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# GC/MS Characterization of The Non-Polar Phytochemical Constituents from Leaves of *Hydrangea macrophylla* Cultivated in Baghdad-Iraq

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## Abstract

*Hydrangea macrophylla* is a medicinal plant with a long history of use in traditional medicine, particularly for its supposed diuretic and antioxidant effects.

**Objective:** The purpose of this study was to analyze the phytochemical contents and identify the key non-polar active chemicals in the leaves of *Hydrangea macrophylla*, also known as French Hydrangea, cultivated in Baghdad, Iraq.

**Methods:** Non-polar compounds were extracted using Soxhlet as hot hexane extraction and subsequently analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

**Results:** Preliminary phytochemical screening revealed the presence of terpenoids, saponins, and flavonoids in the methanol extract of the leaves. The GC-MS analysis of the hexane extract identified seven major peaks corresponding to terpene-based compounds, with the primary constituents being squalene (4.43%), heneicosane (2.7%), heptacosane (2.1%), and hexacosane (2.7%). These compounds are known for their diverse pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects.

**Conclusion:** There were relatively numerous of phytochemicals present in the hexane extract from leaves collected before flowering. These findings highlighted the importance of environmental factors, such as soil conditions and harvest timing, on the chemical composition of the plant. Further studies are recommended to explore the plant's polar constituents and investigate their therapeutic applications.

# توصيف المكونات الكيميائية النباتية الغير قطبية من أوراق نبات الهيدرانجيا الكبيرة المزروعة في بغداد-العراق باستخدام تقنية كروماتوغرافيا الغاز/مطياف الكتلة

نورس خيري فاضل

فرع العقاقير والنباتات الطبية \كلية الصيدلة \الجامعة المستنصرية \العراق بغداد

**الخلاصة**

مقدمة: نبات الهدرانجيا ماكروفيلا (فصيلة الهدرانجيا) هو نبات طبي له تاريخ طويل من الاستخدام في الطب التقليدي، وخاصة لتأثيراته المفترضة كمدر للبول ومضاد للأكسدة. الهدف: كان الغرض من هذه الدراسة تحليل محتويات المواد الكيميائية النباتية وتحديد المواد الكيميائية النشطة غير القطبية الرئيسية في أوراق نبات الهدرانجيا ماكروفيلا، والمعروفة أيضًا باسم الهدرانجيا الفرنسية، المزروعة في بغداد، العراق.طرق العمل: كشف الفحص الكيميائي النباتي الأولي عن وجود التربينويدات والسابونينات والفلافونويدات في المستخلص الميثانولي للأوراق. لإجراء تحليل أكثر تعمقًا، تم استخراج المركبات غير القطبية باستخدام سوكسليت كاستخراج هكسان ساخن وتحليلها لاحقًا باستخدام كروماتوغرافيا الغاز-مطياف الكتلة. النتائج: قد حدد تحليل كروماتوغرافيا الغاز ومطياف الكتلة للمستخلص الهكساني سبع قمم رئيسية تتوافق مع المركبات القائمة على التربين، حيث كانت المكونات الأساسية هي السكوالين (4.43٪)، والهينيكوسان (2.7٪)، والهبتاكوسان (2.1٪)، والهكساكوسان (2.7٪). تُعرف هذه المركبات بأنشطتها الدوائية المتنوعة، بما في ذلك التأثيرات المضادة للأكسدة والالتهابات والمضادة للميكروبات. الاستنتاج: على الرغم من العائد المنخفض نسبيًا لمستخلص الهكسان من الأوراق التي تم جمعها قبل الإزهار، تشير النتائج إلى أن نبات الكوبية الكبيرة يمكن أن يوفر إمكانات علاجية كبيرة، وخاصة في مجالات صحة الكلى وإدارة الإجهاد التأكسدي. وتسلط النتائج الضوء على أهمية العوامل البيئية، مثل ظروف التربة وتوقيت الحصاد، على التركيب الكيميائي للنبات. التوصيات: يوصى بإجراء المزيد من الدراسات لاستكشاف المكونات القطبية للنبات والتحقيق في تطبيقاته العلاجية، وخاصة في علاج حصوات الكلى والمثانة والبروستات

**الكلمات المفتاحية:** نبات القرطاسية، كروماتوغرافيا الغاز/مطياف الكتلة، هكسان.

## Introduction

Medicinal plants have long been recognized for their therapeutic benefits due to the large number of bioactive chemicals they contain, each with a unique biological activity. This makes them a valuable resource for the development of alternative and complementary therapies for a variety of diseases. As concerns about the adverse effects and decreased performance of synthetic pharmaceuticals grow, traditional herbal therapies have regained popularity for their ability to provide safer and more effective treatments. The hunt for new, natural sources of medicine has grown vital, especially given the increasing frequency of chronic diseases and the limitations of conventional pharmacological treatments [(1, 2)].One such plant gaining recognition for its medicinal potential is *Hydrangea*, a genus of climbing woody shrubs belonging to the *Hydrangeaceae* family. Native to the Western Hemisphere and Eastern Asia, *Hydrangea* includes approximately 23 species, many of which are cultivated for ornamental purposes due to their attractive, ball-shaped flower clusters [(3)]. While the plant is primarily known for its ornamental value, *Hydrangea* also holds promise as a therapeutic agent, particularly due to the unique bioactive compounds it contains [(4)].*Hydrangea* is characterized by its simple, opposite leaves, and bisexual flowers, which give rise to capsule-shaped fruits. The plant's active constituents have been widely studied for their potential pharmacological applications. Notably, *Hydrangea* leaves contain phyllodulcin, a sweet compound used as a sugar substitute, as well as hydrangenol, a free isocoumarin isolated from hydrolyzed flowers, leaves, and roots [(5, 6)]. Additionally, two secoiridoid glucosides—hydramacrosides A and B—have been identified as important constituents, particularly in the leaves [(7)]. These compounds contribute to the plant's antioxidant, anti-inflammatory, and other therapeutic properties [(8)].Hydrangea leaves, in particular, has been utilized in traditional medicine for centuries, mainly for its supposed diuretic effects. It has been used to treat urinary tract disorders, including bladder and prostate infections, and is believed to aid in the prevention and treatment of kidney stones [(9)]. Recent studies have shown that *Hydrangea* root extract exhibits significant pharmacological activity, including a reduction in blood urea nitrogen

(BUN) levels in rats with renal damage, suggesting its potential in treating kidney diseases [10]. The plant's antioxidant properties, attributed to the presence of coumarins, have also been explored, with promising results showing a reduction in oxidative stress and related biomarkers such as nitric oxide (NO) and malondialdehyde (MDA) [11]. Thus, the therapeutic potential of *Hydrangea* remains an area of great interest, with ongoing research investigating its bioactive compounds and their role in treating a range of health conditions, from urinary tract disorders to kidney and bladder dysfunction.

## Materials and methods

### Plant Material

The leaves of *Hydrangea macrophylla* (French Hydrangea) were collected from plant houses in two different locations in Baghdad, Iraq, during the month of January. The collected plant material was immediately dried at room temperature in a shaded area to prevent exposure to direct sunlight, which can degrade the plant's active compounds. Once dried, the leaves were finely ground into a powder and weighed to determine the quantity of plant material used for the extraction.

### Preliminary Phytochemical Screening

The methanol extract of *Hydrangea macrophylla* was subjected to qualitative phytochemical screening to identify key secondary metabolites. The following tests were performed [15,16]:

**Tannins**: A small amount of the methanol extract was diluted with distilled water, and a few drops of a 10% ferric chloride solution were added. The formation of a blue-black color indicated the presence of tannins.

As more evidence emerges, *Hydrangea* could play an increasingly important role in the development of natural, plant-based therapeutics with fewer side effects than conventional medications [12]. Terpenoids: These are often non-polar, lipophilic compounds. Various terpenes, like monoterpenes and sesquiterpenes, may be present in *Hydrangea macrophylla*. These include compounds like α-pinene, limonene, and others, which are usually found in the essential oils of the plant [13]. The aim of this study is the qualitative estimation of the phytoconstituents such as terpenoids, saponins, alkaloids and flavonoids concentrated in the leaves of *Hydrangea macrophylla* grown in Baghdad, Iraq and Investigate the constituents of hexane fraction using gas chromatography-mass spectrometry (GC-MS). [14]

**Saponins**: The Froth test was used to detect saponins. In a test tube,

10 mL of sterile distilled water and 2.5 mL of the methanol extract were combined. The mixture was vigorously shaken for 30 seconds, and the test tube was allowed to stand for 30 minutes. The presence of a persistent froth indicated the presence of saponins.

**Flavonoids**: One milliliter of the methanol extract was mixed with two milliliters of ethanol KOH solution. A yellow color formation indicated the presence of flavonoids.

**Terpenoids**: To detect terpenoids, the methanol extract was treated with 0.5 mL of acetic anhydride and 0.5 mL of chloroform. Then, a concentrated solution of sulfuric acid was slowly added. The development of a red color indicated the presence of terpenoids.

### Extraction of Non-Polar Constituents (Hot Hexane Extraction)

To isolate the non-polar constituents from *Hydrangea macrophylla*, a hexane extraction was performed using the Soxhlet apparatus which is a closed system in which hexane solvent circulated the apparatus, boiling in 70 °C to extract the active compounds. A total of 100 grams of powdered plant leaves were extracted with 1000 mL of hexane. The extraction was conducted under continuous reflux until the solvent was exhausted. After extraction, the hexane was evaporated to dryness using a rotary evaporator, and the resulting residue was weighed and stored for further analysis [17].

### GC-MS Analysis of Hexane Extract

The hexane extract of *Hydrangea macrophylla* was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS)(shimadzu SOPS) to identify the non-polar constituents, such as terpenoids. The GC analysis was performed at the AL-Betar Center for Research, Ministry of Industry, Baghdad. The following parameters were used for the GC-MS analysis: Carrier gas: Helium, Injection volume: 1 µL, Split ratio: 2.0, Injection temperature: 250°C. Column temperature program: The temperature was initially set at 80°C, then increased to 310°C at a rate of 10°C per minute. This temperature gradient was used to separate the compounds based on their boiling points and retention times. The GC-MS system provided mass spectra, which were used to identify and characterize the individual compounds in the hexane extract based on their mass-to-charge ratios (m/z). The analysis allowed for the detection of various terpenes and other non-polar constituents present in the plant extract [(18).]

## Result and discussion

### Preliminary Phytochemical Study of Crude Extracts of *Hydrangea macrophylla*

The preliminary phytochemical screening of the methanol extract of *Hydrangea mcrophylla* leaves revealed the presence of several key bioactive compounds. Specifically, the tests indicated the presence of **terpenoids**, **flavonoids** and **saponins**.

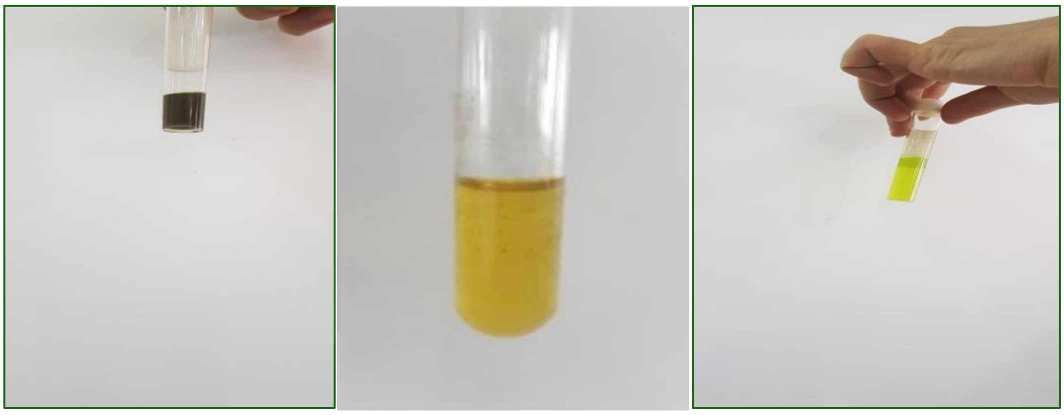
These compounds are known for their diverse pharmacological activities, which may contribute to the therapeutic properties of *Hydrangea macrophylla*. Figure 1 illustrates the results of these phytochemical tests.

### Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of Hexane Extract

The hexane extract of *Hydrangea macrophylla* leaves was subjected to GC/MS analysis to identify its non-polar constituents. The GC/MS chromatogram (Figure2 and 3) GC/MS chromatogram revealed a total of seven peaks, each representing different terpene compounds present in the extract. The individual compounds were identified by comparing their mass spectra with a reference database from the Ibn AL-Betar Center for Research, Ministry of Industry, Baghdad.The identified compounds are listed in Table 1, which provides the retention times, similarity indices, and area percentages for each compound. The major compounds identified in the hexane extract include:

Table 1: Phytochemicals identified by GC-Mass of hexane extract from leaves of *hydrangea macrophylla* leaves.

| Peak No | Compound | Ret time(min) | Similarity index | Area% |
|---|---|---|---|---|
| **1** | Squalene | 30.39 | 99% | 4.43 |
| **2** | Heneicosane | 25.83 | 91% | 2.7 |
| **3** | Hexacosane | 25.83 | 91% | 2.7 |
| **4** | Hexadecenoic acid | 19.81 | 97% | 6.9 |
| **5** | Heptacosane | 27,003 | 81% | 2.1 |
| **6** | Tretetracotone | 19.47 | 87% | 1.5 |
| **7** | Butynol | 4.68 | 74% | 1.46 |



(A) (B) (C)

Figure (1): preliminary phytochemical tests of *Hydrangea macrophylla* extract: (A) terpenoids, (B) flavonoids, (C) saponin.

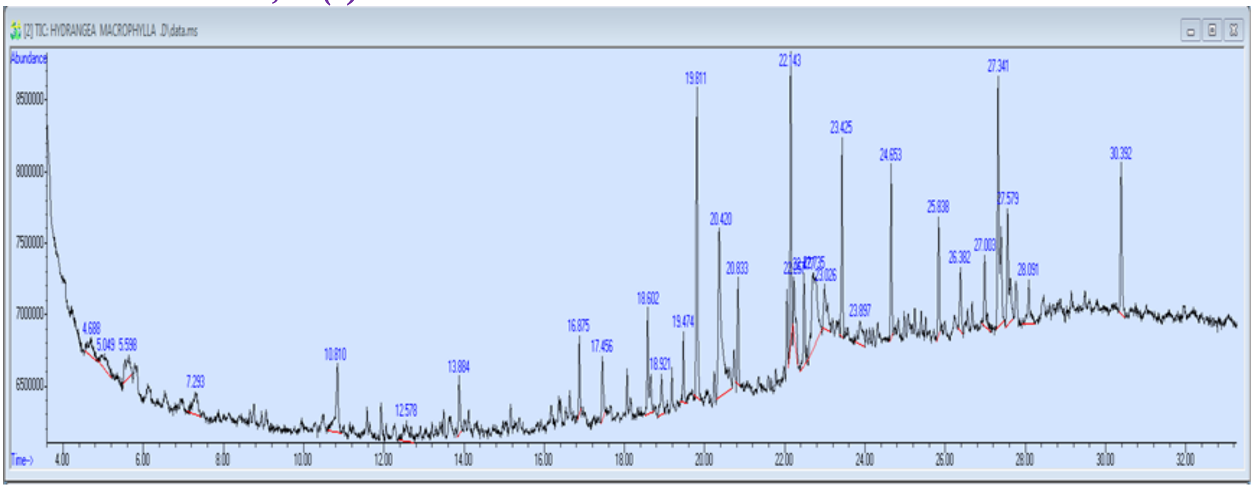
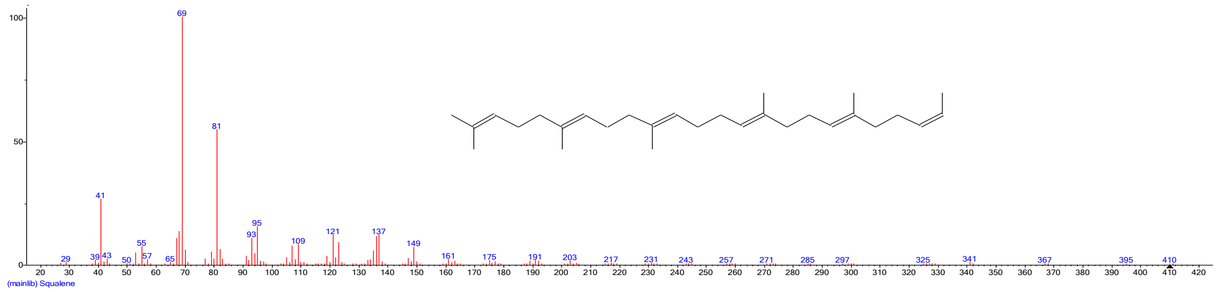
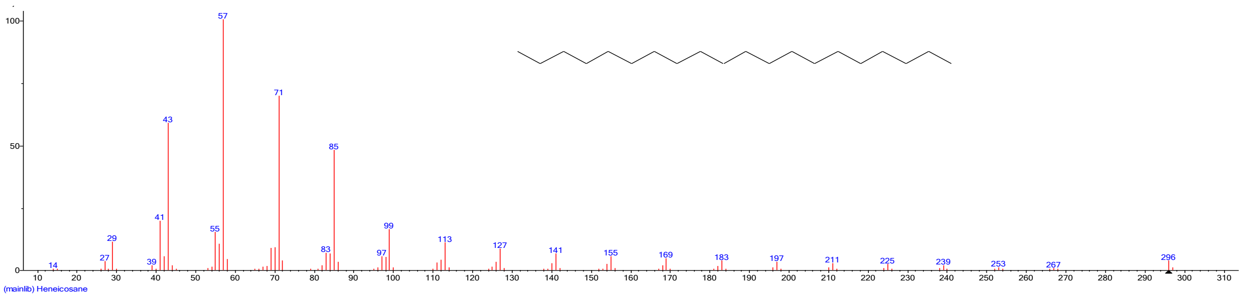



Figure (2): GC/MS analysis of hexane extract from leaves of *hydrangea macrophylla* leaves




(A)




(B)


(C)

**Figure (3)** mass fragmentation pattern of some of the most significant compounds: (A) Squalene, (B) Heniencosane, (C) Tritetracontane, obtained for the leaves of *Hydrangea macrophylla*

The presence of **squalene** in particular has attracted attention due to its wide-ranging applications in both medicine and the food industry. Squalene is known for its antioxidant, anti-inflammatory, and immune-boosting properties, which are beneficial for a variety of health conditions. The compound's significance in *Hydrangea macrophylla* further underscores the therapeutic potential of the plant [19].The diversity of terpenoids found in *Hydrangea macrophylla* is consistent with the plant's medicinal value. Terpenoids are widely recognized for their broad spectrum of biological and pharmacological activities, including antimicrobial, antifungal, insecticidal, anticancer, anti-inflammatory, and anti-diabetic properties. These findings support the increasing interest in terpenoid-rich plants like *Hydrangea macrophylla* for their potential use in the development of novel therapeutics [19].

**Comparison of *Hydrangea macrophylla* Grown in Different Environments**

Interestingly, while the leaves of *Hydrangea macrophylla* were collected prior to flowering to optimize essential oil
concentration, the yield of the hexane extract from plants grown in Baghdad soil was found to be relatively low. This suggests that *Hydrangea macrophylla* flourishes better in more humid regions, where environmental conditions are more conducive to the production of essential oils and secondary metabolites [20]. *Hydrangea macrophylla* is known to exhibit significant variation in its terpene content, percentage of compounds, and chemotypes, depending on factors such as geographical location, soil characteristics, genetic makeup, and environmental conditions [18].

**Conclusion**

The preliminary phytochemical analysis of *Hydrangea macrophylla* leaves has revealed the presence of several bioactive compounds, including terpenoids, saponins, and flavonoids. The presence of terpenoids, in particular, supports the growing interest in *Hydrangea macrophylla* as a source of compounds with GC/MS analysis of the hexane extract further identified several key compounds, including squalene, heneicosane, hexacosane, and heptacosane.

**Recommendation**

Further effort for phytochemical identification for active compounds presents in other parts of plant like (stem, bark and flowers) are required. In addition, studying there antibacterial, antioxidant and hepatoprotective activity of plant and finally Preparation of pharmaceutical formula and in vivo evaluation are required.

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