with p = 0.297 indicating that the data fit well with the model and correctly classifying 74.8% of the overall cases. Accordingly, 89.4% of the subjects who had anxiety were predicted by the model to have anxiety (sensitivity), and 43.6% of the subjects with no anxiety were predicted as not having anxiety (specificity). Of all cases predicted as having anxiety 77.2% were correctly predicted (positive predictive value) while the negative predictive value was 65.67%, meaning 65.67% of the subject were correctly predicted as not having anxiety.

Table 2. Association between depression and anxiety with subject characteristics

Variable	Depression		Anxiety	
	None n (%)	**Yes** n (%)	**None** n (%)	**Yes** n (%)
Gender				
Male	88 (62.4)	53 (37.6)	50 (35.5)	91 (64.5)
Female	111(63.1%)	65 (36.9)	51(29)	125(71)
χ2	0.014		1.516	
p-value	0.904		0.218	
Age				
15 -25	154 (61.8%)	95 (38.2)	68 (27.3)	181 (72.7)
26-51	45 (66.2%)	23 (33.8)	33(48.5%)	35 (51.5%)
χ2	0.428		11.079	
p-value	0.513		0.001*	
Student category				
Undergraduate	163(59.7%)	110(40.3)	72 (26.4)	201 (73.6)
postgraduate	36 (81.8%)	8(18.2%)	29 (65.9)	15 (34.1)
χ2	7.928		27.282	
p-value	0.005*		0.000*	
Type of university				
State	33 (55.9%)	26 (44.1)	19(32.2)	40(67.8)
Federal	166 (64.3%)	92 (35.7)	82(31.8)	176(68.2)
χ2	1.453		0.004	
p-value	0.228		0.950	
Marital status				
Married	20 (83.3%)	4(16.7%)	14 (58.3)	10 (41.7)
Single	179(61.1%)	114(38.9)	87 (29.7)	206 (70.3)
χ2	4.696		8.382	
p-value	0.030*		0.004*	
Residence				
Southern Nigeria	176 (61.8%)	109 (38.2)	89 (31.2)	196 (68.8)
Northern Nigeria	23 (71.9%)	9 (28.1%)	12 (37.5)	20 (62.5)
χ2	1.261		0.521	
p-value	0.261		0.470	
Steady Family income				
Yes	91 (74.6%)	31 (25.4)	54 (44.3)	68 (55.7%)
No	108 (55.4%)	87(44.6)	47 (24.1%)	148 (75.9%)
χ2	11.846		14.049	
p-value	0.001*		0.000*	
Leaving home				
≤5 times	133 (61.0%)	85 (39.0)	58 (26.6%)	160 (73.4%)
> 5 times	66 (66.7%)	33 (33.3)	43 (43.4%)	56 (56.6%)
χ2	0.933		8.881	
p-value	0.334		0.003*	
Afraid of my future				
Agree	185 (62.1%)	113 (37.9)	89 (29.9%)	209 (70.1%)
Disagree	14 (73.7%)	5 (26.3%)	12 (63.2%)	7 (36.8%)
χ2	1.029		9.119	
p-value	0.310		0.003*	
Lost interest				
Agree	98 (56.0%)	77(44.0)	39 (22.3%)	136 (77.7%)
Disagree	101(71.1%)	41(28.9)	62 (43.7%)	80 (56.3%)
χ2	7.676		16.500	
p-value	0.006*		0.000*	
Contemplating dropping out of school				
Agree	47 (51.6%)	44(48.4)	17 (18.7%)	74 (81.3%)
Disagree	152(67.3%)	74(32.7)	84 (37.2%)	142 (62.8%)
χ2	6.764		10.213	
p-value	0.009*		0.001*	
Do you see any positive about the strike?				
Yes	79 (67.5%)	38 (32.5)	50 (42.7%)	67 (57.3%)
No	120(60.0%)	80(40.0)	51 (25.5%)	149(74.5%)
χ2	1.787		10.100	
p-value	0.181		0.001*	
Frequency of exercise				
< 3 days/week	161(61.7%)	100(38.3)	87 (33.3%)	174(66.7%)
> 3 days/week	38 (67.9%)	18(32.1)	14 (25.0%)	42 (75.0%)
χ2	0.751		1.475	
p-value	0.386		0.225	
Place of exercise				
Exercise at home	112 (66.7%)	56(33.3)	57 (33.9%)	111(66.1%)
Exercise out of home	87(58.4%)	62(41.6)	44 (29.5%)	105(70.5%)
χ2	2.315		0.704	
p-value	0.128		0.402	
Hours spent watching television				
≤ 3 hours daily	162(64.5%)	89 (35.5)	85 (33.9%)	166 (66.1%)
> 3 hours daily	37 (56.1%)	29 (43.9)	16 (24.2%)	50 (75.8%)
χ2	1.609		2.229	
p-value	0.205		0.135	
Living condition				
Living alone	44 (60.3%)	29 (39.7)	23 (31.5%)	50 (68.5%)
Living with family	155 (63.5%)	89 (36.5)	78 (32.0%)	166 (68.0%)
χ2	0.254		0.005	
p-value	0.614		0.941	
Do you know any student that died during the strike?				
Yes	79 (61.7%)	49 (38.3)	23 (18.0%)	105 (82.0%)
No	120 (63.5%)	69 (36.5)	78 (41.3%)	111 (58.7%)
χ2	0.103		19.086	
p-value	0.749		0.000*	

*= p- value < 0.05, level of significance for Chi-square test

The variable that added significance to the model were student category (OR =3.545, 95% CI= 1.260- 9.976, p = 0.017), Family income (OR = 0.518, 95% CI= 0.285-0.942, p = 0.031), leaving home (OR =

2.182, 95% CI= 1.199-3.973, p = 0.011), lost interest in my studies (OR = 2.407, 95% CI= 1.267-4.575, p = 0.007) and frequency of exercise (OR = 0.419 95% CI= 0.186-0.944, p = 0.036). The odds of developing anxiety were 3.545 times, higher among students who were undergraduate than postgraduate, students who answered yes to steady family income had 0.518 times less likelihood of developing anxiety. Additionally, students who leave their homes ≤5 times/week had a 2.182 times higher likelihood of anxiety than those who leave home > 5 times/week, and students who agree that they have lost interest in their studies had a 2.407 times higher likelihood of anxiety than those who disagree. The odds of having anxiety was 0.419 times less among students whose frequency of exercise was <3 days/week than those whose frequency of exercise was >3 days/week. All other variables were not significant p > 0.05 see Table 3.

Table 3. The predictors of anxiety and depression among students during the strike

Variables	OR	95% CI for OR		p-value
		lower	upper	
Anxiety				
Gender	1.088	0.595	1.991	0.783
Age (years)	0.989	0.887	1.103	0.840
Age category	1.370	0.398	4.717	0.618
Student category	3.545	1.260	9.976	**0.017***
Type of university	0.850	0.401	1.803	0.672
Marital status	1.169	0.275	4.972	0.832
Residence	0.818	0.296	2.262	0.699
Steady Family income	0.518	0.285	0.942	**0.031***
Leaving home	2.182	1.199	3.973	**0.011***
Afraid of my future	1.091	0.324	3.674	0.888
Lost interest	2.407	1.267	4.575	**0.007***
Contemplating dropping out of school	1.319	0.607	2.870	0.484
Do you see any positive about the strike	0.575	0.315	1.049	0.071
Frequency of exercise (Days)	0.419	0.186	0.944	**0.036***
Duration exercise (minutes)	0.995	0.989	1.001	0.123
Place of exercise	0.762	0.419	1.385	0.372
Hours spent watching television (hours)	0.828	0.380	1.806	0.635
Living condition	0.992	0.478	2.062	0.984
Do you know any student that died during the strike	1.786	0.630	5.063	0.275
If yes, how many?	1.394	0.882	2.204	0.154
Depression				
Gender	1.012	0.592	1.729	0.966
Age (years)	0.969	0.873	1.077	0.563
Age category	0.529	0.177	1.579	0.254
Student category	2.153	0.756	6.134	0.151
Type of university	1.563	0.814	3.001	0.180
Marital status	0.596	0.140	2.530	0.483
Residence	1.492	0.596	3.739	0.393
Steady Family income	0.501	0.291	0.864	**0.013***
Leaving home	1.206	0.692	2.103	0.509
Afraid of my future	0.949	0.290	3.104	0.931
Lost interest	1.688	0.950	2.998	0.074
Contemplating dropping out of school	1.312	0.714	2.410	0.382
Do you see any positive about the strike	1.022	0.590	1.769	0.938
Frequency of exercise	1.431	0.729	2.809	0.298
Duration exercise	1.001	0.996	1.007	0.595
Place of exercise	0.779	0.464	1.307	0.345
Hours spent watching television	0.775	0.419	1.434	0.417
Living condition	0.982	0.523	1.845	0.955
Do you know any student that died during the strike	1.058	0.519	2.157	0.876
If yes, how many	0.922	0.725	1.173	0.511

*= p- value < 0.05, level of significance for Logistic Regression

For depression, the model was significant X^2 (20) = 34.22, p = 0.025. The model was able to explain 14% (Nagelkereke R^2) of the variance in depression. The Hosmer-Lemeshow test showed a non-significant Chi-square with p = 0.586, indicating that the data fit well with the model. The model correctly classifies 65.9% of cases. Additionally, 33.1% of the subjects who had a likelihood of developing major depression were predicted by the model to have a likelihood of major depression (Sensitivity), and 85.4% of the subjects with no likelihood of major depression were correctly predicted by the model to have no depression (specificity). Additionally, of cases predicted as having a likelihood of major depression 57.35% were correctly predicted (positive predictive value) and the negative predictive value was 68.27% meaning that of all cases predicted as not having a likelihood of major depression 68.27% were correctly predicted. The variable that added significance to the model was steady family income (OR =0.501, 95% CI= 0.291-0.864, p = 0.013), lost interest in my studies was nearly but not a significant predictor of depression (OR =1.688, 95% CI= 0.950-2.998, p = 0.074). The odds of having a likelihood of major depression were 1.688 times greater in those who agree that they have lost interest in their studies than those who disagree, while those who answered yes to steady family income had 0.501 times less likelihood of developing major depression.

DISCUSSION

This study aimed to determine the physical, socioeconomic, and psychological impact of the ASUU strike on university students at public universities in Nigeria. Therefore, we determine the associating and predicting factors of anxiety and depression among university students following the disruption of academic activities by strike embarked by university workers in public universities in Nigeria. This report is currently lacking in the literature since they are not fully explored. Psychological distress is experienced to be heightened among students during health distress and we hypothesized that this distress would worsen when student's academic activities are being truncated by strikes embarked by teachers. Probing to explore the physical and psychological impact of strikes especially among students may offer possible ways to avert it. The prevalence of anxiety among the students was 68.8% while that of depression was 37.2%, therefore the students were more anxious

than depressed. The prevalence of psychological distress among students in this study was therefore higher than that reported in a similar study among students in Nigeria, Brazil, India, and Ethiopia.[13-18]

The subject characteristics that were significantly associated with depression were student category, marital status, family income, lost interest in my studies, and contemplating dropping out of school. Undergraduate students showed an increased level of depression than postgraduate, while students who were married were less likely to be depressive than those who were single. Students who had a steady family were less likely to be depressive than those with none. Students who agreed that they have lost interest in their studies or were contemplating dropping out of school were more likely to be depressed than those who disagreed. As regards anxiety, their significant associating characteristics were age, student category, marital status, family income, leaving home, being afraid of my future, lost interest in my studies, contemplating dropping out of school, seeing anything positive about the strike, and knowing a student that died during the strike. Similarly, undergraduate students had a higher level of anxiety than postgraduate while married students were less likely to be anxious than those who were single. Students with a steady family income were less likely to be anxious than those with none while students who leave home less often had an increased level of anxiety than those who leave home more often per week. Accordingly, Students who agree that they were afraid of the future of their education, that they have lost interest in their studies, and that they were contemplating dropping out of school had a higher level of anxiety than those who disagree. However, students who saw something positive about the strike were less likely to be anxious than those who do not, while those who know a student that died during the strike had a higher level of anxiety than those who do not. Similarly, a study conducted among Malaysian university students during the COVID-19 lockdown reported that students with a low family income had higher levels of both anxiety and depression than those with a high family income.[23] However, there was no significant association between anxiety and depression with gender or physical activity level. This finding was inconsistent with a similar study conducted among university student in Northern Italian Bangladesh, and Malaysia which found that the likelihood of developing anxiety and depression were higher in females than males[31,23] and that increase physical activity may be protective against

depression.[32]

In this study, the significant predictors of anxiety were student category, family income, leaving home, loss of interest in my studies, and frequency of exercise. However, family income was the only significant predictor of depression, although lost interest in my studies was nearly but not a significant predictor of depression. Among the stressors of the strike, lack of a steady family income was an outstanding stressor and it may be implicated that students that lost interest in their studies or were afraid of the future of their education, reduced frequency of leaving home, and contemplating dropping out of school. Similar findings were seen during the health distress caused by the COVID pandemic where financial lack stood out as a major stressor and kept students disturbed about how they were going to complete their studies.[33] The disruption of academic activities where students were made to stay at home away from learned course content for a long time and the negative news about the death of a colleague may be implicated in the poor mental health of the students during this strike. Our implication for worsening psychological distress is similar to that of Libert et al. where an abrupt interruption in studies was implicated for the worsening existing mental health as a result of exposure to negative COVID-related information with resultant unsettled accumulated thoughts.[34]

In this study frequency of exercise was a significant predictor of anxiety, where students who exercise less often had less anxiety than those who exercise frequently. This finding was consistent with the report of Mir et al., who found the likelihood of anxiety to be less among inactive than active students.[23] However, the duration of exercise was not a significant predictor of anxiety or depression. This finding may be because the duration, type, and intensity of exercise may elicit different effects on the mental health of individuals.[35] However, the optimal benefit of exercise in alleviating psychological distress is peaked at durations of 30–60 minutes and decline and even worsen at a duration exceeding 3 hours.[35] Contrarily, a lack of exercise aggravates preexisting anxiety and depression to domestic violence sexual violation, substance abuse, and crimes.[36-37] A lifestyle of frequent exercise requires self-disciple, it is also uncultured by many and habitual.[12] Most of this study subject exercised in the comfort of their homes than out of their home and may have been exercised for a longer duration than expected during

the strike. It was previously unknown where most Nigerians exercise especially during the COVID pandemic lockdown[13], however, our studies showed that most student tends to exercise at their home.

Additionally, students who leave home ≤ 5 times/week had higher anxiety than those who leave home > 5 times/week, however students who leave home more often were less than those with a lesser frequency of leaving home. This finding is consistent with a similar study among university students in Malaysia which found that a higher frequency of leaving home results in a positive effect on the psychological states of students during the lockdown.[23] Frequent leaving home make people more sociable, which stimulate freedom and meeting with and making new friends which breeds positive thinking and excitement among student with a result of positive effect on mental health.[23] We therefore recommend that to reduce psychological distress during strikes parents should encourage their children to leave home more often by enrolling them in different skill acquisition programs like tailoring, computer training, and driving school. This would help to protect and strengthen the mental health of students during strikes. The Government may also protect the mental health of the students by honouring agreements reached with the striking workers to prevent the occurrence of strike.

Limitations of this study

This study has provided empirical evidence on the impact of the incessant strikes by identifying the possible associating and predicting factors of mental health among students. This evidence may be implemented as an intervention on preexisting mental health challenges among students through, helpful measures, health education, and promotions for those at risk of developing mental health.

The limitation of this study includes the use of a cross-sectional design and non-probability sampling which may challenge the generalization of results and may impede finding a causal relationship between the characteristics and the psychological state of the student. The direct comparison of the findings of this study with previous studies should be done cautiously. For example, why we determined the level of PA by assessing the frequency in days/week and duration of exercise in minutes/day, previous studies only classify their subjects into active and inactive. Additionally, other studies classified the family income of their subject into low and high while we dichotomized the family income into yes or no. The duration of exercise was

only estimates and may be less or more than reported values and students who showed a likelihood of depression would have been further assessed using PHQ-9, but this was not done. However, this study provided insight into the impact of incessant strikes on the psychological state of university students in Nigeria.

CONCLUSION
Generally, Anxiety and depression were both associated with steady family income, lost interest in my studies, and contemplating dropping out of school, student category, and marital status. Similarly, the significant predictor of anxiety and depression was steady family income. Leaving home, lost interest in my studies, and frequency of exercise also predicted anxiety. Students who exercised less often had less anxiety than those who exercise frequently. Students with a steady family income were less likely to be depressive and anxious than those with none. We therefore recommend that to reduce psychological distress during strikes parents should encourage their children to leave home more often by enrolling them

in different skill acquisition programs like tailoring, computer training, and driving schools. The government should enhance the student's income by providing student bursaries, loans, and financial scholarship programs for students. Therefore, financial support and timely intervention strategies through participation in regular exercise may be useful in preventing and managing any pre-existing mental health among students.

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