

Extraction of oxygenated terpenes from potamogeton species that growing in Tigris river within Mosul City.

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Abstract

In the current study, a continuous soxhlet apparatus with a series of different polar solvent used for the preparation of crude extracts from the shoot of five species belonging to the genus Potamogeton are *P. crispus*, *P. perfoliatus*, *P. pectinatus*, *P. nodosus* and *P. pusillus*. This species noticed in Nineveh province on the right bank of the Tigris River at Hawy Al- Kanissa within Mosul city. That location which chosen for as an aim during the present study. Then phytochemical compounds belong to the oxygenated terpenes: Octyl acetate, Carvyl acetate, Methyl heptenone, 1,8-Cineol, Citronellyl acetate, Citral, α -Terpineol and Linalool monoxide were separated from crude extracts by thin-layer chromatography technique (TLC).

Keywords: Potamogeton, TLC, Terpenoids, Oxygenated terpenes, Aquatic plants, Tigris river.

Introduction

The active compounds of plants such as phenols, volatile oils, alkaloid glycosides and other compounds as important therapeutic agents. However, the content of these compounds varies depending on the growth and environmental conditions, plant parts and stages of development, and genotypes. Studies have indicated that genetic variation is the factor that directly affects the contents of the active ingredients [1]. The chemical compounds responsible for the color, taste and smell in plants are known as secondary plant metabolites or phytochemical, so this term is used in general to refer to the chemical substances that are built secondary during the metabolic processes carried out by the plant and are of vital importance in the life of the plant. It is worth noting that these compounds have been used for a long time as medicinal drugs through the use of medicinal herbs. There are many groups of secondary metabolites in the plant, such as alkaloids, tannins, terpenes, sterols, and others [2].

Terpenes are the largest family of compounds that are built by plants, and the term terpene was given to the compounds in a volatile liquid (terpenine), which was isolated from pine trees, and was originally used to refer to hydrocarbon isomers that have a molecular formula $(C_5H_8)_n$. However, there is a tendency to use a more general term, terpenoids, which also includes the oxygen derivatives of the hydrocarbons that make up the terpenes [3]. Terpenes are among the most widespread and chemically diverse groups of natural products, and although they have a variety of compositions, they are characterized by common features that are easy to identify, and through these features they can be classified, the basic unit of their composition is isoprene or isopentene, which consists

of five Carbon atoms, and based on their content of those units, they contain several basic groups (Table 1).

Regarding the nature of these compounds, the monoterpenes and most of the cisco-terpenoids are essential oils under normal conditions. Because of its ability to evaporate and fragmentation, while Diterpenes have high boiling points, so they are resins, while Triterpenes (contains of 6 isoprene units) includes steroids, Saponins, sterols, and cardiac glycosides, while most of the Tetraterpenes are carotenoids like β -carotene (The most common biological pigment in carrots) which is converted by enzymes into vitamin A [4].

On the other hand, terpenoids can be classified into carbonyl compounds, oxides, peroxides, alcohols, esters, phenols, acids and hydrocarbons. Terpenes are found in most plant species, functionally, terpenes have different physiological, metabolic and synthetic roles, and among those functional terpenes are photosynthetic pigments and plant hormones that have regulatory activities for metabolic processes. In addition, many terpene derivatives have functions related to other organisms such as defense, production and symbiotic [5]. In general, some terpenes are often in the form of lipids dissolved in the cytoplasm of plant cells, while the aromatic types (volatile oils) are observed in special glandular cells located on the surface of the leaves, while carotenoids are specifically associated with chloroplasts in leaves and chromoplasts in petals petal [6].

Sampling site

In order to achieve the objectives of the study, samples were collected from a part of the Tigris River within the city of Mosul, which were distinguished by the abundance of aquatic plants growing there, as shown in (Figure 1), where five

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Table 1. The classification of terpenoids is based on isoprene units.

Class of Terpenoids	Number of Isoprene Units	Number of a Carbon Atoms
Hemi-terpenes	1	5
Mono-terpenes	2	10
Sesqui-terpenes	3	15
Di-terpenes	4	20
Ses-terpenes	5	25
Tri-terpenes	6	30
Tetra-terpenes	8	40
Poly-terpenes	> 8	(C ₅ H ₈) _n

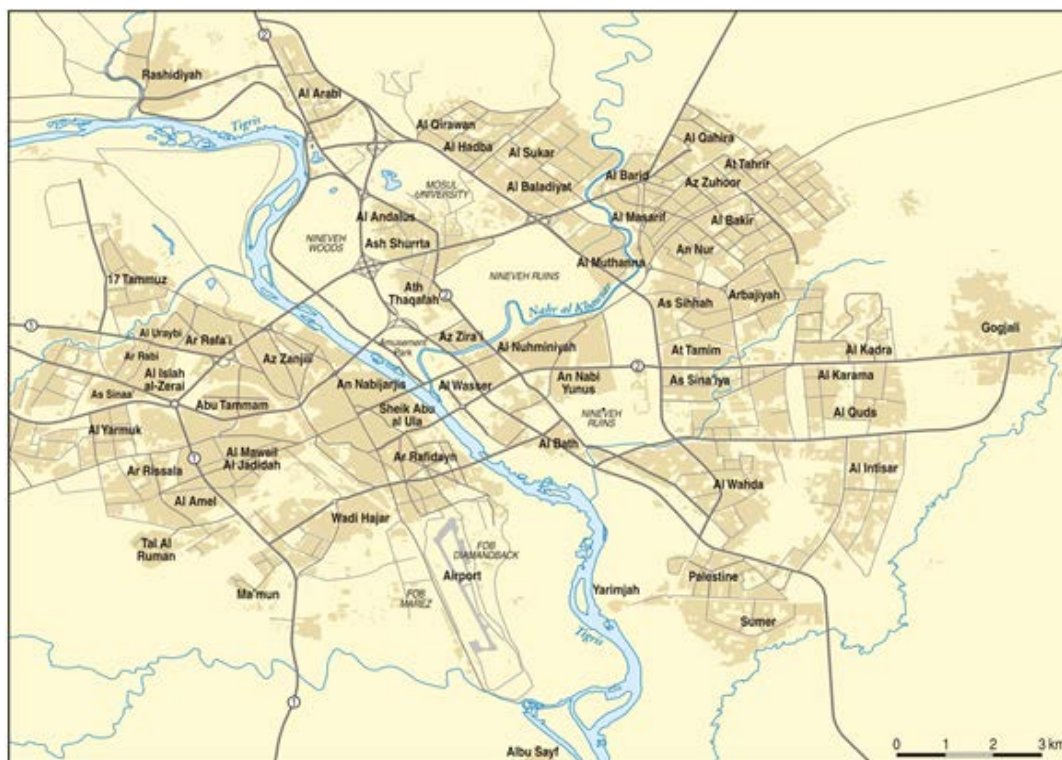


Figure 1. A map of Mosul city showing the location of sample collection at the right coast.

species belonging to the genus *Potamogeton* were collected. They are: *Potamogeton crispus*, *P. perfoliatus*, *P. pectinatus*, *P. nodosus*, and *P. pusillus*.

Materials and Methods

Methods of extraction

The extraction process was carried out using the Continuous Soxhlet Apparatus, after drying the samples in the shade and then grinding and weighing them [6]. Four different polar solvents were used in the separation process: petroleum ether 60-80°C, benzene, chloroform, and ethanol 95%. Although the current study did not aim to quantify the compounds under study, However, we were keen to prepare the crude in equal concentrations in order to give an equal opportunity for the appearance of compounds during the separation process, So 16 grams of every vegetable powder dissolved in a liter of solvents mentioned earlier. After the extraction, the solutions were concentrated to 160 ml (i.e. 1/10 ml) using (Rotary Vacuum Evaporator). Crude extracts were then stored in the refrigerator pending the separation on the TLC layer [7].

Methods of separation

The separation process was done with the thin layer chromatography technique, by using sheets of German silica gel with the following specifications: Aluminum sheets, 20 × 20 × 0.2 cm, silica gel 60 and the separation was carried out after the plate was activated in the oven under 70°C and for 10 minutes.

Methods of identification

Based on the Rate of flow (Rf) values of compounds, calculated according to the following formula:

Rate of flow (Rf Value) = Distance traveled by the spots / Distance traveled by the solvent

Results and Discussion

Oxygen compounds are derivatives of hydrocarbons to which the taste and smell of volatile oil is due. In most cases, they have a medical and physiological effect as well. They are either in the form of alcohols, aldehydes, ketones, phenols, esters, or any other form of oxygen-containing groups in their composition. These compounds and other plant chemicals can be diagnosed using thin layer chromatography technique, which gives a

good and direct picture of the number of these compounds and their polar properties, because different active compounds of plant give different R_f values in chemical separation systems, and these values are important in understanding the polarity of those compounds [6], who also mentioned that terpenoids can be isolated from plant tissues using petroleum ether or by chloroform, and then it can be separated after that by silica or aluminum plate chromatography technique by using the same solutions.

Studies indicated that silica gel layers give an efficient separation for most terpenes, so the principle stated [8,9] was adopted in the current study to separate a number of oxygen terpenes mediated by the thin layer chromatography

technique, which includes the use of a solution consisting of ethyl acetate-chloroform (10-90, v/v) as a development.

The compounds were separated from the petroleum ether extracts of the species under study, by placing samples of them on the separation plate using fine capillary tubes. After drying the plate and spraying it with a reagent consisting of vanillin and concentrated sulfuric acid, the flow rate of the separated compounds was calculated; this amounted to 56 compounds (**Table 2**). Eight of them were diagnosed by comparing the flow rate of each of them with the values of the corresponding standard compounds, and these compounds belonged to the group of oxygen terpenes, and their structural formulas are as shown in (**Figures 2 and 3**).

Table 2. Values of the Rate of flow (R_f) of the compounds that were shown on the thin layer chromatography plate during the process of separating some oxygen terpenes of the genus *Potamogeton* using Ethyl acetate-chloroform (10-90, v/v) as a mobile phase.

Crude extractions	Rate of flow (R _f)													
	96	93	91	77	63	55	31	23	9	3				
<i>P. crispus</i>	96	93	91	77	63	55	31	23	9	3				
<i>P. perfoliatus</i>	63	31	23	9	2									
<i>P. pectinatus</i>	96	93	91	77	63	55	31	26	16	9	3			
<i>P. nodosus</i>	96	93	91	86	84	80	77	74	67	31	26	16	4	3
<i>P. pusillus</i>	96	93	91	77	63	55	31	26	16	9	3			

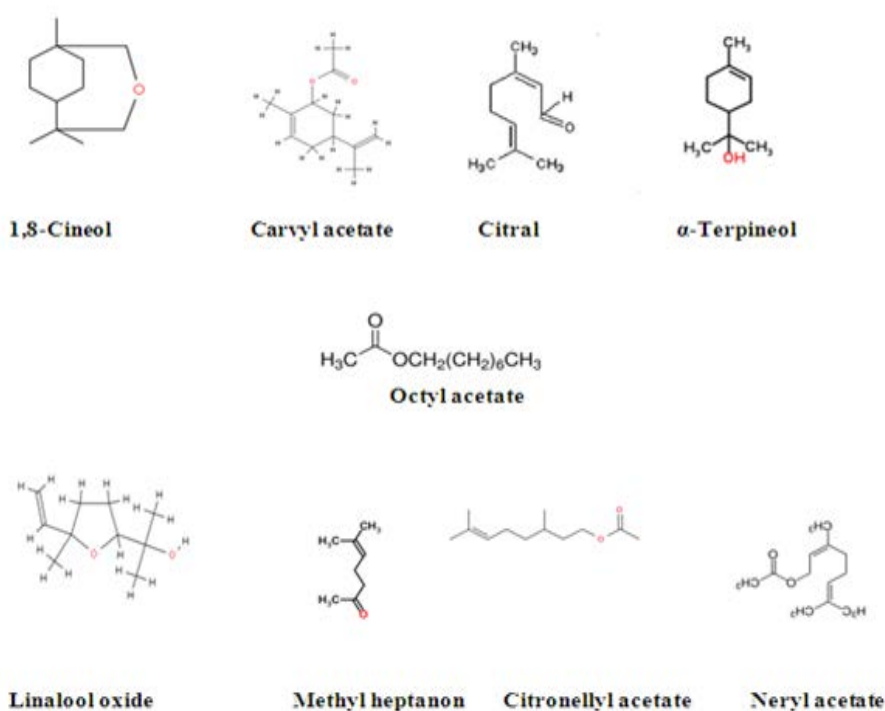


Figure 2. Oxygen Terpenes that were separated and diagnosed during the study.

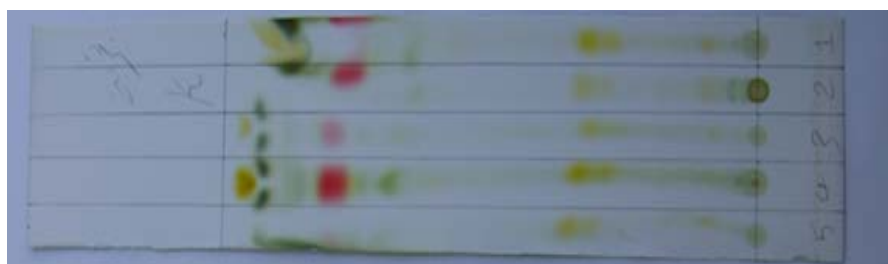


Figure 3. Thin layer chromatography plate during the process of separating some oxygen terpenes of the genus *Potamogeton* using the solvent (Ethyl acetate-chloroform., 10-90, v/v) as a mobile phase.

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Table 3. Values of the flow rate of some standard oxygen terpenes and the compounds diagnosed in the petroleum ether extracts of the genus *Potamogeton* using the solvent (Ethyl acetate-chloroform (10-90,v/v) as a mobile phase.

compounds diagnosed	Rate of flow (Rf)							
	Octyl acetate	Carvyl acetate	Methyl heptenone	1,8-Cineol	Citral	Citronellyl acetate	α -Terpineol	Linalool monoxide
<i>P. crispus</i>	96	77	-	-	63	55	31	23
<i>P. perfoliatus</i>	-	-	-	-	63	-	31	23
<i>P. pectinatus</i>	96	77	-	-	63	55	31	23
<i>P. nodosus</i>	96	77	74	67	-	-	31	23
<i>P. pusillus</i>	96	77	-	-	63	55	31	23
Standards	98	79	74	68	64	56	34	20

It was noted that the values of the flow rate of the diagnosed compounds were very close to or identical to their counterparts in the standard compounds. So the spots that showed a flow rate equal to (23 and 31) were diagnosed as Linalool monoxide and α -Terpineol depending on the value of the flow rate (20 and 34) for the corresponding standard compounds. As for the compounds with Rf values equal to (55, 63, 67, 74, 77 and 96) they were considered: Octyl acetate, Carvyl acetate, Methyl heptenone, 1,8-Cineol, Citronellyl acetate and Citral, respectively, based on the standard flow rate values (56, 64, 68, 74, 79 and 98) shown in (Table 3).

Conclusion

The results also showed that the *P. perfoliatus* had the lowest number of compounds that appeared on the separation plate, and it amounted to only five compounds, while the *P. nodosus* had the most number, reaching 14 compounds, and the extracts of this species were also distinguished by containing the compound 1,8-Cineol Which was not observed in the extracts of the other species under study.

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