Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 953-965.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

BULL. PHARM. SCI. Assiut Univ.

CHEMICAL COMPOSITION OF ESSENTIAL OILS, OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION AND EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF *ANAGYRIS FOETIDA*

Hamza Aliboudhar[1*], Zineb Lakache[2], Daouia K. Benmaarouf[3], Nacéra Tigrine-Kordjani[1]

[1]*Laboratory of Functional Organic Analysis, Faculty of Chemistry, University of Sciences and Technology Houari Boumediene (USTHB), El-Alia, Bab-Ezzouar, Algiers, Algeria*
[2]*Laboratory of Ethnobotany and Natural Substances, Department of Natural Sciences, Ecole Normale Superieure (ENS), Kouba, Algiers, Algeria*
[3]*Animal Health and Production Research Laboratory, Ecole Nationale Supérieure Vétérinaire, Algiers, Algeria*

The experimental study on the secondary metabolites of Anagyris foetida is very limited. Driven by the need to identify and evaluate bioactive compounds in Anagyris foetida, this study investigates the extraction and optimization of essential oils and phenolic compounds from various plant parts using different techniques. The antioxidant and anti-inflammatory potentials of the extracts were subsequently evaluated. Essential oils extracted from the leaves and aerial parts by hydrodistillation and microwave-assisted hydrodistillation, and analyzed by gas chromatography coupled with mass spectrometry, revealed distinct compositions, with significant variations in the number and percentage of the 33 identified compounds between extraction methods. The optimization of phenolic compound extraction involved various methods and parameters to achieve high total phenolic and flavonoid contents. The highest levels were obtained through reflux heating in ethyl acetate at 60 °C for 4 hours. The extracts demonstrated moderate antioxidant activity, with IC50 values of 346.56 ± 33.67 µg/mL for the leaves and 287.14 ± 30.31 µg/mL for the stems. Anti-inflammatory activity was observed, with the leaves showing higher inhibition of paw edema compared to the stems across all tested doses. Notably, the concentration of 300 mg/mL yielded the highest reduction percentages: 38.92% for the leaves and 20.53% for the stems. This study highlights Anagyris foetida as a valuable source of bioactive compounds with promising pharmacological applications, which can explain some traditional uses

Keyword: *Anagyris foetida, essential oil, phenolic compounds, antioxidant activity, anti-inflammatory activity*

INTRODUCTION

Secondary metabolites play a protective role and offer various benefits for human health. These compounds, which can be utilized individually or in combination with others, are commonly employed in pharmaceuticals, dyes, perfumes, pigments, pesticides, and food additives. Among the most frequently encountered and utilized secondary metabolites are essential oils, phenolic compounds, and alkaloids. In recent years, there has been heightened interest in these compounds, with ongoing efforts to explore new sources and reduce reliance on synthetic agents that often lead to undesirable side effects. Consequently, plants remain a secure and natural reservoir of novel, safe, and effective bioactive compounds.

This paper investigates the potential health benefits of *Anagyris foetida*, a perennial shrub from the Fabaceae family native to the Mediterranean and Middle East. Characterized by trifoliate leaves and clusters of 3-10 yellow flowers with black markings, this plant thrives

Received : 11/5/2024 & Accepted : 27/6/2024

*Corresponding author: Hamza Aliboudhar, E-mail: : haliboudhar@yahoo.fr

in clay ravines, arid hillsides, dry and sunny locales, and mountainous regions[1-3].

Beyond its ecological role, *Anagyris foetida* holds promise for its nutritional value and medicinal properties due to the presence of secondary metabolites [4]. *Anagyris foetida* has been used in traditional medicine for centuries, with documented evidence of its use for various ailments [4-6]. Local knowledge suggests its leaves and seeds were employed as purgatives, emetics, laxatives, and vermifuges [7-11] to treat digestive issues like constipation, vomiting, and intestinal worms [12-13]. Additionally, historical accounts mention its use in respiratory care [14], eczema management, and renal diseases [7-8]. In some contexts, it was also used as an emmenagogue and abortifacient [15-16]. Notably, traditional practices even explored its potential role in diabetes management [17]. Recent research has provided scientific validation for some of these traditional uses. Studies have shown that extracts from *Anagyris foetida* can enhance the efficacy of amoxicillin against antibiotic-resistant bacteria [18-19]. Emerging evidence also suggests potential applications in cancer treatment [20]. However, it is crucial to exercise caution and avoid self-medication due to the presence of toxic compounds in *Anagyris foetida* [21-22].

Extracting secondary metabolites is an essential step for understanding a plant's chemical composition. This process is influenced by various factors, including temperature, solvent type, and extraction technique [23-24]. Essential oils, phenolic compounds and alkaloids known for their medicinal properties and diverse applications, are frequently targeted for extraction. Our team recently published a study on the extraction and identification of alkaloids from *Anagyris foetida*, revealing a diverse composition across leaves, stems, and seeds. Chromatographic analyzes allowed the identification of six alkaloids in the leaves and stems and five in the seeds, include sparteine, N-methylcytisine, cytisine, 5,6-dehydrolupanine, lupanine, and anagyrine. Notably, these samples exhibited promising antioxidant activity [25].

This study represents the first comprehensive investigation into the extraction, identification, and quantification of essential oils and phenolic compounds from different parts of *Anagyris foetida*. We further evaluate their antioxidant and anti-inflammatory activities. We employed hydrodistillation and microwave-assisted hydrodistillation for essential oil extraction from aerial parts and leaves, followed by analysis using gas chromatography coupled with flame ionization detection (GC-FID) and mass spectrometry (GC–MS). Optimization of phenolic compound extraction involved determining the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in leaves and stems under various conditions like solvent, time, agitation, and temperature. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess antioxidant activity, while the carrageenan-induced mouse paw edema inhibition method evaluated anti-inflammatory activity. This groundbreaking investigation aims to bridge the existing knowledge gap regarding the potential applications of *Anagyris foetida.*

MATERIALS AND METHODS

Plant material

Anagyris foetida was harvested in May 2019 at the fruiting stage from the region of Si Mustapha, located 55 kilometers east of Algiers (36°43′29″N, 3°36′55″E). Fresh plant material was directly used for essential oil extraction, while remaining samples were separated, dried in the dark at room temperature, and ground for further experimentation.

Extraction of essential oils

Microwave-Assisted Hydro Distillation Process (MAHD)

A total of 200 g of fresh plant material were placed in a flask containing 80 mL of water. The extraction was conducted at atmospheric pressure for an optimized duration of 30 minutes and at 800W power in a multimode reactor fixed at 2450 MHz. The flask was connected to a Clevenger-type apparatus located outside the microwave oven. After extraction, the essential oil was collected in a separating funnel. The resulting mixture underwent liquid-liquid extraction with diethyl ether. Subsequently, the diethyl ether was removed under a stream of nitrogen, and the essential oil was stored at 4°C until analysis. All experiments were performed in triplicate.

Hydro Distillation Process (HD)

A total of 200 g of fresh plant material was subjected to hydro distillation (HD) using a Clevenger-type apparatus with 2 L of water for

an optimized duration of 3 hours. The essential oil was collected following the same procedure as described for the MAHD process. All experiments were conducted in triplicate.

Chromatographic analyses

Gas Chromatography-Flame Ionization Detector (GC-FID) analysis

A GC-FID system equipped with a fused-silica-capillary column containing a non-polar stationary phase HP5MS (30m x 0.25mm x 0.25 µm film thickness) was employed for the GC analysis. The column temperature program was initiated at 60°C for 8 minutes, followed by an increase at a rate of 4°C/min to 250°C, and maintained at 250°C for 25 minutes. A volume of 0.2 mL was injected into the split-less GC inlet held at 250°C. Nitrogen served as the carrier gas, flowing through the column at a rate of 1.5 mL/min. The temperature of the FID was set at 320°C.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The essential oils were additionally analyzed by GC–MS using 7890/5975 MSD Agilent system equipped with the same column (HP-5MS). The GC–MS operation involved helium as the carrier gas at a flow rate of 1.5 mL/min in split-less mode. An injected volume of 0.2 mL was used at an injection temperature of 250°C. The oven temperature program starting at 60°C for 8 minutes, increasing at a rate of 4°C/min to 250°C, and holding at 250°C for 25 minutes. The ionization mode utilized was electronic impact at 70 eV, operating in scan mode with a solvent delay of 3 minutes. The identification was established by comparison of the mass spectral fragmentation patterns with those stored in the database NIST 2014 and Wiley 7. The retention indices of the volatile extract constituents compared with those of the published index data [26] confirmed the identification.

Retention index calculation

The homologous n-alkane series C_6–C_{28}, injected in GC and GC–MS under the same conditions as the essential oils, were employed to calculate the retention indices. Relative amounts of individual components were based on peak areas obtained without FID response factor correction. Three replicates were conducted for each sample, and the average of these three values, along with the standard deviation, were determined for each identified component.

Optimization of extraction of phenolic compounds

Extraction of phenolic compounds

Extraction is the most important step in the chemical isolation of compounds, a family of compounds or a mixture of compounds. Its purpose is to maximize the content of target compounds in the extract. In this context, several experiments were carried out to optimize the operating conditions for extracting phenolic compounds from stems and leaves of *Anagyris foetida* under the effect of several factors: solvent, time, magnetic stirring and temperature.

Extraction solvent

Difference of phenolic compounds structures that result in different solubility properties of those compounds led us to use different solvents with decreasing polarity: ethanol, ethyl acetate and chloroform. The extraction was performed by immersing 5g of the plant materials in 50 mL of each solvent for 6 h at room temperature. After filtration and concentration, solutions of 1mg/ml of the obtained extract were prepared in ethanol and the total phenolic compounds (TPC) and total flavonoid contents (TFC) were determined.

Optimization of extraction conditions

After determining the optimum extraction solvent, a series of experiments was done to optimize the extraction time, extraction temperature and the extraction method.

Extraction time

Ten g of powdered each plant material was immerged in 100mL of ethanol at room temperature at different extraction times (1h, 2h, 3h, 4h and 6h). The final extract filtered and the solvent was removed under vacuum and the obtained extracts were stored until use.

Magnetic stirring

After determining the optimal extraction time, an experiment was performed to study the effect of magnetic stirring on TPC and TFC. For this, 10 g of each sample were immersed in the optimal solvent for 4 h an at room temperature. After filtration and concentration, the extracts were stored until use.

Extraction temperature

Plant material (10 g) was heated (heating reflux) with 100mL of ethyl acetate for 4h at different temperatures: 25, 40, 50, 60 and 80°C. After refluxing, solutions were filtered and concentered and the final extracts were stored until use. Subsequently, TPC and TFC were determined.

Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC was determined using the Folin–Ciocalteu reagent method [27]. Briefly, 0.2 mL of the extract was mixed with 0.8 mL of distilled water and 0.1 mL of Folin–Ciocalteu reagent. The mixture was incubated at room temperature for 3 minutes. Subsequently, 0.3 mL of Na_2CO_3 solution (20%) was added, and the mixture was further incubated at room temperature for 120 minutes. The absorbance of the mixture was measured at 765 nm. TPC was expressed as mg of gallic acid equivalents (GAE) per gram of dry matter. The standard curve equation of gallic acid was $y = 0.0082x + 0.0971$ ($R^2 = 0.9959$).

TFC was determined using the aluminum chloride colorimetric method [28]. One milliliter of each extract was mixed with 1 mL of 2% aluminum trichloride ($AlCl_3$). After incubation at room temperature for 10 minutes, the absorbance was measured at 440 nm. TFC was expressed as mg of quercetin equivalents (QE) per gram of dry matter. The standard curve equation of quercetin was $y = 0.0438x – 0.0296$ ($R^2 = 0.9964$).

Antioxidant Activity: Scavenging Activity of DPPH

Antioxidant activity was evaluated on the extracts of stems and leaves exhibiting the highest values of TPC and TFC. The DPPH assay was performed according to the method described by Burits & Bucar [29]. Specifically, 975 mL of 60 mM DPPH was mixed with 25 mL of the sample solution. The standard solution was stored separately in the dark for 30 minutes, and the optical density was measured at 517 nm. Synthetic antioxidant butylhydroxytoluene (BHT) was used as the standard solution. The percentage of inhibition of DPPH radical was calculated, and the IC50 (concentration at which the antioxidant activity inhibition ratio reached 50%) was determined.

Anti-inflammatory activity

The anti-inflammatory activity was assessed by measuring the inhibition of carrageenan-induced mouse paw edema [30]. Prior to the experiment, animals were fasted for 12 hours and weighed. Male albino mice, with an average weight of 25-28g, were randomly and equally divided into ten groups of four mice each. The initial thickness of each mouse's hind paw was measured using a digital caliper before the oral administration of the ethyl acetate extract of the tested samples (leaves and stems). The mice were treated as follows:

- Control group: received a solution of physiological saline
- Reference group: received an aspirin solution at a concentration of 100 mg/kg
- Treatment group 1: received the leaves extract solution at a concentration of 100 mg/kg
- Treatment group 2: received the leaves extract solution at a concentration of 200 mg/kg
- Treatment group 3: received the leaves extract solution at a concentration of 300 mg/kg.
- Treatment group 4: received the leaves extract solution at a concentration of 400 mg/kg.
- Treatment group 1': received the stems extract solution at a concentration of 100 mg/kg
- Treatment group 2': received the stems extract solution at a concentration of 200 mg/kg
- Treatment group 3': received the stems extract solution at a concentration of 300 mg/kg.
- Treatment group 4': received the stems extract solution at a concentration of 400 mg/kg.

After one hour of treatment administration, mice received a subcutaneous injection of 5 µL of a 1% carrageenan solution into the aponeurosis of the left hind paw. Paw thickness was then measured every hour for four hours. The percentage reduction of edema was calculated using the formula:

$$\%\ \text{Reduction of edema} = \frac{(\Delta T - \Delta E)}{\Delta T} \times 100$$

Where:

ΔT: the difference between the average thickness of the hind paws (right - left) for the control group (physiological saline solution 0.9% NaCl).

ΔE: the difference between the average thickness of the hind paws (right - left) for the test group (methanolic extract or control +).

The experimental tests were in accordance with the Guide for the Care and Use of Laboratory Animals [31].

Statistical analysis

Experimental results represented as mean ± standard error (SE) were subjected to statistical analysis performed by using one-way analysis of variance (ANOVA in XLSTAT software on Microsoft Excel 2010; differences at $p<0.05$ were considered statistically significant).

RESULTS AND DISCUSSION

Results

Essential oils

Extraction yield

The extraction yield was determined based on the dry plant material after accounting for sample moisture content, which was found to be 66% for leaves and 60% for the aerial part (leaves + stems). The extraction yields were approximately 0.026% and 0.020% for the leaves sample and 0.031% and 0.029% for the aerial part sample obtained via the HD and MAHD processes, respectively. The obtained extraction yields indicate that the extraction processes, both HD and MAHD, were effective in extracting essential oils from *Anagyris foetida*. These yields suggest that the plant material contains relatively low concentrations of essential oils compared to the total dry weight of the plant.

Quantitative and qualitative analysis of essential oil

This study investigated the chemical composition of essential oils derived from *Anagyris foetida* aerial parts and leaves using Hydrodistillation (HD) and Microwave-Assisted Hydrodistillation (MAHD) techniques. Both methods identified a diverse array of secondary metabolites belonging to various chemical families: alcohols, aldehydes, ketones, esters, alkanes, acids, phenols, and terpenoids.

Table 1 presents the qualitative and semi-quantitative chemical composition of the essential oils obtained. A total of 33 compounds representing 95.8 ± 2.1% and 30 compounds representing 91.0 ± 3.2% were identified in the aerial part samples using HD and MAHD, respectively. For the leaves, 32 and 29 compounds representing over 90% of the oil composition were identified using HD and MAHD, respectively.

A significant difference was observed in the chemical composition of the essential oils from both samples obtained by the different processes, despite the common major families and constituents. The major compounds in the aerial part samples included 2-hexen-1-ol (E) (18.8 ± 2.0 for HD vs. 11.8 ± 2.1 for MAHD), heptadecane (6.7 ± 0.8 for HD vs. 17.0 ± 1.4 for MAHD), phytol (16.2 ± 1.7 for HD vs. 7.0 ± 1.0 for MAHD), 3-hexen-1-ol (Z) (12.4 ± 1.1 for HD vs. 8.2 ± 1.2 for MAHD), phenol, 3-(1,1-dimethylethyl)-4-methoxy (4.7 ± 0.8 for HD vs. 9.0 ± 1.5 for MAHD), 2-propanol, 1-(1-methylethoxy) (3.9 ± 0.8 for HD vs. 5.1 ± 0.8 for MAHD), and oxacycloheptadecan-2-one (3.9 ± 0.8 for HD vs. 9.9 ± 1.1 for MAHD).

In the leaves samples, the major compounds were phytol (13.7 ± 1.9 for HD vs. 7.3 ± 1.1 for MAHD), 3-hexen-1-ol (Z) (12.2 ± 2.1 for HD vs. 3.2 ± 0.6 for MAHD), heptadecane (9.6 ± 1.3 for HD vs. 17.8 ± 1.3 for MAHD), phenol, 3-(1,1-dimethylethyl)-4-methoxy (5.3 ± 0.8 for HD vs. 8.6 ± 0.9 for MAHD), oxacycloheptadecan-2-one (4.6 ± 0.9 for HD vs. 11.9 ± 1.5 for MAHD), and 2-propanol, 1-(1-methylethoxy) (3.0 ± 0.6 for HD vs. 4.5 ± 0.7 for MAHD). Moreover, in the case of the leaves essential oils, certain compounds presented a high amount only in one extract, such as 2-hexen-1-ol (E) (0.3 ± 0.2 for HD vs. 9.1 ± 0.9 for MAHD), α-terpineol (9.3 ± 1.1 for HD vs. 1.8 ± 0.6 for MAHD), and 3-hexen-1-ol, benzoate (Z) (5.0 ± 0.8 for HD vs. 1.6 ± 0.5 for MAHD).

Furthermore, some compounds were extracted only by the HD process, such as 2-undecanone (0.7 ± 0.2 in the aerial part and 0.6 ± 0.2 in leaves), cis-jasmone (0.3 ± 0.1 in the aerial part and 1.3 ± 0.4 in leaves), and isophytol with a considerable percentage (3.9 ± 0.9 in the aerial part and 4.7 ± 0.9 in leaves). Additionally, one compound, 6,10,14-trimethyl-2-pentadecanone (0.4 ± 0.1 for HD vs. 0.3 ± 0.1 for MAHD), was detected only in the aerial part, indicating it is produced in the stems.

Table 1: Chemical composition of essential oils from aerial part and leaves of *Anagyris foetida* obtained by hydro-distillation (HD) and microwave-assisted hydro-distillation (MAHD) techniques.

N°	Compound	RI [a]	RI [b]	Aerial Part		Leaves	
				HD % [c]	MAHD % [c]	HD % [c]	MAHD% [c]
	Alcool						
1	2-Propanol, 1-(1-methylethoxy)	813	814	3.9 ± 0.8	5.1 ± 0.8	3.0 ± 0.6	4.5 ± 0.7
2	2-Hexen-1-ol (E)	851	854	18.8 ± 2.0	11.8 ± 2.1	0.3 ± 0.2	9.1 ± 0.9
3	3-Hexen-1-ol (Z)	863	864	12.8 ± 1.1	8.2 ± 1.2	12.2 ± 2.1	3.2 ± 0.6
4	Linalool	1101	1101	0.9 ± 0.3	1.5 ± 0.3	1.78 ± 0.5	1.5 ± 0.3
5	α-Terpineol	1193	1191	1.8 ± 0.4	1.3 ± 0.3	9.3 ± 1.1	1.8 ± 0.6
6	4-Terpineol	1169	1170	0.4 ± 0.2	1.1 ± 0.6	0.8 ± 0.3	3.5 ± 0.8
7	3-Hexen-1-ol, benzoate, (Z)	1574	1573	1.4 ± 0.4	0.7 ± 0.3	5.0 ± 0.8	1.6 ± 0.5
8	Phytol	2121	2122	16.2 ± 1.7	7.0 ± 1.0	13.7 ± 1.9	7.4 ± 1.1
9	1-Docosanol	2439	2456	3.2 ± 0.6	2.3 ± 0.6	1.3 ± 0.4	3.5 ± 0.7
	Aldehydes						
10	Nonanal	1105	1105	1.6 ± 0.5	2.3 ± 0.5	2.0 ± 0.6	1.5 ± 0.55
11	Hexadecanal	1844	1844	1.2 ± 0.3	0.3 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
12	Octadecanal	2034	2033	0.7 ± 0.2	1.2 ± 0.3	0.5 ± 0.2	0.8 ± 0.4
13	Tetracosanal	2630	2632	1.4 ± 0.44	1.7 ± 0.6	1.4 ± 0.6	1.4 ± 0.5
	Ketones						
14	2-Undecanone	1288	1288	0.7 ± 0.25	-	0.6 ± 0.2	-
15	6,10, 14-trimethyl-2-pentadecanone	1847	1848	0.4 ± 0.1	0.3 ± 0.1	-	-
16	Oxacycloheptadecan-2-one	1939	1932	3.9 ± 0.8	9.7 ± 1.1	4.6 ± 0.9	11.9 ± 1.5
	Esters						
17	3-Hexen-1-ol, acetate (Z)	1010	1008	1.0 ± 0.3	0.4 ± 0,2	1.3 ± 0.5	1.5 ± 0.4
18	Hexadecanoic acid, methyl ester	1931	1933	0.5 ± 0.2	0.8 ± 0.3	0.6 ± 0.2	0.5 ± 0.2
	Alkanes						
19	2,6-Dimethyldecane	1120	1121	1.3 ± 0.3	2.0 ± 0.4	1.1 ± 0.3	1.9 ± 0.6
20	Hexadecane	1604	1600	0.3 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.3
21	Heptadecane	1703	1700	6.7 ± 0.8	17.0 ± 1.4	9.6 ± 1.3	17.8 ± 1.3
22	Octadecane	1805	1800	0.3 ± 0.1	0.7 ± 0.3	0.5 ± 0.1	0.7 ± 0.3
23	Heneicosane	2101	2100	0.9 ± 0.3	0.7 ± 0.3	0.7 ± 0.2	0.6 ± 0.3
24	Docosane	2202	2200	0.4 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	0.4 ± 0.2
25	Tricosane	2304	2300	1.8 ± 0.4	1.7 ± 0.5	1.3 ± 0.6	1.6 ± 0.7
26	Tetracosane	2404	2400	0.6 ± 0.2	0.4 ± 0.2	0.9 ± 0.3	0.6 ± 0.3
	Acids						
27	Oleic acid	2146	2147	1.0 ± 0.4	1.2 ± 0.4	1.1 ± 0.5	0.8 ± 0.2
	Phenols						
28	Phenol, 3-(1,1-dimethylethyl) - 4-methoxy	1480	1490	4.7 ± 0.8	9.0 ± 1.5	5.3 ± 0.8	8.6 ± 0.9
	Terpenoids						
29	β-Cyclocitral	1221	1220	1.7 ± 0.43	0.5 ± 0.1	0.6 ± 0.3	0.9 ± 0.2
30	β-Damascenone	1384	1384	1.7 ± 0.5	0.8 ± 0.3	3.8 ± 0.9	1.2 ± 0.5
31	cis-Jasmone	1405	1405	0.3 ± 0.1	-	1.3 ± 0.4	-
32	β-Ionone	1482	1482	0.2 ± 0.1	0.3 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
33	Isophytol	1944	1943	3.9 ± 0.9	-	4.7 ± 0.9	-
Total identified (%)				**95.8 ± 2.1**	**91.0 ± 3.2**	**92.1 ± 1.9**	**90.7 ± 2.9**

Percentage values are expressed as the mean ± standard deviation (n = 3).
[a] Retention indices given in the literature (NIST on non-polar HP5MS^{TM} or DB5 capillary column).
[b] Retention indices with respect to C5–C28 n-alkanes calculated on non-polar HP5MSTM capillary column.
[c] Percentage calculated by GC-FID on non-polar HP5MS^{TM} capillary column.

The results indicate that the essential oils from *Anagyris foetida* are predominantly composed of oxygenated compounds, including alcohols, aldehydes, ketones, ethers, oxides, and esters. These oxygenated compounds accounted for 83.8% and 67.4% in the aerial parts, and 76.9% and 66.4% in the leaves, obtained through Hydrodistillation (HD) and Microwave-Assisted Hydrodistillation (MAHD), respectively. The remaining components were mainly alkanes. The significant differences in extraction yields and the qualitative and semi-quantitative chemical composition of the essential oils can be attributed to the varying extraction mechanisms and heating modes of the methods used. These findings underscore the importance of selecting appropriate extraction techniques to achieve the desired essential oil profiles.

Alcohols emerged as the most abundant class of compounds, with 2-hexen-1-ol (E) and phytol being the most prominent across all samples. Additionally, notable amounts of aldehydes (nonanal), ketones (oxacycloheptadecan-2-one), esters (3-hexen-1-ol, acetate (Z)), and terpenoids (isophytol, β-damascenone) were detected. The MAHD method, in particular, yielded higher concentrations of ketones and alkanes, such as heptadecane, compared to HD.

The variation in the qualitative and quantitative composition of the essential oils highlights the significant impact of the extraction method (HD vs. MAHD) on the quality of the extracted oils. Overall, these results emphasize the importance of selecting appropriate extraction techniques to obtain the desired essential oils [32].

The study revealed notable differences in the composition of essential oils obtained from the aerial parts and leaves, despite sharing the same major chemical families. In the aerial parts, key compounds included 2-hexen-1-ol (E), phytol, heptadecane, and oxacycloheptadecan-2-one, with concentrations varying based on the extraction method. The leaves exhibited similar trends with phytol, heptadecane, and oxacycloheptadecan-2-one being prominent, but also showed higher levels of specific compounds like α-terpineol and 3-hexen-1-ol, benzoate (Z) when extracted primarily by MAHD. Notably, some compounds, such as isophytol and cis-jasmone, were exclusively obtained using HD.

The rich diversity of identified compounds suggests potential applications for these essential oils in various fields. The presence of specific metabolites aligns with the traditional uses of *Anagyris foetida* and hints at its potential for pharmacological applications, particularly related to its antioxidant, antimicrobial, and therapeutic properties. Further research is needed to explore these possibilities and fully realize the potential of these essential oils.

Optimization of extraction of phenolic compounds

Optimal solvent

To extract the maximum amount of phenolic compounds present in the leaves and stems of *Anagyris foetida*, three solvents of increasing polarity were selected: chloroform, ethyl acetate and ethanol. Quantitatively, *Anagyris foetida* can be considered rich in polyphenols and flavonoids. The results indicate that leaves contain more phenolic compounds than stems across all obtained extracts. The total polyphenol and flavonoid contents vary depending on the solvent used. As shown in **Fig. 1**, ethyl acetate proved to be the most efficient solvent for extracting these secondary metabolites from the plant under study, exhibiting a notable difference in the amounts of TPC and TFC. The highest reported values of TPC are 124.8 ± 6.9 and 95.5 ± 4.7 mg GAE/g, and those of TFC are 60.9 ± 5.8 and 17.2 ± 3.2 mg EQ/g in leaf and stem samples, respectively. The other solvents yielded lower values, with a slight preference observed for chloroform.

Optimization of extraction conditions

Optimization of extraction conditions for phenolic compounds from the leaves and stems of *Anagyris foetida* involved a series of experiments focusing on two essential factors: time and temperature, in addition to the use of magnetic stirring, subsequent to determining the optimal extraction solvent. Initially, extractions were conducted at room temperature over varying durations ranging from 1 to 6 hours to assess the impact of extraction time on the yields of TPC and TFC. The results revealed a gradual increase in TPC and TFC with extraction time until reaching peak levels, followed by a slight decline. The most favorable contents were observed at 4 hours, with TPC values of 128.4 ± 7.4 and 97.9

± 6.7 mg GAE/g, and TFC values of 61.8 ± 5.3 and 17.7 ± 3.3 mg QE/g in the leaves and stems, respectively **(Fig. 2).**

Furthermore, the effect of magnetic stirring was examined, leading to a decrease in the TPC both samples, while the TFC showed improvement **(Table 2)**. Notably, the decline in polyphenol levels was significant, dropping from 128.4 ± 7.4 to 83.2 ± 6.6 mg GAE/g in leaves and from 97.9 ± 6.7 to 77.7 ± 5.2 mg GAE/g in stems. This reduction may be attributed to the breakdown or degradation of certain molecules during magnetic stirring. On the other hand, the flavonoid levels experienced a slight enhancement, from 61.8 ± 5.3 to 69.3 ± 4.4 mg QE/g in leaves and from 17.7 ± 3.3 to 19.6 ± 3.9 mg QE/g in stems.

In general, extraction by maceration is more advantageous than magnetic stirring in the case of the *Anagyris foetida*. Therefore, conventional maceration in ethyl acetate and for an extraction time of 4h were chosen as optimum conditions for subsequent experiments.

Subsequently, the effect of extraction temperature was investigated, with extractions conducted by reflux heating in ethyl acetate for 4 hours and at different temperatures: 40, 50, 60 and 80 ° C, also compared with the result obtained at room temperature (25 °C).

As illustrated in **Fig. 2** (c and d), the TPC and TFC increase significantly with temperature between 25 to 50 °C, while there was no noticeable difference between 50 and 60 ° C, a remarkable decrease was observed from 60 to 80 °C. Moderate heating facilitated the rapid and efficient release of biomolecules; however, higher temperatures led to decreased TPC and TFC contents, possibly due to the degradation of thermo-sensitive compounds. This observation was noticed with the two studied samples.

Conclusively, 60 °C was identified as the optimal temperature for phenolic compound extraction, resulting in TPC values of 192.6 ± 8.4 and 143.8 ± 6.3 mg GAE/g, and TFC values of 87.7 ± 5.8 and 26.5 ± 5.3 mg QE/g extracted from leaves and stems, respectively. The study also revealed a positive and linear correlation between the extraction of polyphenols (TPC) and flavonoids (TFC), and they present the same graphic appearance, therefore the same extraction mechanism. This correlation was consistent across both parts of the plant, leaves, and stems, during investigations into the influence of extraction time and temperature. This result is reflected by the fact that the content of flavonoids varies proportionally with the content of phenolic compounds.

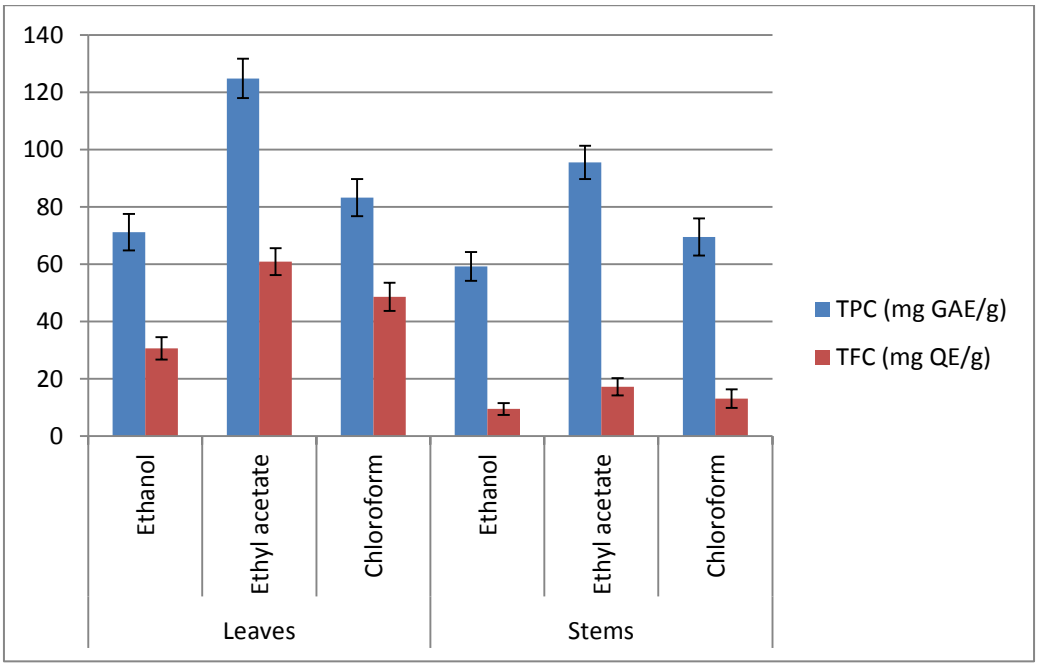

Fig. 1: Influence of various solvents on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) from leaves and stems of *Anagyris foetida.*

Table 2: Influence of the magnetic stirring on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of leaves and stems of *Anagyris foetida.*

	Leaves		Stems	
	Without magnetic stirring	With magnetic stirring	Without magnetic stirring	With magnetic stirring
TPC (mg GAE/g)	128.4 ± 7.4	83.2 ± 6.6	97.9 ± 6.7	77.8 ± 5.2
TFC (mg QE/g)	61.8 ± 5.3	69.3 ± 4.4	17.7 ± 3.3	19.6 ± 3.9

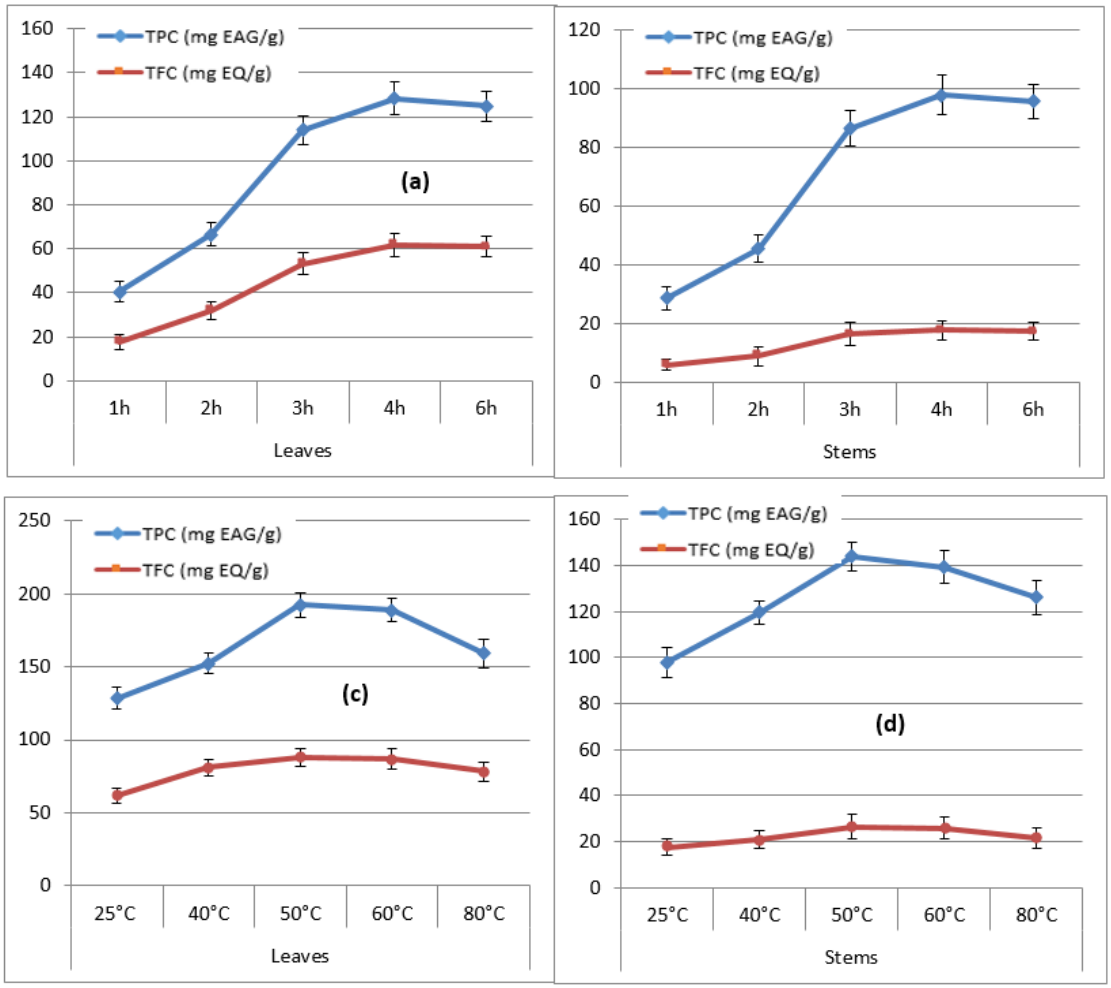

Fig. 2: Influence of extraction time (a and b) and extraction temperature (c and d) on Total Phenolic Content (TPC) (mg GAE/g) and Total Flavonoid Content (TFC) (mg QE/g) of leaves and stems of *Anagyris foetida*.

Antioxidant activity

Antioxidant activity was assessed in this study using the DPPH radical scavenging test to evaluate the extracts from leaves and stems of *Anagyris foetida*. This method is indicative of the ability of antioxidants to inhibit lipid oxidation and prevent oxidative damage [33]. The tested extracts exhibited scavenging activity against the DPPH radical, with IC50 values of 346.6 ± 33.7 µg/mL and 287.1 ± 30.3 µg/mL in leaves and stems, respectively. Although these values are higher than that of the BHT standard (IC50 = 18.1 ± 1.1 µg/mL), indicating a lower yet moderate activity, *Anagyris foetida* emerges as a potential source of natural compounds with significant antioxidant properties.

Usually, antioxidant activity is attributed to the presence of phenolic compounds. However, in this case, despite the lower levels of TPC and TFC in the stems extract, it exhibited superior activity. This discrepancy underscores the influence of the distinct

chemical compositions of the extracts. It suggests that the stems may contain one or more compounds with more potent antioxidant properties, which may be absent or present at lower levels in the leaves.

Anti-inflammatory activity

The acute inflammation induced by carrageenan injection in mice serves as a standard model for evaluating the anti-inflammatory properties of natural agents[34]. In our study, we investigated the anti-inflammatory activity of ethyl acetate extracts from the leaves and stems of *Anagyris foetida*. The results are depicted in **Fig. 3**, showing the kinetics of edema reduction in the left hind paws of mice across various tests, including a positive control and ethyl acetate extracts, compared to a negative control (physiological saline solution). Following carrageenan injection, visible inflammation was observed across all groups, gradually diminishing over the four-hour experimental period, with the negative control exhibiting the highest swelling thickness.

A remarquable difference was observed between the leaves and stems samples. Leaves exhibited significantly higher percentages of inflammation inhibition compared to the roots across all tested doses. Notably, the concentration of 300 mg/mL demonstrated the highest reduction percentages, measuring 38.92 ± 3.09 and 20.53 ± 2.72 mg/mL, respectively.

We also identified a positive correlation between the contents of polyphenols and flavonoids in the leaves and stems with the observed anti-inflammatory activity. Elevated total polyphenol and flavonoid content corresponded to stronger anti-inflammatory effects, potentially due to the presence of bioactive compounds known for their anti-inflammatory properties.

Interestingly, there was a notable difference in the anti-inflammatory effect between the leaves and stems extracts. The leaves extract demonstrated a more pronounced reduction in paw edema compared to the stems extract, suggesting that the leaves of *Anagyris foetida* may contain higher concentrations of bioactive compounds with anti-inflammatory properties compared to the stems.

Furthermore, the concentration of the extract also played a significant role in determining its anti-inflammatory efficacy. Higher concentrations of the extract resulted in greater reduction percentages of paw edema, indicating a dose-dependent effect. A significant increase was observed from the 100 mg/kg to 200 mg/kg concentrations, and then to 300 mg/kg. However, at a concentration of 400 mg/kg, lower reduction percentages were noted, possibly due to receptor saturation or potential toxicity at higher concentrations.

Overall, these results suggest that both leaves and stems extracts of *Anagyris foetida* possess anti-inflammatory properties, with the leaves extract exhibiting a more potent effect. Further studies are warranted to elucidate the specific bioactive compounds responsible for the observed anti-inflammatory activity and to explore their potential mechanisms of action.

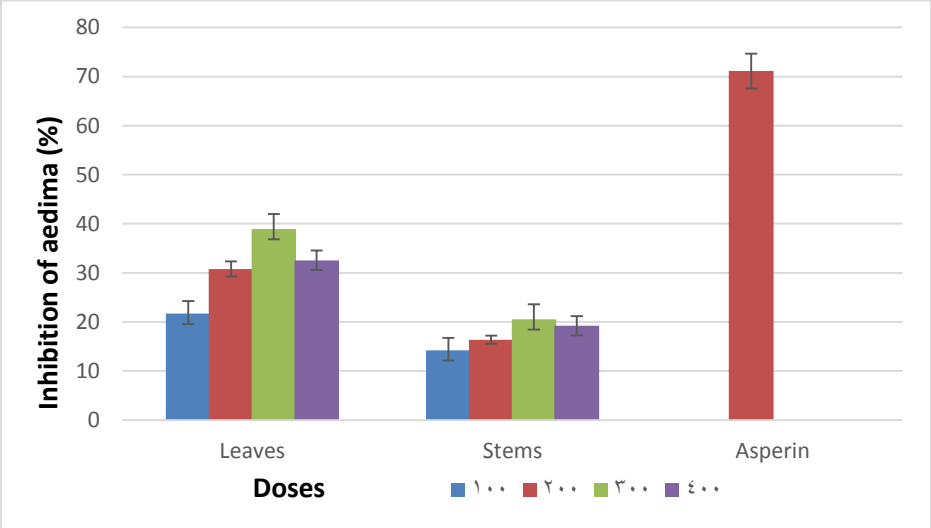

Fig. 3: Anti-inflammatory effect of leaves and stems of *Anagyris foetida* in carrageenan-induced paw edema.

Conclusion

This study provides the first comprehensive investigation into the essential oils and phenolic compounds of *Anagyris foetida*, analyzing extracts from different plant parts and evaluating their potential health benefits. The essential oil profiles from both aerial parts and leaves, obtained using HD and HDMO techniques, displayed significant variations in composition, highlighting the diverse range of oxygenated compounds present. These compositional differences suggest the oils may have diverse qualities and applications.

Optimization of the extraction process for phenolic compounds revealed that refluxing ground leaves and stems in ethyl acetate at 60°C for 4 hours yielded the highest content of phenolics and flavonoids. Furthermore, extracts exhibited moderate antioxidant activity against the DPPH radical, suggesting *Anagyris foetida* as a potential source of natural antioxidants.

The investigation into anti-inflammatory activity using a carrageenan-induced inflammation model in mice showed promising results, particularly for leaf extracts. Notably, leaves exhibited a higher reduction in inflammation compared to stems at all tested doses. These findings warrant further research to explore the complete pharmacological potential of this plant and its bioactive compounds. While this study provides valuable insights, future research could explore in vivo models and the mechanisms behind the observed activities.

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Bull. Pharm. Sci., Assiut University, Vol. 47, Issue 2, 2024, pp. 953-965.

نشرة العلوم الصيدلية
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التركيب الكيميائي للزيوت الأساسية، تحسين استخراج المركبات الفينولية، وتقييم الخصائص المضادة للأكسدة والمضادة للالتهابات لنبات ANAGYRIS FOETIDA

حمزة علي بوظهر[1*] – زينب لكاش[2] – ضاوية ك. بن معروف[3] – نصيرة تيغرين – كرجاني[1]

[1]مختبر تحليل العضوية الوظيفي، كلية الكيمياء، جامعة العلوم والتكنولوجيا هواري بومدين، العلية، باب الزوار، الجزائر العاصمة، الجزائر

[2]مختبر الإثنوبوتاني والمواد الطبيعية، قسم العلوم الطبيعية، المدرسة العليا للأساتذة، القبة، الجزائر العاصمة، الجزائر

[3]مختبر الصحة الحيوانية وإنتاج الحيوانات، المدرسة الوطنية العليا للطب البيطري، الجزائر العاصمة، الجزائر

الدراسة التجريبية حول المستقلبات الثانوية لنبات أناغيريس فويتيدا محدودة جدًا. بناءً على الحاجة إلى تحديد وتقييم المركبات النشطة بيولوجيًا لهذا النبات، تركز هذه الدراسة على استخراج الزيوت الأساسية والمركبات الفينولية من أجزاء مختلفة باستخدام تقنيات متنوعة لتحسين شروط الاستخراج. بعد ذلك، تم تقييم القدرات المضادة للأكسدة والمضادة للالتهابات لمستخلصاته. كشفت الزيوت الأساسية المستخرجة من الأوراق والأجزاء الهوائية بواسطة التقطير المائي والتقطير المائي بمساعدة الميكروويف، والمحللة بواسطة كروماتوجرافيا الغاز المقترنة بمطياف الكتلة، عن تركيبات مميزة، مع تحديد ٣٣ مركبًا أظهرت اختلافات ملحوظة في العدد والنسبة بين طرق الاستخراج. تضمنت عملية تحسين استخراج المركبات الفينولية استخدام طرق ومعايير متنوعة لتحقيق محتويات عالية من الفينولات الكلية والفلافونويدات. تم الحصول على أعلى المستويات من خلال التسخين بالانعكاس في خلات الإيثيل عند درجة حرارة ٦٠ درجة مئوية لمدة ٤ ساعات. أظهرت المستخلصات نشاطًا مضادًا للأكسدة معتدلًا، حيث كانت قيم IC50 هي ٣٤٦.٥٦ ± ٣٣.٦٧ ميكروغرام/مل للأوراق و٢٨٧.١٤ ± ٣٠.٣١ ميكروغرام/مل للأجزاء الهوائية. لوحظ نشاط مضاد للالتهابات، حيث أظهرت الأوراق تثبيطًا أعلى لوذمة القدم مقارنة بالأجزاء الهوائية عبر جميع الجرعات المختبرة. بشكل ملحوظ، أظهر تركيز ٣٠٠ ميكروغرام/مل أعلى نسب تقليل: ٣٨.٩٢% للأوراق و٢٠.٥٣% للأجزاء الهوائية. تسلط هذه الدراسة الضوء على نبات أناغيريس فويتيدا كمصدر قيم للمركبات النشطة بيولوجيًا ذات التطبيقات الدوائية الواعدة، مما يمكن أن يفسر بعض الاستخدامات التقليدية.