

## SCREENING OF SECONDARY METABOLITES AND INVESTIGATION OF PLANTS USED IN TREATMENT OF UPPER RESPIRATORY TRACT INFECTIONS IN NORTHERN CYPRUS BASED ON ETHNOBOTANICAL APPROACH

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### Abstract

Ethnobotany and drug discovery are interconnected as it has played a pivotal role in the discovery of effective drugs for treatment of diseases in both humans and animals. Plants have historically played a crucial role in the discovery of therapeutic agents for upper respiratory tract infections, serving as a rich source of bioactive compounds with antimicrobial, anti-inflammatory, and immunomodulatory properties that justify their continued exploration. The upper respiratory tract is the most frequently infected part of the respiratory system due to the direct exposure to the external environment. The research was aimed at selecting plants frequently used in the Northern Cyprus for treatment of upper respiratory tract infection and to screen their chemical constituents and antibacterial activities. The information on the plants were collected by administration of structured questionnaire to 60 respondents in Avtepe, Kuruova and Kaleburnu villages of the Karpaz region of Northern Cyprus base on their sociodemographic information. The collected plants were evaluated for their phytochemical constituents using qualitative screening and LC-MS. The disk diffusion, MIC and MBC assays were performed in order to determine the antimicrobial effects of collected plant extracts against the pathogenic bacteria involved in the upper respiratory system infections. The plants collected cuts across a number of families including Lamiaceae, Zingiberaceae, Asteraceae, Apiaceae, Malvaceae, Amaryllidaceae, Asparagaceae, Cupressaceae, Oleaceae, Myrtaceae, Fabaceae and Rutaceae. During the research, seventeen (17) plants were mention by the respondents to be effective in the treatment of upper respiratory tract infections and the most frequently used plants belongs to the Lamiaceae (*Salvia fruticosa* (35.29%), *Mentha spicata* and (12.94%) *Thymus capitatus* (10.59%). Water is the popular solvent used for extraction (80%) and preparation in the form of tea is the most frequently used by the respondents (85%) among other methods. According to the respondents, the plants are collected mostly from the wild sources (76.67%). The leaves were the most frequently used part of the plants as it constituted 80% of the responses. The extracts (20 mg/mL) were screened for antimicrobial activity using the disk diffusion and micro-dilution on *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. typhi*, and *K. pneumoniae*. The phytochemical screening revealed the presence of saponins, tannins, flavonoids, Steroids/Triterpenoids and cardiac glycosides. Gallic acid, protocatechuic acid, 2,5-Dihydroxybenzoic acid, caffeic acid, p-coumaric acid, jaceosidin, apigenin, luteolin, quercetin and others were identified in appreciable amounts in the selected plant extracts by LC-MS/MS. The zone of inhibition of growth produced ranged between  $4.66 \pm 0.47$  mm to  $20 \pm 0.1$  mm on the microorganisms. The standard antibiotic (ofloxacin 5 $\mu$ ) produced zone of  $25.66 \pm 0.94$  mm. The MIC produced by the extracts ranged between 5 mg/mL and 10 mg/mL. In the MBC only *Thymus capitatus* extract was found to be bactericidal on *E. coli* and *P. aeruginosa* with value of 10 mg/mL and 5 mg/mL respectively. This survey has identified important plants with potential application in treatment upper respiratory tract infections and other bacterial infections.

**Key words:** Antimicrobial-resistance; Ethnobotany; Upper-respiratory; North-Cyprus; LC-MS/MS and phytochemicals

### Introduction

Ethnobotany involves the study of plants and their complex relationship with humans (Iwu, 2002). It plays a pivotal role in the investigation of plants with proven track record of being beneficial to the human and has helped in the discovery of new drugs and therapies (Okogun, 2002). The information obtained from the plants through ethnobotanical studies are important for the culture of the people (Martin, 2007). Ethnobotany is a tool that gives valuable information about the herbal medicines used in phytotherapies (Martin, 2007; Guarrera, 2003). The use of herbal medicine have been on the increase in pharmaceutical research there by affecting the economy in many positive ways (Barata *et al.*, 2016). Medicinal plants can be accessed easily by the indigenous people and serves as raw material for preparation of home remedies for certain ailments.

The medicinal plants have been drawn from many families including the Lamiaceae which is widely distributed around the Mediterranean and Southwest Asia. It contains 236 genera and has around 6900 to 7200 species (Xu & Chang, 2017). *Thymus capitatus* belong to the Lamiaceae family and has been used in treatment of respiratory tract infections (cough, bronchitis and asthma) due its expectorant activity (Akrou *et al.*, 2010). *Salvia fruticosa* Miller has been used medicinally in the form infusions and inhalation for treatment of cough, mouth inflammation and cold (Boukhary *et al.*, 2016). *Mentha spicata* is an important medicinal plant used as diuretic, spasm reliever, gastro intestinal and respiratory activities in the form of decoction or tincture (Mahboubi, 2021). Humans are mostly affected by respiratory tract infections all over the world (Bryce *et al.*, 2005).

The active plant constituents exhibit biological activities in different ways depending on how they are administered (Rao *et al.*, 2022). Volatile plant compound including p-cymene,  $\gamma$ -terpinene, 1,8-cineole, camphor, camphene,  $\alpha$ -terpineol,  $\beta$ -myrcene,  $\beta$ -pinene,  $\alpha$ -pinene, are carvone, limonene and carveol have been reported to demonstrated antimicrobial activities (Hanoglu *et al.*, 2017; Badalamenti *et al.*, 2023; Chauhan *et al.*, 2009). The non-volatile plant compounds have contributed to the antibacterial activities of several plant extracts and they include: flavonoids such as quercetin, catechin, naringenin, cyanidin-glycoside and daidzein (Archivio *et al.*, 2007; Dai & Mumper, 2010). The phenolic acids consist of benzoic acid and cinnamic acid. The Benzoic acid contains gallic acid and cinnamic acid contains ferulic acid, coumaric acid, caffeic acid and quinic acid. Phenolic acids are common in vegetable fruits (Dai & Mumper, 2010). Tannins are phenolic compounds consisting of hydrolysable and condensed tannins (Khanbabaee & Van Ree, 2001).

Currently, the world is facing the challenge of bacterial resistance to antibiotics and the WHO has estimated that, globally, it has been forecast that 10 million deaths due to antimicrobial resistance each year may occur by the year 2050 (Tang *et al.*, 2023; Walsh *et al.*, 2023). One of major factors which contribute to the rise in antimicrobial resistance is overuse and misuse of antibiotics (Tang *et al.*, 2023; Halilu *et al.*, 2023; Korkmazer & Halilu, 2023).

The upper respiratory tract infections include the otitis media, mastoiditis, sinusitis and pharyngitis (Hall-Stoodley *et al.*, 2006). Some common bacteria that are involved in the upper respiratory infections include: *Streptococcus*

*pneumonia*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (DeMuri & Wald, 2012). Antimicrobial resistance has been reported as global health challenge (Salam *et al.*, 2023). Due to this reason, there is the need to search and develop more potent antimicrobial agents from plants. This research has for the first time put forward an ethnobotanical base method of selection of plants for treatment upper respiratory tract infection in the Northern-Cyprus and has investigated their chemical constituents and antibacterial activities against pathogenic bacteria involved in upper respiratory.

## Materials and Methods

**Selection of participants:** The participants were randomly selected from Avtepe, Kuruova, and Kaleburnu villages in Karpaz region of the Northern Cyprus. The local people with specific knowledge about the plants used for treatment of upper respiratory tract infections were included in the survey. Informants who lack the knowledge of the plants, those living outside these villages and individuals who could not give an informed consent were excluded from the study.

**Data collection and survey area:** Structured questionnaires were administered orally to sixty (60) people based on their sociodemographic status (Buwa-Komoreng *et al.*, 2019). The data on the medicinal plants used in treatment of the upper respiratory tract infections were collected from the local people living in Avtepe (Fig. 1), Kuruova (Fig. 2) and Kaleburnu (Fig. 3) villages in Karpaz region of the Northern Cyprus.

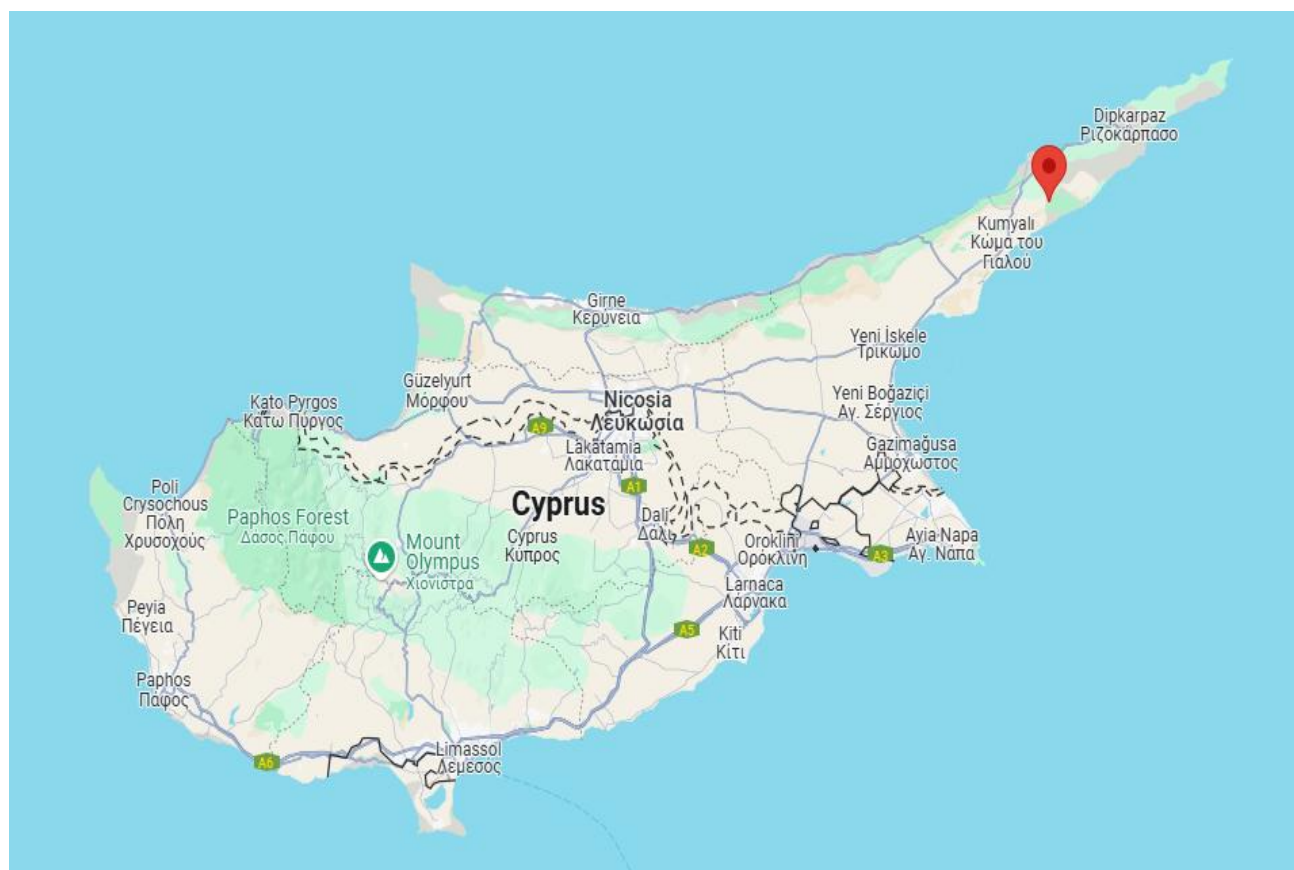


Fig. 1. Avtepe village in Cyprus map (coordinates: 35°29'29.0"N 34°13'04.0"E).



Fig. 2. Kuruova village in *Cyprus* map (coordinates: 35°30'39.0"N 34°16'25.0"E).



Fig. 3. Kaleburnu village in *Cyprus* map (coordinates: 35°31'21.0"N 34°18'07.0"E).

**Plant collection and identification:** The three most frequently mentioned medicinal plants by the respondents (*T. capitatus*, *S. fruticosa* and *M. spicata*) were collected from the surveyed communities in May 2023. The plants were identified by Asst. Prof. Dr. Emmanuel Mshelia Halilu from Faculty of Pharmacy, Cyprus International University. After identification, the voucher specimen of each plant was prepared and then assigned a specimen number: for *T. capitatus* (CIU/PHARM/LAMI/001); *S. fruticosa* (CIU/PHARM/LAMI/010) and *M. spicata* (CIU/PHARM/LAMI/003) and then kept at the Herbarium of the Faculty for future reference.

**Drying and extraction:** The leaves of *T. capitatus*, *S. fruticosa* and *M. spicata* were collected, and then air-dried separately at room temperature for 14 days. The plants were powdered with the aid of a blender (Qaralleh, Abboud *et al.*, 2009). The plant materials (20g) each were separately extracted by maceration with 160 mL of 80% ethanol for 48 hours and then filtered. The extracts concentrated using rotary evaporator (30°C and 60 rpm) and then dried in a low temperature air drying oven at 30°C (Arumugam *et al.*, 2010).

**Qualitative phytochemical screening:** The dried extract of each plant was reconstituted in water and then screened for the presence of steroids/triterpenoids, cardiac glycosides, saponins, phenolics, tannins, flavonoids, anthraquinones, and alkaloids using the method outlined by Halilu *et al.*, (2008); Shaikh & Patil (2020).

**Quantitative LC-MS analysis of the crude extracts:** The LC-tandem mass spectrometry LC-MS/MS was executed on a 6460 system Agilent with a triple quadrupole MS designed with an electrospray ionization interface. The analysis was carried out according to the method outlined by Bayram *et al.*, (2021).

**Antimicrobial studies:** The clinical isolates of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. typhi*, and *K. pneumoniae* were obtained from the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

**Preparation and sterilization of culture media:** The Mueller Hinton agar (38g) was prepared according to the manufacturer's specifications. The agar was dissolved in 1000 ml of distilled water in 1 L flask. Dehydrated medium was stirred with a glass rod to prevent clumping. The media heated with beaker on electric burner to dissolve. The medium was dispensed into 20 ml McCartney bottles and sterilized by autoclave for 15 minutes at 121°C. The sterilized medium cooled and poured into sterilized petri dishes.

**Preparation and standardization of inoculum:** The wire loop was used to take the colonies of each organism from the refrigerated organisms at 8°C of inoculation. Each organism separately inoculated in 5 ml nutrient broth in a test tube at 37°C or 24 hours. The culture standardized by dilution method in normal saline approximately  $10^6$

cfu/mL to yield microbial suspension. The procedure described a method of preparing the desired inoculum by comparison with 0.5 McFarland standard.

**Sensitivity test:** The agar well diffusion method was used with 1 mL of the inoculum flooded to the surface of dried agar plate by using the Bunsen burner. The inoculated plates were allowed to dry for 15 minutes. The cork borer mark 8 mm in diameter was used to bore (cups) four holes on each plate separately. The wells were filled with 0.1 ml of the solution 20 mg/mL of each plant extract. The standard antibiotics (Ofloxacin 5ug) was used as positive control. After the introduction of the extract, the plate allowed to stand for 1 hour for pre diffusion time. The plates incubated for 24 hours at 37°C. The presence of zone of inhibition observed for each plate. The zones of inhibition were measured in millimeters using a ruler. The experiment was done in triplicate.

**Minimum inhibitory concentration (MIC):** The MIC was determined using the dilution method. The stock solution of the extract (20 mg/mL) was serially diluted using 2-fold into 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.3125 mg/mL, 0.15625 mg/mL, 0.078125 mg/mL, 0.0390625 mg/mL and 0.01953125 mg/mL in 5 mL test tube containing nutrient broth. The extract (5 mL; 20 mg/mL) was added to the first test tube containing 5 mL sterile nutrient broth and the contents and the serial dilution process continued to the 9<sup>th</sup> number of tube. After the 9<sup>th</sup> tube was mixed 5 ml of broth was discarded. 10<sup>th</sup> and 11<sup>th</sup> test tubes receive no plant extracts and it was served as positive and negative control. With using sterile 1 ml pipette, 0.1 ml of standardized suspension of the test organism were inoculated in the test tube containing 5 ml of the various concentration of plant extracts in nutrient broth and to the positive control tube. The MIC assay test tubes were incubated at 37°C for 24 hours. The least concentration that clearly inhibited the bacterial growth was taken as the MIC.

**Minimum bactericidal concentration (MBC):** A loopful of broth from each test tube not showing growth (turbidity) in MIC determination was inoculated into sterile nutrient agar plate and incubated at 37°C for 24 hours. The absence of growth on the surface the medium was examined on plate. The lowest concentration with no visible growth recorded as the MBC.

**Data analysis:** The ethnobotanical survey results have been presented in terms of frequencies and percentages. The antibacterial test results (zone of inhibitions) were in triplicates and the data were expressed as means  $\pm$  standard deviation (SD) using Excel.

**Ethical approval and informed consent:** An approval concerning the ethnobotanical survey was sought through the Cyprus International University ethical committee. The participants were made to carefully read the consent form before accepting to be part of the study and those who voluntarily agreed to participate in the survey were selected.



## Results

**Survey:** The study shows that medicinal plants have been employed by the locals who lived around the Karpaz region of Northern Cyprus used plants for the treatment upper respiratory tract infections. This has been revealed by the data obtained from the sixty 60 respondents from the three (3) villages who participated in the survey.

**Participants' age interval:** The age range of the participants was from 18 to 100 years old. The majority of the participants were between age 51 and 70 years old. This accounted for 45% of the of respondent who participated in the survey (Table 1).

**Participants' gender:** The data showed that, there were more male participants accounting for 58.3% of the total respondents than the female (Table 2).

**Participants' nationality:** The survey showed that, most of the participants are Turkish Cypriot which accounted for 93.33% and only around only 6.67% are from Turkey (Table 3). The result also showed that, most of the people living in these villages have Turkish Cypriot ethnicity.

**Participants education levels:** The education level of the majority of participants were those who attended primary school which accounted for 45% of the total participants (Table 4). This showed participants with age interval between 51-70 years, and those above 71 years old have lower education level.

**Participants' occupation:** The participants came from different occupational background (Table 5). The retired people and the housewives have the highest percentage among the participants which accounted for 26.66% and 21.66% respectively.

**Medicinal plants used for upper respiratory tract infections:** The results showed that 17 medicinal plants are used for treatment of upper respiratory tract infections. The result reveal that *S. fruticosa*, *M. spicata* and *T. capitatus* are the most frequently used for treatment of upper respiratory tract infection among the participants accounting for 35.29%, 12.94% and 10.59 respectively (Table 6).

**Plants used for treatment of upper respiratory tract infections and other diseases:** *S. fruticosa*, *Mentha spicata* and *T. capitatus* are widely used in the villages around the Karpaz peninsula as herbal medicine for respiratory tract infections and they account for 55 % of the plants (Table 7). There is also high percentage of report of their usage for abdominal pain (due to common cold) which accounts for about 24%.

**Parts of plants used for treatment of upper respiratory:** The results showed that different parts of the plants have been used for the treatment of the upper respiratory tract infection (Table 8). The leaves accounts for the most frequently used plant part with 80% of the respondents using it.

**Table 1. Age Interval of the participants.**

Age	Frequency	Percentage (%)
18-30	12	20
31-50	11	18.33
51-70	27	45
71-100	10	16.67
Total	60	100

**Table 2. Gender of the participants.**

Gender	Frequency	Percentage (%)
Male	35	58.33
Female	25	41.67
Others	0	0
Total	60	100

**Table 3. Nationality of the participants.**

Nationality	Frequency	Percentage (%)
Cypriot	56	93.33
Turkish	4	6.67
Others	0	0
Total	60	100

**Table 4. Education of the participants.**

Education level	Frequency	Percentage (%)
Primary School	27	45
Secondary School	2	3.33
High School	18	30
University	13	21.67
Total	60	100

**Table 5. Occupation of the participants.**

Occupation	Frequency	Percentage (%)
Retired	16	26.66
Housewife	13	21.66
Self-Employment	5	8.33
Sales Marketer	5	8.33
Student	4	6.65
Teacher	2	3.33
Accountant	2	3.33
Academician	1	1.67
Municipality Worker	1	1.67
Driver	1	1.67
Real Estate	1	1.67
Shepherd	1	1.67
Construction Contractor	1	1.67
Electrician	1	1.67
Painter	1	1.67
Graphic Designer	1	1.67
Mechanical and Medical Engineer	1	1.67
Carpenter	1	1.67
Advocate	1	1.67
Farmer	1	1.67
Total	60	100

**Table 6. Medicinal plants used for treatment of upper respiratory tract infections.**

S.No.	Common english	Scientific names	Family	Part of plant used	Preparation method	Frequency	Percentage (%)
1.	Sage	<i>Salvia fruticosa</i> Mill.	Lamiaceae	Leaves	Tea	30	35.29
2.	Mint	<i>Mentha spicata</i> L.	Lamiaceae	Leaves	Tea	11	12.94
3.	Thymus	<i>Thymus capitatus</i> L.	Lamiaceae	Leaves	Tea Other (Dried)	9	10.59
4.	Ginger	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Root	Tea	7	8.24
5.	Daisy	<i>Matricaria chamomilla</i> L.	Asteraceae	Flowers	Tea	5	5.87
5.	Fennel	<i>Foeniculum vulgare</i> Mill.	Apiaceae	Leaves	Tea	4	4.71
6.	Linden	<i>Tilia cordata</i> Mill.	Malvaceae	Leaves	Tea	3	3.53
7.	Garlic	<i>Allium sativum</i> L.	Amaryllidaceae	Root	Raw	3	3.53
8.	Anise	<i>Pimpinella anisum</i> L.	Apiaceae	Leaves	Tea	2	2.35
9.	Parsley	<i>Petroselinum crispum</i> Mill.	Apiaceae	Leaves stem	Raw Tea	2	2.35
10.	Asparagus	<i>Asparagus officinalis</i> L.	Asparagaceae	Stem	Raw	2	2.35
11.	Common Juniper	<i>Juniperus communis</i> L.	Cupressaceae	-	-	2	2.35
13.	Olive	<i>Olea europaea</i> L.	Oleaceae	Fruit	Oil	1	1.18
14.	Eucalyptus	<i>Eucalyptus camaldulensis</i> (Hook.) Blakely	Myrtaceae	Leaves	Other (Inhalation)	1	1.18
15.	Carob	<i>Cretonia siliqua</i> L.	Fabaceae	-	-	1	1.18
16.	Lemon	<i>Citrus limon</i> (L.) Burm. f	Rutaceae	-	-	1	1.18
17.	Rosemary	<i>Salvia Rosmarinus</i> L.	Lamiaceae	-	-	1	1.18
Total						85	100

**Table 7. Plants used for treatment of upper respiratory tract infections and other diseases.**

Disease	Frequency	Percentage (%)
Upper respiratory tract infection	36	50.70
Lower respiratory tract infection	3	4.23
Abdominal pain	17	23.94
Urinary tract infection	6	8.45
Stress – Sleep	4	5.63
Blood pressure	3	4.23
Skin and hair care	1	1.41
Headache	1	1.41
Total	71	100

**Table 8. Plants parts used for treatment of upper respiratory.**

Part of plant	Frequency	Percentage (%)
Leaves	48	80
Root	6	10
Fruit	1	1.67
Stem	3	5
Flower	2	3.33
Total	60	100

**Table 9. Frequency and percentage of methods of preparation of pants.**

Technique	Frequency	Percentage (%)
Tea	51	85
Oil	1	1.67
Raw	6	10
Cream	0	0
Others	2	3.33
Total	60	100

**Table 10. Frequency and percentage of sourcing of plants.**

Obtain	Frequency	Percentage (%)
Cultivated	14	23.33
Collected from the wild	46	76.67
Total	60	100

**Table 11. Frequency and Percentage of Solvents used for extraction of plants.**

Solvent	Frequency	Percentage (%)
Raw	12	20
Water	48	80
Alcohol	0	0
Others	0	0
Total	60	100

**Table 12. Frequency and percentage of Route of administration of plant extract.**

Administration	Frequency	Percentage (%)
Oral	58	96.66
Topical	1	1.67
Inhalation	1	1.67
Parenteral	0	0
Other	0	0
Total	60	100

**Table 13. Frequency and percentage of administration regiment of plant.**

Usage per day	Frequency	Percentage (%)
1	29	48.33
2	22	36.67
3	6	10
More	3	5
Total	60	100

**Table 14. Percentage yield of plant extract.**

Plant	Percentage yield (%)
<i>T. capitatus</i>	10.095 ± 2.25
<i>M. spicata</i>	9.93 ± 1.40
<i>S. fruticosa</i>	12.933 ± 0.507

**Table 15. Phytochemical analyses.**

Phytochemicals	<i>T. capitatus</i>	<i>M. spicata</i>	<i>S. fruticosa</i>
Saponins	+	+	+
Phenolics	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Anthraquinones	-	-	-
Steroids/Triterpenoids	+	+	+
Cardiac glycosides	+	+	+
Alkaloids	+	+	+

**Methods of preparation of plants for treatment of upper respiratory:** The result showed that the most widely used method of preparation of the plants among the respondent is tea accounting for 85% (Table 9).

**Sourcing of plants for treatment of upper respiratory:** The result revealed that the respondents frequently collect the plants from the wild accounting for 76 % and cultivated sources 23.33% (Table 10).

**Solvents used for extraction of plants for treatment of upper respiratory:** Water is the most commonly used solvent for the preparation of the plant accounting for 80% while others used it raw accounting for 20% (Table 11).

**Route of administration of plant extract for treatment of upper respiratory:** The oral route of administration is the most familiar route as 96% of the respondents used it (Table 12).

**Administration regiment of plant extract for treatment of upper respiratory:** The administration of the plant extracts differs greatly per day but most of the respondents used the plant extracts 1 or 2 times daily which accounts for 48.33% and 36.67% respectively (Table 13).

**Extraction of plants for phytochemical and antibacterial studies:** The percentage yields of the plants from 3 repeated extraction are presented in Table 14 as Mean  $\pm$  SD.

**Qualitative phytochemical screening:** The results indicated the presence of saponins, flavonoids and other phytochemicals (Table 15). Anthraquinone was found to be absent in all the three plants.

**Quantitative analysis of phenolic phytochemicals using LC-MS/MS in *S. fruticose*:** The concentrations of the phytochemicals in ng/g showed that jaceosidin, 2, 5-dihydroxybenzoic acid, protocatechuic acid and caffeic acid had the highest concentration (Table 16).

**Quantitative analysis of phenolic phytochemicals using LC-MS/MS in *M. spicata*:** The concentrations of the phytochemicals in ng/g showed that jaceosidin, caffeic acid, Luteolin and 2,5-dihydroxybenzoic acid had the highest concentration (Table 17).

**Quantitative analysis of phenolic phytochemicals using LC-MS/MS in *T. capitatus*:** The concentrations of the phytochemicals in ng/g showed that 2,5-dihydroxybenzoic acid, syringic acid, hesperidin, jaceosidin, naringenin, and caffeic acid (Table 18).

**Antimicrobial studies:** The zone of inhibition produced by the plant extracts (*T. capitatus*, *M. spicata* and *S. fruticosa*) on the microorganisms ranged between  $5.66 \pm 0.47$  and  $10.33 \pm 1.24$  (Table 19).

Table 16. Phytochemicals and their quantity in *S. fruticose*.

Compound	Retention time	Final concentration (ng/g)	Pharmacological activities of compounds
Gallic acid	1.660	1810.3976	Antioxidant, anti-inflammatory, and antineoplastic (Kahkeshani <i>et al.</i> , 2019)
Protocatechuic acid	1.830	20918.8922	Antioxidant (Semaming <i>et al.</i> , 2015)
2,5-Dihydroxybenzoic acid	2.083	25296.5886	Anti-inflammatory, antigenotoxic, hepatoprotective, neuroprotective, antimicrobial, antioxidant (Abedi <i>et al.</i> , 2020)
Caffeic acid	3.657	20833.2027	Anti-inflammatory, anti-viral, anti-cancer, anti-hypertensive, anti-thrombosis, anti-fibrosis (Sadhukhan <i>et al.</i> , 2018)
Syringic acid	3.682	7379.3333	Antioxidant, antimicrobial, anti-inflammatory, antiendotoxic (Bartel <i>et al.</i> , 2023)
Salicylic acid	3.700	2249.3216	Anti-inflammatory (Randjelovic <i>et al.</i> , 2015)
<i>p</i> -Coumaric acid	3.988	1079.9525	Antioxidant, anti-tumor, antibacterial, antiaging (Chen <i>et al.</i> , 2024)
Trans-Ferrulic acid	4.038	4042.2078	Antioxidant, anti-inflammatory, anti-fibrotic, anti-cancer (Zhai <i>et al.</i> , 2023)
Hesperidin	4.040	510.8147	Antioxidant, anti-inflammatory, anti-cancer, antiviral, protective cardiovascular disorders, eurodegenerative properties (Pyrzynska <i>et al.</i> , 2022)
Ethyl gallate	4.063	185.8778	Antioxidant, anti-inflammatory anti-cancer (Muddu <i>et al.</i> , 2023)
Quercetin	4.221	251.2972	Antioxidant, antiviral, antihypertensive, anti-inflammatory (Carillo-Martinez <i>et al.</i> , 2024)
Abscisic acid	4.291	3102.7409	Anti-diabetic (Bassaganya-Riera <i>et al.</i> , 2010)
Luteolin	4.264	6904.7967	Antioxidant, antimicrobial, anti-inflammatory, neuro protective, anti-diabetic, anti-cancer (Taheri <i>et al.</i> , 2021)
Naringenin	4.315	101.7330	Anti-inflammatory, antioxidant, anti-tumor (Shilpa <i>et al.</i> , 2023)
Kaempferol	4.264	623.9382	Anti-inflammatory, antifungal, antiprotozoal, anti-carcinogenic (Periferakis <i>et al.</i> , 2022)
Jasmonic acid	4.385	14469.2676	Anti-inflammatory, anti-cancer (Jarocka-Karpowicz <i>et al.</i> , 2021)
Apigenin	4.384	715.1479	Antioxidant, anti-inflammatory, anti-amyloidogenic, neuroprotective, muscle relaxation and sedation (Salehi <i>et al.</i> , 2019)
Genistein	4.392	584.8914	Antioxidant, anti-inflammatory, antibacterial, antiviral (Sharifi-Rad <i>et al.</i> , 2021)
Jaceosidin	4.398	38136.4420	Antioxidant, anti-inflammatory, antibacterial, anti-allergic, anti-cancer (Nageen <i>et al.</i> , 2021)

Table 17. Phytochemicals and their quantity in *M. spicata*.

Compound	Retention time	Final concentration (ng/g)	Pharmacological activities of compounds
Gallic acid	1.685	594.3325	Antioxidant, anti-inflammatory, and antineoplastic (Kahkeshani <i>et al.</i> , 2019)
Protocatechuic acid	1.822	6078.6895	Antioxidant (Semaming <i>et al.</i> , 2015)
2,5-Dihydroxybenzoic acid	2.075	14827.2413	Anti-inflammatory, antigenotoxic, hepatoprotective, neuroprotective, antimicrobial, antioxidant (Abedi <i>et al.</i> , 2020)
Caffeic acid	3.657	28359.4354	Anti-inflammatory, anti-viral, anti-cancer, anti-hypertensive, anti-thrombosis, anti-fibrosis (Sadhukhan <i>et al.</i> , 2018)
Syringic acid	3.690	8339.9809	Antioxidant, antimicrobial, anti-inflammatory, antiendotoxic (Bartel <i>et al.</i> , 2023)
Chlorogenic acid	3.670	2181.7724	Anti-inflammatory, antioxidant, anti-tumor, neuroprotection (Nguyen <i>et al.</i> , 2024)
Salicylic acid	3.700	4627.2480	Anti-inflammatory (Randjelovic <i>et al.</i> , 2015)
<i>p</i> -Coumaric acid	3.979	4975.7338	Antioxidant, anti-tumor, antibacterial, antiaging (Chen <i>et al.</i> , 2024)
Rutin	3.929	28.6302	Antioxidant, anti-tumor, anti-inflammatory, anti-diarrheal, anti-mutagenic, cardioprotective, immunomodulatory (Arjumand <i>et al.</i> , 2011)
Trans-Ferrulic acid	4.030	5877.8541	Antioxidant, anti-inflammatory, anti-fibrotic, anti-cancer (Zhai <i>et al.</i> , 2023)
Hesperidin	4.048	3898.3750	Antioxidant, anti-inflammatory, anti-cancer, antiviral, protective cardiovascular disorders, neurodegenerative properties (Pyrzynska <i>et al.</i> , 2022)
Indole-3-acetic acid	4.115	3393.0865	Anti-inflammatory, antioxidant (Ji <i>et al.</i> , 2020)
Quercetin	4.221	5.0160	Antioxidant, antiviral, antihypertensive, anti-inflammatory (Carillo-Martinez <i>et al.</i> , 2024)
Absciscic acid	4.282	2934.4272	Anti-diabetic (Bassaganya-Riera <i>et al.</i> , 2010)
Luteolin	4.264	26834.2157	Antioxidant, antimicrobial, anti-inflammatory, neuro protective, anti-diabetic, anti-cancer (Taheri <i>et al.</i> , 2021)
Naringenin	4.315	321.0444	Anti-inflammatory, antioxidant, anti-tumor (Shilpa <i>et al.</i> , 2023)
Kaempferol	4.272	2483.1305	Anti-inflammatory, antifungal, antiprotozoal, anti-carcinogenic (Periferakis <i>et al.</i> , 2022)
Apigenin	4.392	1607.3862	Antioxidant, anti-inflammatory, anti-amyloidogenic, neuroprotective, muscle relaxation and sedation (Salehi <i>et al.</i> , 2019)
Genistein	4.392	1367.2389	Antioxidant, anti-inflammatory, antibacterial, antiviral (Sharifi-Rad <i>et al.</i> , 2021)
Jaceosidin	4.372	72035.4218	Antioxidant, anti-inflammatory, antibacterial, anti-allergic, anti-cancer (Nageen <i>et al.</i> , 2021)
CAPE	4.551	423.9505	Antioxidant, anti-inflammatory, anti-cancer, anti-fungal, antibacterial (Taysi <i>et al.</i> , 2023)

Table 18. Quantity of metabolites in *T. capitatus* base on LC-MS.

Compound	Retention time	Final concentration (ng/g)	Pharmacological activities of compounds
Gallic acid	1.702	231.6715	Antioxidant, anti-inflammatory, and antineoplastic (Kahkeshani <i>et al.</i> , 2019)
Protocatechuic acid	1.830	9493.3997	Antioxidant (Semaming <i>et al.</i> , 2015)
2,5-Dihydroxybenzoic acid	2.075	69370.2950	Anti-inflammatory, antigenotoxic, hepatoprotective, neuroprotective, antimicrobial, antioxidant (Abedi <i>et al.</i> , 2020)
Caffeic acid	3.657	12806.8601	Anti-inflammatory, anti-viral, anti-cancer, anti-hypertensive, anti-thrombosis, anti-fibrosis (Sadhukhan <i>et al.</i> , 2018)
Syringic acid	3.690	34168.2279	Antioxidant, antimicrobial, anti-inflammatory, antiendotoxic (Bartel <i>et al.</i> , 2023)
Chlorogenic acid	3.670	264.7416	Anti-inflammatory, antioxidant, anti-tumor, neuroprotection (Nguyen <i>et al.</i> , 2024)
Salicylic acid	3.692	10645.8989	Anti-inflammatory (Randjelovic <i>et al.</i> , 2015)
<i>p</i> -Coumaric acid	3.971	1724.3483	Antioxidant, anti-tumor, antibacterial, antiaging (Chen <i>et al.</i> , 2024)
Rutin	3.879	65.7913	Antioxidant, anti-tumor, anti-inflammatory, anti-diarrheal, anti-mutagenic, cardioprotective, immunomodulatory (Arjumand <i>et al.</i> , 2011)
Trans-Ferrulic acid	4.021	3384.4645	Antioxidant, anti-inflammatory, anti-fibrotic, anti-cancer (Zhai <i>et al.</i> , 2023)
Hesperidin	4.040	28222.3327	Antioxidant, anti-inflammatory, anti-cancer, antiviral, protective cardiovascular disorders, neurodegenerative properties (Pyrzynska <i>et al.</i> , 2022)
Quercetin	4.229	1564.5588	Antioxidant, antiviral, antihypertensive, anti-inflammatory (Carillo-Martinez <i>et al.</i> , 2024)
Absciscic acid	4.291	711.3490	Anti-diabetic (Bassaganya-Riera <i>et al.</i> , 2010)
Luteolin	4.264	8496.0798	Antioxidant, antimicrobial, anti-inflammatory, neuro protective, anti-diabetic, anti-cancer (Taheri <i>et al.</i> , 2021)
Naringenin	4.324	13863.8942	Anti-inflammatory, antioxidant, anti-tumor (Shilpa <i>et al.</i> , 2023)
Kaempferol	4.272	759.2223	Anti-inflammatory, antifungal, antiprotozoal, anti-carcinogenic (Periferakis <i>et al.</i> , 2022)
Jasmonic acid	4.402	4124.1378	Anti-inflammatory, anti-cancer (Jarocka-Karpowicz <i>et al.</i> , 2021)
Apigenin	4.392	2170.3045	Antioxidant, anti-inflammatory, anti-amyloidogenic, neuroprotective, muscle relaxation and sedation (Salehi <i>et al.</i> , 2019)
Genistein	4.383	2330.1408	Antioxidant, anti-inflammatory, antibacterial, antiviral (Sharifi-Rad <i>et al.</i> , 2021)
Jaceosidin	4.322	17854.3136	Antioxidant, anti-inflammatory, antibacterial, anti-allergic, anti-cancer (Nageen <i>et al.</i> , 2021)



Table 19. Zone of Inhibition of plant extracts.

Bacteria	Zone of inhibition (mm) Mean $\pm$ SD				
	<i>T. capitatus</i>	<i>M. spicata</i>	<i>S. fruticosa</i>	Ofloxacin	DMSO %10
<i>S. aureus</i>	20 $\pm$ 0.1	10.33 $\pm$ 1.24	20 $\pm$ 0.81	25.66 $\pm$ 0.94	0
<i>B. subtilis</i>	14 $\pm$ 0.81	0.0	20 $\pm$ 0.81	24 $\pm$ 0.81	0
<i>E. coli</i>	13.66 $\pm$ 0.47	4.66 $\pm$ 0.47	15 $\pm$ 0.81	23.33 $\pm$ 0.47	0
<i>P. aeruginosa</i>	19.66 $\pm$ 0.47	11.66 $\pm$ 0.47	14.33 $\pm$ 1.24	23.33 $\pm$ 0.47	0
<i>S. typhi</i>	19.66 $\pm$ 1.24	17.66 $\pm$ 1.24	15.33 $\pm$ 1.24	23.66 $\pm$ 0.47	0
<i>K. pneumoniae</i>	14.66 $\pm$ 0.47	5.66 $\pm$ 0.47	16 $\pm$ 0.81	22.33 $\pm$ 0.47	0

Table 20. MIC of plant extracts.

Bacteria	MIC (mg/mL)		
	<i>T. capitatus</i>	<i>M. spicata</i>	<i>S. fruticosa</i>
<i>S. aureus</i>	10	10	10
<i>B. subtilis</i>	10	0	5
<i>E. coli</i>	10	5	10
<i>P. aeruginosa</i>	5	10	10
<i>S. typhi</i>	10	10	10
<i>K. pneumoniae</i>	10	5	10

**Minimum inhibitory concentration:** The plant extracts inhibited the growth of the organisms with minimum inhibitory concentration ranging from 5 mg/mL to 10 mg/mL. The results revealed that all the extracts inhibited the growth of the bacteria strains except *M. spicata* which could not inhibit the growth of *B. subtilis* (Table 20).

**Minimum bactericidal concentration:** The MBC results showed that only *T. capitatus* had bactericidal activity on *P. aeruginosa* and *E. coli*. *M. spicata* and *S. fruticosa* are mainly bacteriostatic on the microorganisms (Table 21).

## Discussion

Ethnobotany plays a pivotal role in the discovery of potent drugs from medicinal plants used by the indigenous people in treatment of diseases (Heinrich & Gibbons, 2001). Ethnobotanical studies are still relevant as 80% of the world's population depends on natural products mainly plants and their products for treatment of illnesses (Dias *et al.*, 2012; Chaachouay & Zidane, 2024).

The gathering and documentation of information on medicinal plants used specifically for treatment of the upper respiratory tract infections through ethnobotanical survey in the Karpaz region of Northern Cyprus is the first attempt where the data was directly collected from the local inhabitants of the communities. The data showed that the majority of the respondents who have information about the applications of these plants are people with ages above 51 years constituting a total of 61.67% of the total respondents. This trend is expected, because the younger generation may not be interested in the traditional knowledge about the use of these plants (Weckmüller *et al.*, 2019; Mekonnen *et al.*, 2022).

The gender of the respondents revealed some effects on their knowledge about the plants, as the results showed that 58.33% of the male respondents have some knowledge about these plants, then followed by the females who constituted 41.67%. These finding differ from the studies of Caballero-Serrano *et al.*, (2019) which showed that women have knowledge about medicinal plants more than men. This difference may be due to the community and the

Table 21. MBC of plant extracts.

Bacteria	MBC (mg/mL)		
	<i>T. capitatus</i>	<i>M. spicata</i>	<i>S. fruticosa</i>
<i>S. aureus</i>	-	-	-
<i>B. subtilis</i>	-	-	-
<i>E. coli</i>	10	-	-
<i>P. aeruginosa</i>	5	-	-
<i>S. typhi</i>	-	-	-
<i>K. pneumoniae</i>	-	-	-

interest in the medicinal plants by the women and men (Weckmüller *et al.*, 2019).

The questionnaire results revealed that Turkish Cypriots had more knowledge about these plants, accounting for 93.33%, while only around 6.67% of the Turkish nationals from Turkey had knowledge of the plants. This trend is expected as the Turkish Cypriot are indigenous people and are expected to know this plants. This finding is in agreement with Caballero-Serrano *et al.*, (2019) who stated that the indigenous people tend to have more knowledge about the medicinal plants in their locality (Joshi *et al.*, 2020).

The level of education of the respondents had some effect on their knowledge about the medicinal plants. Those with primary school education had the highest percentage accounting for 45%, of the total respondents. This trend is expected because the highly educated individuals tend to be cautious and have disbelieve about the efficacy of the traditional botanical drugs. As it can be seen that those with university degree accounted for 21.67% of the respondents (Tamene *et al.*, 2024).

The occupation and age of the respondents probably had some influence on their usage of the medicinal plants. The retired (26.66%) people and the housewives (21.66%) have the highest percentage among the participants. These may be attributed to their source of income. Many retired individuals do not have money to afford to buy drugs and therefore, resort to the use of plants in their surroundings for their health care needs (Arjona-García *et al.*, 2021; Tamene *et al.*, 2024).

The *Salvia fruticosa*, *Mentha spicata* and *Thymus capitatus* have been shown to be the most frequently used medicinal plants for treatment of upper respiratory tract infection among the participants accounting for 35.29%, 12.94% and 10.59 respectively. The aforementioned plants belonging to the Lamiaceae family, and their use in treating upper respiratory tract infections especially through their essential oil composition have been well documented. These plants have been reported for treatment of lower respiratory tract infections, abdominal pain and urinary tract infections (Bruno *et al.*, 2023; Kianmehr *et al.*, 2023).

The leaves have been observed to be the most frequently used plant part for the preparation of medicament accounting for 80% then followed by the roots 10%. According to Wachtel-Galor & Benzie (2011), the herbs can be prepared and taken in many ways and forms. The most probable reason for the highest percentage of the usage of the leaves is due to the fact that they can be easily procured from the plants because they are easily accessible, as against the roots that must be dug from the ground (Uzun & Koca, 2020). Scientifically, the leaves are active site of photosynthesis and the site for production of the secondary metabolites (Uzun & Koca, 2020).

Nature has been the source of the medicinal plants and it still provides the largest quantity of the plants for the service of man. The survey results revealed that the respondents frequently collect the plants from the wild accounting for 76% and cultivated sources 23.33%.

The method of preparation of the plants among the respondent is tea accounting for 85%. The reason for the highest percentage of tea is because herbal tea can be easily prepared by infusion, decoction or by maceration at room temperature, thereby forming a different composition of extractable constituents (Jäger *et al.*, 2011).

The oral route of administration is the most familiar route as 96% of the respondents used it. The oral route is the most preferred route, due to its advantages, such as non-invasiveness, patient compliance and convenience of drug administration (Alqahtani *et al.*, 2021). Many factors govern the oral drug absorption which include drug solubility, mucosal permeability, and stability in the gastrointestinal tract environment (Alqahtani *et al.*, 2021).

The administration of the plant extracts differs greatly on a daily basis but most of the respondents used the plant extracts once (48.33%) or twice (36.67%) daily. The probably reason for this may be due to perceive toxicity that may arise due to lack of standard dosage of the herbal medicine and the sensitivity of the upper respiratory tract (Ekor, 2014).

The qualitative phytochemical screening has revealed the presence of tannins, flavonoids, steroids/triterpenoids, saponins, cardiac glycosides and alkaloids. The presence of the chemicals has been shown to exist in the Lamiaceae family. The LC-MS/MS profiling of the phenolic compounds in *T. capitatus*, *S. fruticosa* and *M. spicata* leaves has revealed the presence of polyphenols such as luteolin quercetin, kaempferol, apigenin, catechol, chlorogenic acid and caffeic acid. These compounds have been identified in the Lamiaceae family (Nasirkandi *et al.*, 2023).

The antibacterial activity demonstrated by the plant extracts spans through a broad spectrum of bacteria (Gram positive and Gram negative) known to be implicated in the upper respiratory tract infection. The zone of inhibition produced on the bacterial strains ranged between  $5.66 \pm 0.47$  and  $10.33 \pm 1.24$ . On comparing the result of the current of *T. capitatus*, with the findings of Qaralleh *et al.*, (2009), on *T. capitatus* obtained from Jordan in 2009; the Cyprus *T. capitatus* showed higher antibacterial activity on *E. coli* and *S. aureus* and almost same diameter of zone of inhibition on *P. aeruginosa* (Qaralleh *et al.*, 2009). The antibacterial activity demonstrated by the *S. fruticosa* in 2021, in Libya with 100 mg/mL concentration showed diameters of 12 mm with *B. subtilis*, 0 mm with *S. aureus*, 11 mm with *E. coli*

and 13 mm with *P. aeruginosa*. The experiment conducted with the Northern Cyprus *S. fruticosa* at 20 mg/mL concentration produced the following zone of inhibition on *B. subtilis* 20 mm; 20 mm on *S. aureus*, 15 mm on *E. coli* and 14 mm with *P. aeruginosa*. The result indicated that the Northern Cyprus *S. fruticosa* is more potent than Libyan *S. fruticosa* especially on *S. aureus* (Abdulla-Eltawaty *et al.*, 2021). Among the three plants, *M. spicata* demonstrated the least activity on the tested bacteria (Table 19). The extracts demonstrated of 5 mg/mL to 10 mg/mL on the bacteria. The MBC results showed that only *T. capitatus* had bactericidal activity on *P. aeruginosa* and *E. coli*. *M. spicata* and *S. fruticosa* are mainly bacteriostatic on the microorganisms.

## Conclusion

The ethnobotanical survey is the first attempt to gather and document information on medicinal plants mainly used for treatment of the upper respiratory tract infections in the Karpaz region of Northern Cyprus. The data revealed that the most frequently used plants for treatment of upper respiratory tract infections are *S. fruticosa*, *T. capitatus* and *M. spicata*. The most frequent used plant part is the leaves which is mostly prepared in the form of tea. The plant extracts have demonstrated antibacterial of wide range of bacteria including those implicated in upper respiratory tract infections (*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. typhi*, and *K. pneumoniae*). The *T. capitatus* extract demonstrated bacteriostatic activity on *E. coli* and *P. aeruginosa*. The phytochemical, Jaceosidin, 2, 5-dihydroxybenzoic acid, protocatechuic acid and caffeic acid luteolin, syringic acid, hesperidin and naringenin are the most abundant in the plants. The antibacterial activity demonstrated by the plant extracts may be attributed to these phytochemicals.

## Author contributions

Mehmetali Beyaz: Conducted the ethnobotanical survey and drafted the manuscript

Emmanuel Mshelia Halilu: Conducted the phytochemical analysis and edited the manuscript

Övgü İşbilen: Carryout the antibacterial studies and proof read the manuscript.

**Declaration of competing interest:** The authors hereby declare that they have no conflict of interest

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