GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Essential oil-based phytochemical identification of the Algerian north-central species of *Thymus algeriensis*

Islem Boukara [1], Issam Habbouche [1] and Bachir Nabti [1,2,*]

[1] *Faculty of Pharmacy, Laboratory of Pharmacognosy, University of Algiers 1, Algiers, Algeria.*
[2] *Hussein Dey University Hospital Center, Algiers, Algeria.*

GSC Biological and Pharmaceutical Sciences, 2023, 23(03), 075–082

Publication history: Received on 13 April 2023; revised on 31 May 2023; accepted on 03 June 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.3.0206

Abstract

Thymus algeriensis is a Lamiales member especially widespread through the Mediterranean basin, including the northern African region. Algeria counts numerous variants of the species, thriving in disparate locations and climates. The usage of aromatic species' essential oils for the variants identification and the applications adaptation is a practice known as chemotype determination, objective of this study, with the Algerian north-central *Thymus algeriensis* as target. Gas chromatography coupled to mass spectrometry was used to analyze the Clevenger apparatus-extracted essential oil, and leaves staining was performed for the morphological identification. The species chemotype was Linalool dominant, highlighting the difference to adjacent territories growing variants reported in the literature.

Keywords: *Thymus algeriensis*; GC-MS; Chemotype; Essential oil; Clevenger extraction; Hydro-distillation

1. Introduction

Thymus represents an important genus in terms of abundance and usage in the Mediterranean basin, including Algeria, due to its members' adaptability and hybridization potential; these properties resulted in widespread distribution and an apparent morphological identification difficulty.(1,2)

The most abundant *Thymus* species in Algeria are *Thymus fontanesii*, *Thymus commutatus*, *Thymus hirtus*, *Thymus munbyanus*, and *Thymus algeriensis*.(3) This later, prevalent in the central and central-western area of the Algerian coastline, is culturally used as a cooking aromatizing herb and a component of traditional therapeutic infusions for its essential oil virtues.(4)

Thymus algeriensis essential oil origins from the typical glandular trichomes localized mainly on the underside of the leaves. These comprise a unicellular basis, topped by a secreting cell, and a vacuole serving as a stocking then releasing compartment.(5,6) It is noteworthy to mention that climatological variations and topographical setups discrepancies (i.e., light exposition and altitude) were established as factors affecting the qualitative and quantitative composition of various *Thymus* species' essential oils.(7,8)

In order to ensure the suitability, effectiveness, and security of aromatic plants and their derived products usage, the chemotype determination represents a compulsory step to undertake.(9) Additionally, the standardization and validation of gas chromatography (GC) separation techniques, mass spectrometry (MS) detection, and the essential oils extraction procedures allowed the appearance of a classical Clevenger hydro-distillation and GC-MS coupled sequence in the current chemotype determination studies.(10)

* Corresponding author: NABTI Bachir

Considering the Algerian north-central *Thymus algeriensis* chemotype data gap, and the climatological and topological variations alongside the coastline, qualitative disparities to eastern and western species are to be expected. If confirmed, these dissimilarities would represent a key identifier and driver for the local-oriented therapeutic and commercial usages of the species variants.

Therefore, this study aims to determine the chemotype of the north-central *Thymus algeriensis* species through the analysis of its essential oil by the mean of mass spectrometry coupled gas chromatography (GC-MS) following a Clevenger hydro-distillation extraction.

2. Material and methods

2.1. Sample collection and preparation

The vegetal material was collected during the dry period of November, by cutting the upper stem part of healthy wild specimens, growing at 150 meters (m) altitude in the Bougara region of the Algerian state of Blida. The fresh material was spread dried in an amber and ventilated chamber for 3 months prior to the extraction. The stems integrity was conserved during the drying period.

2.2. Histological identification

Randomly sampled fresh stems were conserved in an equal ratio mixture of absolute ethanol (Sigma-Aldrich®), Glycerin (Lab Alley®, 99.5%), and distilled water for a week to permit a proper imbibing prior to the preparation and staining of thin cuts according to the Longeron inclusion double staining method described below:

- Thin cuts were prepared from the pre-soaked leaves.
- The fresh cuts were transferred into a bleach solution for 20 minutes (This step removes the cellular content), then rinsed with distilled water.
- The residual bleach was neutralized by imbibing the cuts in a 1% solution of acetic acid before rinsing them with distilled water.
- The cuts were then immersed into methylene blue for 5 to 10 minutes (This step stains the lignified walls in green), then rinsed with distilled water.
- The cuts were immersed into a Congo red solution for 5 to 10 minutes (This step stains the cellulose walls in pink/red), then rinsed with distilled water.
- The stained cuts were mounted and observed under an optical microscope.

The dry leaves' ground powder was stained using the Gazet Du Chatelier reagent prepared according to Baytop's procedure(11). The observations were made using an optical microscope (Equilab®) and aimed the verification of the species morphological traits.

2.3. Essential oil extraction

The dehydrated stems were manually reduced into 3 to 5 millimeters (mm) length pieces promptly prior to the extraction to maintain their integrity and optimize the extraction yield. A laboratory-scale hydro-distillation Clevenger apparatus was used for the extraction. A 3:1 ratio of vegetal sample to distilled water was observed during the extraction, with a controlled weight of 90 grams (g) for each operation, and an optimized duration of 90 minutes (min). The extracted oil was collected into ambered glass containers and kept at +4 °C until the analysis.

The extraction procedure was conducted as follows:

- Weighted stem parts were introduced into the main container.
- Distilled water was added to immerse the vegetal material and reach a 3:1 ratio. The mix should not exceed 2/3rd of the container capacity.
- The entire extraction Clevenger apparatus was then assembled, and the joints sealed.
- The purge valve was closed after an arbitrary amount of distilled water was added to the lower part of the oil container to monitor the pressure inside the assembly.
- The cooling compartment was connected to a continuous, slow water flow.
- The main container was then heated at medium-low temperature until the water vapor saturated the container and started extracting the essential oil into the collection compartment through the condensation module.

- 90 minutes were timed at the first vapor emission for an entire extraction cycle. The heating was stopped at the end of that period, and the assembly let to cool down for a few minutes.
- The essential oil was then collected into an ambered container, sealed, and conserved at 4 °C until the analysis.

The extraction yield was calculated using the following equation:

$$x = (a \times 100) / b.$$

x: Extraction yield (% v/w). a: volume of the extracted oil in milliliters (mL). b: weight of the initial material (g).

2.4. Gas chromatography analysis

An HP Agilent 2890 GC-MS system including a quadrupole mass spectrometry detection system coupled to a gas chromatography separation module was used for the analysis of the extracted essential oil, on a 30 meters HP-5MS column, with a gradient temperature mode, and an acquisition span of 127 minutes. The analysis was carried out at the facilities of the Research Centre for Physical and Chemical Scientific and Technical Analysis (C.R.A.P.C) -Algiers-. The detailed separation and detection parameters are described in (**Figure. 1**) and (**Table. 1**).

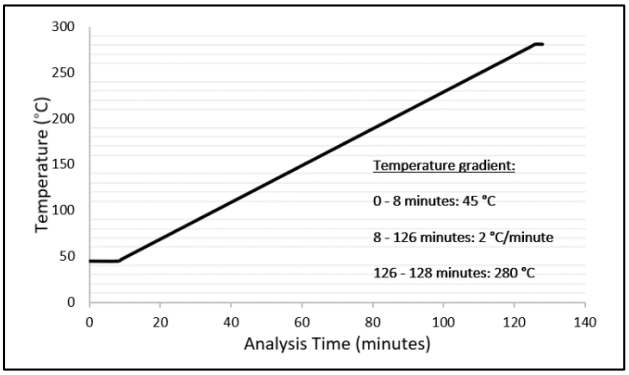

Figure 1 Gas chromatography separation oven temperature gradient details.

Table 1 GC-MS analytical conditions and equipment details.

Equipment	**Chromatograph: HP Agilent 2890**
Injector	- Temperature: 280 °C - Injection mode: Split 20:1 - Injection volume: 0.2µl
Column	- Type: HP-5MS - Length: 30 m - Internal diameter: 0.25 mm - Internal film thickness: 0.25 µm - Stationary phase: 5% phenyl, 95% dimethylpolysiloxane
Oven temperature gradient	Isotherm: 45 °C for 8 minutes Gradient: 2 °C/min, from 45 °C to 280 °C Isotherm: 280 °C for 2 minutes
Analysis duration	127 minutes
Carrier gas	Helium (0.5ml/min)
Mass detector	- Analysis mode: scan TIC (30 ~ 550 M/Z) - Interface temperature: 300 °C - Ionization mode: EI (70 EV) - Detector type: Quadrupoles - Source temperature: 230 °C

3. Results and discussion

3.1. Sample collection and preparation

A total quantity of nine (09) kg of fresh vegetal material was collected, and 3.83 kg of dry material was retrieved, denoting a loss of 57.37% of humid weight, sign of an effective drying procedure.

3.2. Histological identification

The histological analysis revealed a typical downward curvature of the leaves, and the exclusive presence of low-density, large-size glandular trichomes on their inferior face, alongside tarped, elongated, bent capitate hairs (**Figure. 2a**) (**Figure. 2b**). The dorsal face of the leaves was characterized by the presence of small conical capitate hairs, and absence of glandular trichomes (**Figure. 2b**). The powder analysis revealed similar characteristic fragments (**Figure. 2c**) (**Figure. 2d)**.

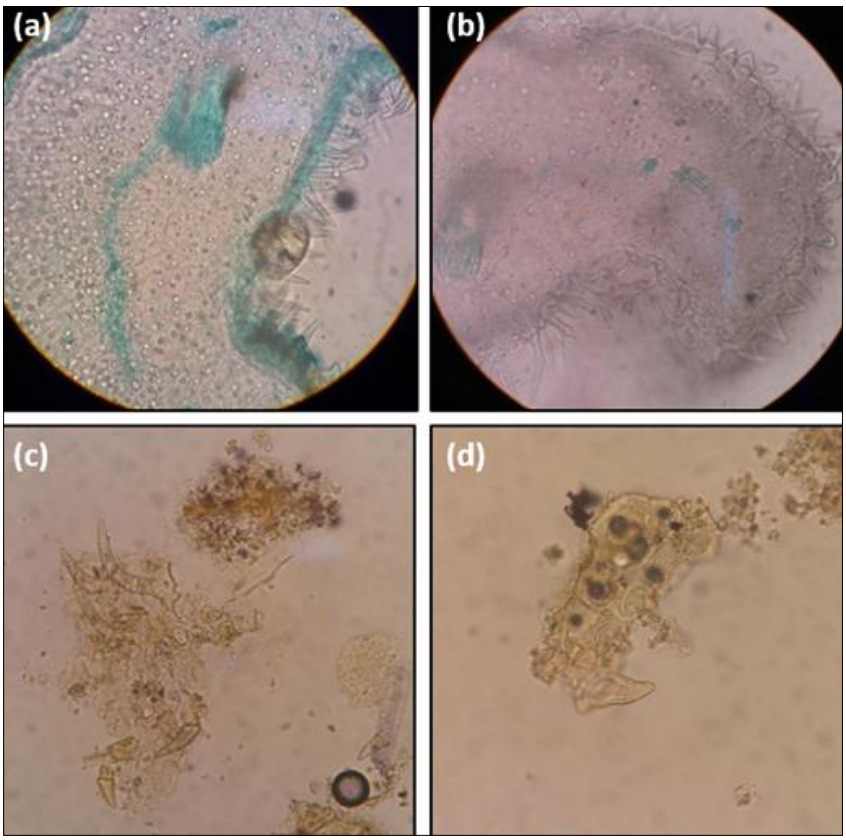

Figure 2 Histological characteristics of *Thymus algeriensis* leaves*.* (a) Glandular trichomes and downwards capitate hairs; (b) Elongated and conical capitate hairs; (c) (d) Leaves powder histological features*.* The cuts were stained according to the protocols described in the methodological section. The observations were made on a x20 magnification.

3.3. Essential oil extraction and GC-MS analysis

The calculated average extraction yield based on seven (7) extraction replicates was estimated to 1.77 ± 0.27 % (v/w). The daily recoveries are described in (**Table. 2**). The obtained average extraction recovery value was higher than those described previously by Dob et al., 2006 in Algeria and Amarti et al., 2009 in Morocco (i.e., 1.13% and 1.2% respectively).(12,13)

The GC-MS analysis revealed the presence of 36 compounds marked by the predominance in a decreased trend of linalool, thymol, cymene, caryophyllene, isolimonene and camphor (**Figure. 3**) (**Table. 3**). The total ions chromatogram (TIC) is given in (**Figure. 4**).

Table 2 Daily extraction yield. g: Gram. mL: milliliter.

Weight (g/operation)	Extract volume (mL)	Yield (mL/100g)
90	1.7	1.88
90	1.7	1.88
90	1.1	1.22
90	1.6	1.77
90	1.5	1.66
90	1.8	2
90	1.8	2

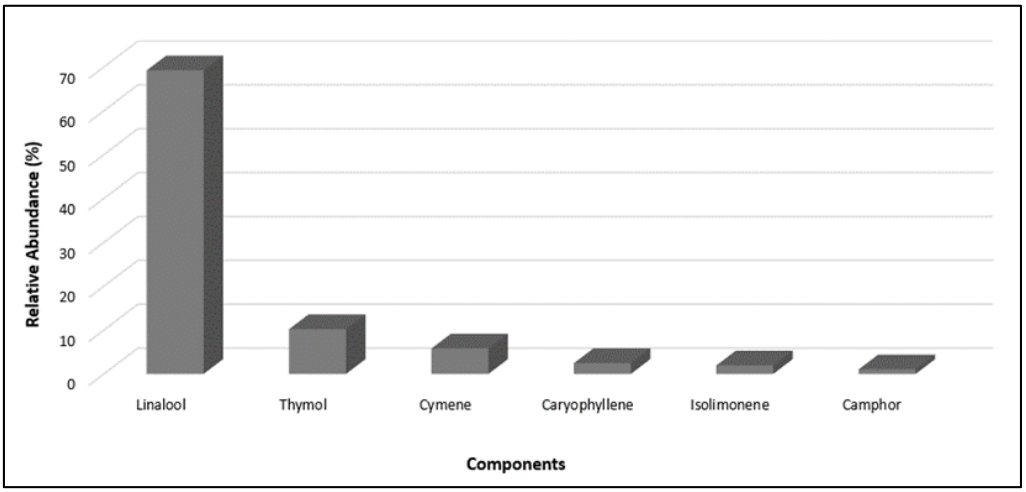

Figure 3 Graphical representation of the obtained major components of *Thymus algeriensis* essential oils

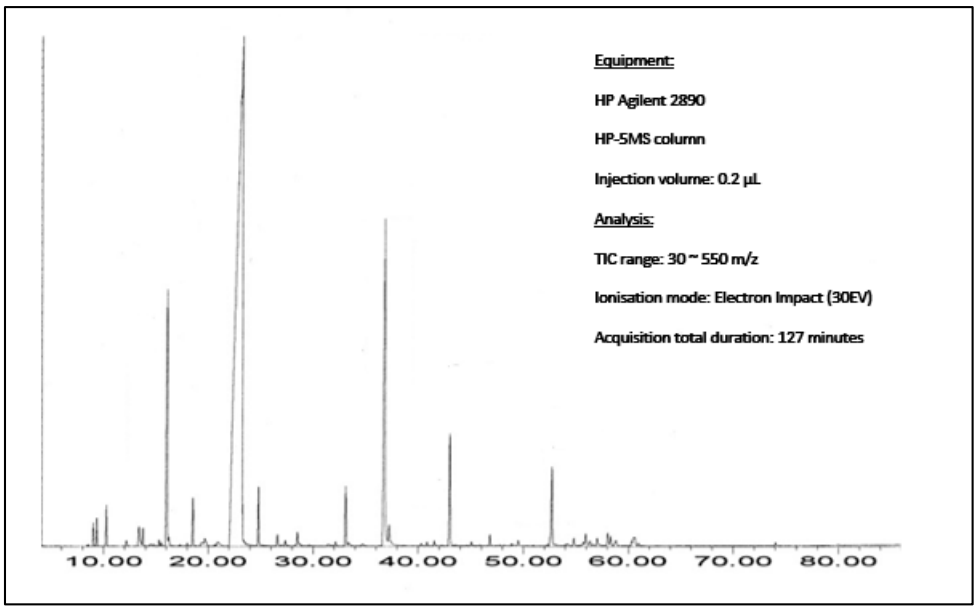

Figure 4 *Thymus algeriensis* essential oil analysis Total Ion Chromatogram (TIC). X-axis: Time in minutes. Y-axis: Signal. The detailed conditions are mentioned in the figure.

Based on the chemical analysis results, the current species is characterized by a Linalool chemotype (69.179%); result corresponding to Dob et al., 2006 chemical characterization of the Algerian north-eastern *Thymus algeriensis* variant (i.e., 43.3% Linalool), and unmatching the eastern Moroccan species characterized by a Camphor dominant chemotype (i.e., 27.7%) in the study of Armarti et al., 2009.(12,13) and western Moroccan species characterized by a chrysanthenone and camphor chemotype with 47.71% and 21.45% abundance proportions respectively.(14, 15) These observations clearly reflect the climatological and topological impacts on inter-species cellular metabolism variations and highlight the importance of chemotype determination before any use is made out of the plant extracts.

Table 3 List of the components and their relative abundances in the extracted essential oil. The identification was carried out via comparison to the NIST (National Institute of Standards and Technology) database. RT: Retention Time. AUC: Area Under the Curve. %: Percentage.

Peak	Chemical designation	RT	AUC	%
1	Bicyclohex-2-ene,2-methyl-5-(1methylethyl	9.049	13212719	0.389
2	2,6,6,-trimethylbicyclo hept-2-ene	9.374	15336771	0.452
3	Bicyclo heptane.2.2-dimethyl-3-methylene	10.288	24105269	0.71
4	Bicyclo hexane.4-methylene-1-(1-methylethyl)	12.202	4507874	0.133
5	1-octen-3-ol	13.374	21737542	0.64
6	1,6-octadiene,7-methyl-3-methylene	13.745	12092561	0.356
7	1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)	15.301	3801091	0.112
8	1-methyl-2-(1methylethyl)	16.062	196781130	5.796
9	Cyclohexene,1-methyl-4-(1methylethenyl)	16.208	8071079	0.238
10	1,4-cyclohexadiene,1-methyl-4-(1-methylethyl)	18.532	30732022	0.905
11	Alpha-(4-methyl-3-pentenyl)oriranemethanol	19.671	12363474	0.364
12	1,6-octadien-3-ol,3,7-dimethyl-Linalool	23.088	1762947146	51.924
13	1,6-octadien-3-ol,3,7-dimethyl	23.214	365840568	10.775
14	1,6-octadien-3-ol,3,7-dimethyl-Linalool	23.274	220008759	6.48
15	Bicyclo heptane-2-ol,1,7,7-trimethyl	24.81	35250896	1.038
16	Bicyclo heptane-2-ol, 1,7,7-trimethyl	26.598	8806689	0.259
17	3-cyclohexen-1-ol, 4-methyl-1-(1-methyethyl)	27.36	3924051	0.116
18	3-cyclohexene-1-methanol	28.512	8549073	0.252
19	Benzen,1-methoxy4-methyl-2-(1-methylethyl)	32.121	3268633	0.096
20	1,6-octadien-3-ol,3,7-dimethyl-2-aminobenzoate	33.088	41545571	1.224
21	Phenol,5-methyl-2-(1-methylethyl)	36.823	345482323	10.175
22	Phenol,5-methyl-2-(1-methylethyl)	37.24	24728728	0.728
23	2,6-octadien-1-ol,3,7-dimethyl-acetate	41.531	3868751	0.114
24	Bicyclo undec-4-ene,4,11,11-trimethyl-8-methylene	43.008	83011138	2.445
25	1-cyclopenta(1,3) cyclopropa(1,2)benzen	46.816	8773712	0.258
26	Naphthalene,1,2,3,5,6,8-hexahydro-4,7-dimethyl-1-(1-methylethyl)	49.517	3717484	0.109
27	5-oxatricyclo dodecane,12-trimethyl-9-methylene	52.742	65359766	1.925
28	Naphthalene,2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)	54.815	5830898	0.172
29	Tricyclodecane	55.947	11022585	0.325

30	1-naphthalene,1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)	57.067	5086917	0.15
31	Naphthalene,2,6-bis-(1-methylethyl)	58.066	11452577	0.337
32	Naphthalene,bis(1-methylethyl)	58.345	7257803	0.214
33	5,10-dihydro-6,7-dimethyl-4H-benzocyclohepta-furane	58.821	7143766	0.21
34	Naphthalene, 2,6-bis(1-methylethyl)	60.318	3386193	0.1
35	Naphthalene, 2,6-bis(1-methylethyl)	60.477	5970014	0.176
36	Naphthalene, 2,6-bis(1-methylethyl)	60.643	10292144	0.303

4. Conclusion

The present study, through the usage of GC-MS, determined the Algerian north-central *Thymus algeriensis* chemotype. This later is characterized by a predominant Linalool component (i.e., 69.179%), a reiterated trait among the African northern coastal variants. Similarly, the extraction yield of 1.77% highlighted the species potential for medical and commercial usages our data aims to direct in the future. Similar studies would benefit the establishment of an accurate chemical identification of the local close species and genus.

Compliance with ethical standards

Acknowledgements

We thank all members of the Pharmacognosy laboratory for their support in carrying out this work.

Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in connection with this document.

References

[1] Gosselet J (1832 1916). Elementary Course in Botany: Description of families and useful species. Plant anatomy and physiology. By M. J. Gosselet. 1878 [cited 2023 Mar 28]. Available from: https://gallica.bnf.fr/ark:/12148/bpt6k143843z

[2] Mallaoui Z, Bahlouli O saad. Valorization of the aromatic essence of thyme (*Thymus vulgaris L.*) and lavender (*Lavandula stoechas L.*) in anti-infective aromatherapy. [Internet] [Thesis]. 2017 [cited 2023 Mar 28]. Available from: https://di.univ-blida.dz/jspui/handle/123456789/6646

[3] Mebarki N. *Thymus fontanesii* essential oil extraction and application to the development of an antimicrobial drug form. [PhD Thesis]. Boumerdès; 2010.

[4] Boudjou, Touzani. Ethnobotanical survey of medicinal plants used to treat respiratory diseases in the region of El Bayadh (Algeria). Journal of Ethnopharmacology. 2021.

[5] Lee Y, Ding P. Physiological Production of Essential Oil in Plants - Ontogeny, secretory structures and seasonal variations: Review. 2016 Jan 1;2:1–11.

[6] Milenković L, Ilić ZS, Šunić L, Tmušić N, Stanojević L, Stanojević J, et al. Modification of light intensity influence essential oils content, composition and antioxidant activity of thyme, marjoram and oregano. Saudi Journal of Biological Sciences. 2021 Nov 1;28(11):6532–43.

[7] Mohammadian A, Karamian R, Mirza M, Sepahvand A. Effects of altitude and soil characteristics on essential of *Thymus fallax* Fisch.et C.A. Mey. in different habitats of Lorestan province. Iranian Journal of Medicinal and Aromatic Plants Research. 2014 Sep 23;30(4):519–28.

[8] Li Y, Craker LE, Potter T. EFFECT OF LIGHT LEVEL ON ESSENTIAL OIL PRODUCTION OF SAGE (*Salvia officinalis*) AND THYME (*Thymus vulgaris*). Acta Hortic. 1996 Aug;(426):419–26.

[9] Kryvtsova M, Hrytsyna M, Salamon I, Skybitska M, Novykevuch O. Chemotypes of Species of the Genus *Thymus L*. in Carpathians Region of Ukraine—Their Essential Oil Qualitative and Quantitative Characteristics and Antimicrobial Activity. Horticulturae. 2022 Dec;8(12):1218.

[10] Boukhalfa D, Nabti B, Boukhalfa D, Nabti B. Physicochemical, chromatographic and spectral analyzes of essential oils of Hoggar Myrtle of Tamanrasset in southern Algeria. GSC Biological and Pharmaceutical Sciences. 2023;22(2):128–35.

[11] Baytop ÖT. A few modifications to the Gazet Du Chatelier reagent. Materiae Vegetabiles. 1956 Jun 1;2(1):31–2.

[12] Dob T, Dahmane D, Benabdelkader T, Chelghoum C. Studies on the essential oil composition and antimicrobial activity of *Thymus algeriensis* Boiss. et Reut. International Journal of Aromatherapy. 2006 Jan 1;16(2):95–100.

[13] Amarti F, Satrani B, Ghanmi M, Farah A, Aafi A, Aarab L, et al. Chemical composition and antimicrobial activity of the essential oils of *Thymus algeriensis* (Boiss. & Reut.) and *Thymus ciliatus* (Desf.) (Benth.) from Morocco. Biotechnologie, Agronomie, Société et Environnement. 2010 Jan 1;14.

[14] El Ouahdani K, Es-safi I, Mechchate H, Al-zahrani M, Qurtam AA, Aleissa M, et al. *Thymus algeriensis* and *Artemisia herba-alba* Essential Oils: Chemical Analysis, Antioxidant Potential and In Vivo Anti-Inflammatory, Analgesic Activities, and Acute Toxicity. Molecules. 2021 Jan;26(22):6780.

[15] Nabti B, Cherifi W, Rabia B. Overview on the role of aromatherapy in therapeutic practices in Algeria. GSC Biological and Pharmaceutical Sciences. 2023; 22(03): 010-015. https://doi.org/10.30574/gscbps.2023.22.3.0092.