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Comprehensive phytochemical analysis and antioxidant potential of *Mentha spicata* L. leaves and stems: insights from the region of Tiaret, Algeria

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Abstract

Medicinal and aromatic plants play a pivotal role in traditional and modern medicine due to their rich secondary metabolite content and associated therapeutic properties. This study investigates the phytochemical composition and antioxidant activity of aqueous and ethanolic extracts of *Mentha spicata* L. (spearmint) leaves and stems, collected from Tiaret region of Algeria. Results revealed that leaves exhibited significantly higher polyphenol and tannin concentrations than stems, irrespective of the solvent. Ethanolic extracts generally demonstrated greater efficiency in extracting polyphenols and flavonoids, with the highest flavonoid content observed in ethanolic leaf extracts (159.74 mg QE/g). Conversely, aqueous extracts were more effective in extracting tannins, with leaves containing 27.07 mg CE/g. Antioxidant activity, measured as IC_{50} values, showed a strong correlation with the phytochemical content. Ethanolic extracts of the leaves exhibited the highest antioxidant potential, surpassing both stem extracts and their aqueous counterparts. These findings highlight *M. spicata* as a promising natural source of bioactive compounds for applications in the therapeutic, food, and cosmetic industries. Furthermore, they emphasize the importance of targeted extraction strategies to optimize the utilization of its bioactive potential, thereby contributing to eco-friendly innovations and biodiversity conservation.

Keywords:

Mentha spicata, bioactive compounds, polyphenols, flavonoids, tannins, antioxidant activity.

1. Introduction

Medicinal and aromatic plants have long been regarded as cornerstones of traditional medicine, playing an integral role in human health and well-being [1, 2]. Beyond their traditional uses, these plants have become essential for modern pharmaceutical industries, functional foods, and cosmetic formulations due to their diverse bioactive compounds [3]. The increasing prevalence of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions has intensified the global interest in identifying natural sources of therapeutic agents with minimal side effects. According to the World Health Organization [4, 5], approximately 80% of the global population relies on medicinal plants as their primary source of healthcare, underscoring their significance in global health systems.

The therapeutic potential of these plants is largely attributed to their chemical diversity, particularly the presence of secondary metabolites such as phenolics, flavonoids, alkaloids, and terpenoids. These compounds exhibit a wide range of biological activities, including antimicrobial [6], anti-inflammatory [7], antitumor [8], and antioxidant properties [9]. Among these, antioxidants have gained considerable attention for their ability to mitigate oxidative stress, a key contributor to cellular damage and the development of chronic diseases. By neutralizing reactive oxygen species (ROS), antioxidants play a crucial role in protecting cells from oxidative damage, thus offering potential for both preventive and therapeutic applications.

Algeria, known for its exceptional biodiversity and diverse ecosystems, ranging from coastal plains to arid deserts, boasts a rich and unique flora. This includes a large number of endemic and medicinally important plant species [10, 11]. The sustainable exploration and valorization of these natural resources represent a promising avenue for advancing both scientific research and economic development in the region. Medicinal and aromatic plants in Algeria are widely used in traditional remedies, yet many remain underexplored scientifically, representing an

untapped reservoir of bioactive compounds with potential applications in the pharmaceutical, cosmetic, and agri-food industries [12, 13].

Among the plants of significant interest is *Mentha spicata* L. (spearmint), a widely cultivated and economically important species of the *Mentha* genus. Known for its characteristic aroma and therapeutic potential, *M. spicata* is extensively used in traditional medicine to treat a variety of ailments, including gastrointestinal disorders, cancer, respiratory issues, and inflammation [14, 15].

Its essential oils and extracts have demonstrated a broad spectrum of pharmacological activities, including antimicrobial, anti-inflammatory, spasmolytic, and antioxidant effects [16, 17 &18]. These properties combined with its wide availability, make *M. spicata* an ideal candidate for further scientific exploration.

The biological activity of *M. spicata* and its therapeutic efficacy are largely attributed to its secondary metabolites, particularly phenolic compounds [19], flavonoids, and tannins [20]. These compounds not only contribute to its antioxidant capacity but also enhance its potential as a natural preservative and functional ingredient in various industries. However, despite its widespread use and promising properties, there is a need for comprehensive studies that systematically evaluate the phytochemical composition and biological activities of its various parts, including leaves and stems, under different extraction conditions.

In the present study, we aim to bridge this gap by investigating the phytochemical profile and antioxidant activity of *M. spicata* extracts obtained from the leaves and stems using aqueous and ethanolic solvents. The antioxidant activity was assessed through the *in vitro* DPPH assay, a widely recognized method for evaluating free radical scavenging capacity. This work seeks to provide a deeper understanding of the bioactive potential of *M. spicata*, while highlighting its value as a natural resource with applications in therapeutic and industrial domains.

The findings of this study will not only contribute to the scientific understanding of *M. spicata* but will also support its valorization as a sustainable and versatile resource. This aligns with global efforts to develop eco-friendly and health-promoting products, fostering innovation in the pharmaceutical, cosmetic, and food industries while promoting biodiversity conservation.

2. Materials and methods

2.1. Plant materials

Plant material used in this study consisted of the aerial parts (leaves and stems) of *M. spicata*, collected at the flowering stage in June from Tiaret region, located in northwest Algeria. This region is characterized by a semi-arid climate and rich biodiversity, which contributes to the chemical diversity of the plant species found there. The collected plant material was taxonomically identified by a specialist, and a voucher specimen was deposited in our research laboratory for future reference.

2.2. Preparation of extracts

The collected leaves and stems were carefully separated to ensure precise analysis of their individual bioactive properties. The plant material was air-dried under controlled conditions (20–25 °C), avoiding direct exposure to sunlight to prevent the degradation of light-sensitive compounds. After drying, the samples were finely ground into powder using an electric grinder to enhance the efficiency of subsequent extraction processes. The powders were stored in opaque, airtight containers at room temperature to prevent oxidation and contamination.

2.2.1. Aqueous and ethanolic extracts

Aqueous extracts were prepared by macerating 50 g of powdered plant material in 500 mL of distilled water in glass flasks. The flasks were sealed and subjected to continuous stirring at

room temperature in complete darkness for 24 hours to maximize the extraction of bioactive compounds. The resulting mixture was filtered through Whatman filter paper to remove plant residues. The filtrates were then concentrated under vacuum using a rotary evaporator and dried in an incubator at 37 °C until a stable dry residue was obtained.

Ethanolic extracts were prepared using the same protocol, substituting distilled water with 70% ethanol. Ethanol was chosen as the solvent due to its ability to solubilize a broader range of secondary metabolites, including phenolic and flavonoid compounds. The dried extracts were stored in clean, sealed containers at 4 °C to preserve their chemical stability until further analysis.

2.2.2. Quantification of phenolic compounds

- Total polyphenols

The total polyphenol content was quantified using the Folin-Ciocalteu method [21], a widely recognized technique for assessing the total hydroxyl group content in plant extracts.

Stock solutions were prepared by dissolving 1 mg of plant extract in 1 mL of distilled water. For each sample, 200 µL of the stock solution were transferred to glass hemolysis tubes, followed by the addition of 1 mL of 10-fold diluted Folin-Ciocalteu reagent. The tubes were incubated in the dark at room temperature for 5 minutes to allow the reaction to proceed. Subsequently, 800 µL of 7.5% sodium carbonate (Na_2CO_3) solution were added, and the tubes were vortexed to ensure uniform mixing. After 30 minutes of incubation, the absorbance was measured at 765 nm using a UV-visible spectrophotometer.

A calibration curve was generated using standard solutions of Gallic acid (0–1000 µg/mL). Results were expressed as milligrams of Gallic acid equivalents (mg GAE) per gram of dry extract.

- Flavonoids

Flavonoid content was quantified using the aluminum chloride colorimetric method [22]. This

method exploits the ability of aluminum chloride ($AlCl_3$) to form stable complexes with the hydroxyl groups of flavonoids.

One milliliter of extract was mixed with 1 mL of 2% $AlCl_3$ solution prepared in methanol. The mixture was vortexed and incubated in the dark for 15 minutes. The absorbance was measured at 430 nm against a blank solution.

Quercetin was used as the standard to construct a calibration curve (0–1000 µg/mL). Flavonoid content was expressed as milligrams of quercetin equivalents (mg QE) per gram of dry extract.

- Condensed tannins

Condensed tannins were measured using the vanillin-HCl assay, which is specific for the reaction between vanillin and high-molecular-weight proanthocyanidins [23].

For each sample, 50 µL of extract were added to 1500 µL of 4% vanillin solution prepared in methanol. The mixture was vigorously vortexed, followed by the addition of 750 µL of concentrated HCl. The reaction mixture was incubated at room temperature for 20 minutes in the dark to prevent interference from light-induced degradation.

The absorbance was measured at 500 nm. Catechin was used as a standard to construct a calibration curve (0–1000 µg/mL), and results were expressed as milligrams of catechin equivalents (mg CE) per gram of dry extract.

2.2.3. Evaluation of antioxidant activity using the DPPH assay

The antioxidant potential of the extracts was assessed using the DPPH˙ (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, a reliable method for evaluating the free radical scavenging ability of bioactive compounds [24].

A stock solution of DPPH˙ was prepared by dissolving 2 mg of DPPH in 100 mL of methanol, and the solution was stirred for 15 minutes to ensure complete dissolution. The solution was filtered and stored in a light-proof container to prevent degradation.

Plant extracts were diluted to various concentrations using methanol. For each concentration, 200 µL of extract were mixed with 2 mL of DPPH˙ solution in Eppendorf tubes. The mixtures were prepared in triplicate and incubated in the dark at room temperature for 30 minutes. The absorbance was recorded at 517 nm using a UV-visible spectrophotometer. A blank solution containing DPPH˙ without extract was used as a control. The percentage of DPPH˙ inhibition was calculated using the formula:

Radical scavenging (%) = 100 (1- A_{sample}/A_{blank}).

Where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test extracts. The IC_{50} value (the concentration of extract required to inhibit 50% of the DPPH˙ radical) was determined from the dose-response curve. Lower IC_{50} values indicate higher antioxidant activity, reflecting the superior ability of the extracts to neutralize free radicals. Results were expressed in micrograms per milliliter (µg/mL).

3. Results

The analysis of secondary metabolites and antioxidant activity in the leaves and stems of *M. spicata* provides a comprehensive understanding of the bioactive potential of different plant parts and extraction solvent.

3.1. Polyphenol content

The total polyphenol content, expressed as gallic acid equivalents (mg/g), demonstrated significant variations depending on the plant organ and extraction solvent. Leaves exhibited the highest concentrations of polyphenols, with 192.45 mg/g in the aqueous extract and 327.12 mg/g in the ethanolic extract, highlighting their superior phytochemical richness. Stems also showed considerable polyphenol levels, with 151.85 mg/g and 206.68 mg/g in the aqueous and ethanolic extracts, respectively (Fig. 1).

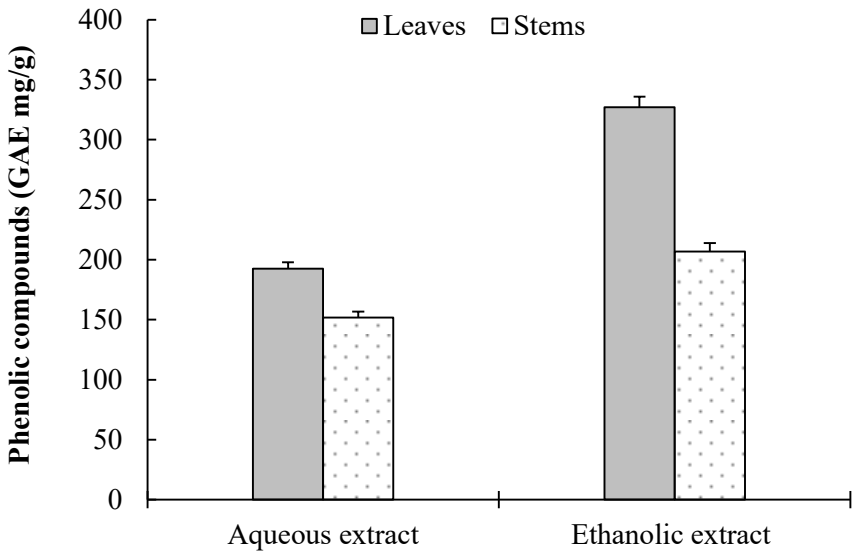

Fig. 1. Variation of the phenolic content of aqueous and ethanolic extracts of *M. spicata.*

3.2. Flavonoid content

Flavonoids, quantified as quercetin equivalents (mg/g), followed a similar trend, with higher concentrations in ethanolic extracts compared to aqueous ones. Leaves contained significantly more flavonoids in the ethanolic extract (159.74 mg/g) than in the aqueous extract (76.01 mg/g), highlighting their potential as a rich source of bioactive flavonoids. In stems, flavonoid content was relatively closer between the two solvents, with values of 121.25 mg/g and 144.69 mg/g for aqueous and ethanolic extracts, respectively (Fig. 2).

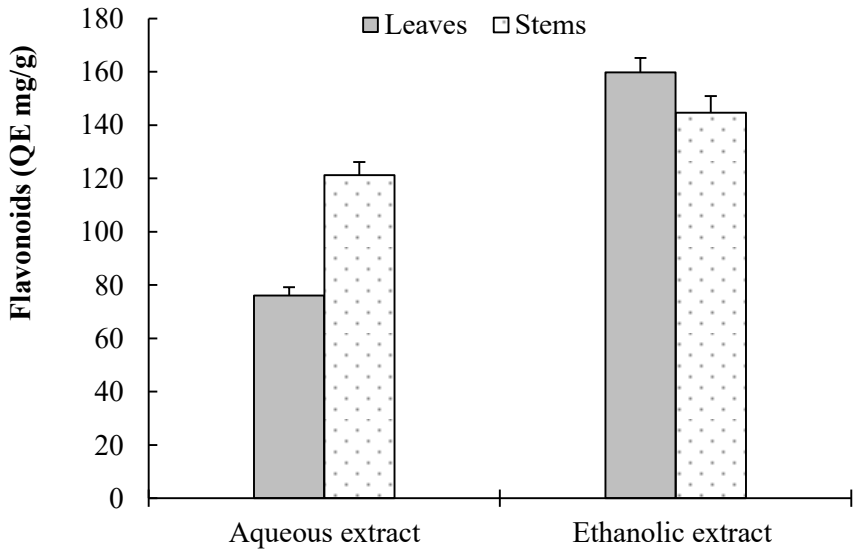

Fig. 2. Variation of the flavonoid content of aqueous and ethanolic extracts of *M. spicata*.

3.3. Condensed tannin content

The condensed tannin content, expressed as catechin equivalents (mg/g), showed a contrasting trend. Aqueous extracts were found to be more efficient in extracting tannins compared to ethanolic extracts. Leaves had a higher tannin concentration in aqueous extracts (27.07 mg/g) than in ethanolic extracts (18.62 mg/g). A similar pattern was observed in stems, with 18.98 mg/g and 11.24 mg/g for aqueous and ethanolic extracts, respectively (Fig. 3).

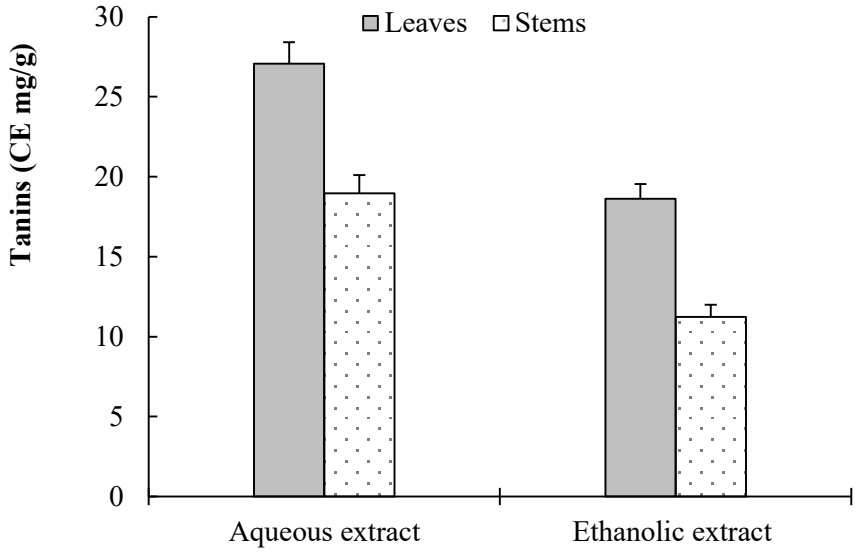

Fig. 3. Variation of the condensed tannins content of aqueous and ethanolic extracts of *M. spicata*.

3.4. Antioxidant activity

The results of the antioxidant activity assessment, expressed by IC_{50} values, highlight the combined influence of the studied organ and the extraction solvent on antioxidant efficacy. Ethanolic extracts, particularly those from the leaves, exhibit significantly higher antioxidant activity, as evidenced by their lower IC_{50} (56.68 µg/mL for the ethanolic extract compared to 72.22 µg/mL for the aqueous extract). In contrast, a different trend is observed for the stems. The aqueous extract from the stems demonstrates superior antioxidant activity (IC_{50} of 177.99 µg/mL) compared to the ethanolic extract (IC_{50} of 322.19 µg/mL) (Fig. 4).

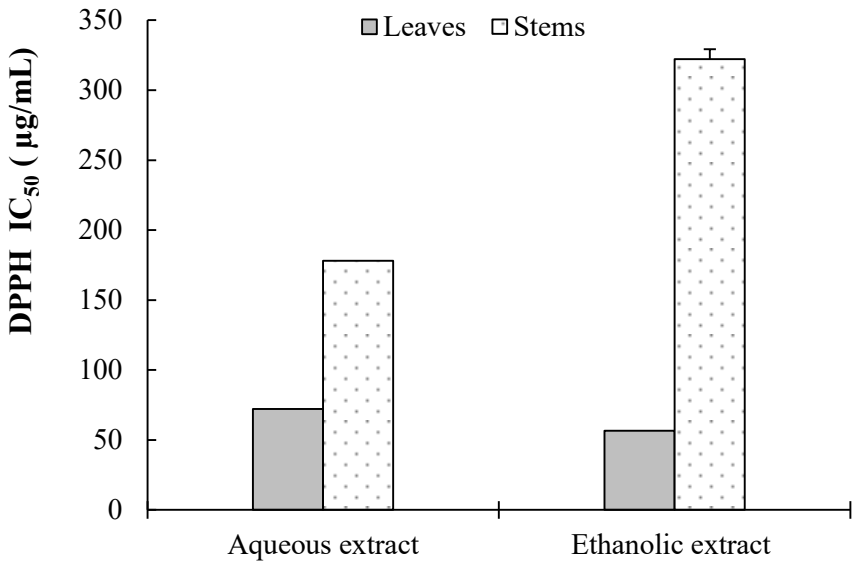

Fig. 4. Variation of the antioxidant activity as revealed by the DPPH assay of aqueous and ethanolic extracts of *M. spicata*.

4. Discussion

M. spicata, a plant widely used in Algerian traditional medicine for its numerous therapeutic virtues, was the focus of this study. The objective was to analyze its phytochemical compounds and evaluate its antioxidant activity to better understand the relationship between its biological properties and bioactive compounds. The obtained results reveal clear metabolic differences between the leaves and stems of *M. spicata*, reflecting their distinct accumulation within the plant. The leaves, rich in flavonoids and tannins, represent a valuable source of bioactive compounds [25], while the stems provide complementary structural and defensive support. Aqueous extracts of the leaves exhibit superior antioxidant activity, whereas ethanolic extracts of the stems significantly outperform those of the leaves.

The leaves demonstrate a markedly higher content of polyphenols and tannins compared to the stems, regardless of the solvent used. Regarding flavonoids, ethanolic extracts of the leaves show significantly higher concentrations than those of the stems, although this difference is less pronounced in aqueous extracts. The abundance of flavonoids in the leaves, also reported by Khoddam et al. [26] for other *M.* species, can be attributed to their central

role in the biosynthesis and accumulation of these metabolites. Flavonoids, known for their hydroxyl-rich chemical structures, are particularly effective in neutralizing free radicals, thereby enhancing the antioxidant capacity of the extracts [27, 28 & 29].

Tannins, while less concentrated in some extracts, significantly contribute to biological activity, particularly in aqueous extracts where their solubility is optimized. Their polyhydroxylated structure facilitates extraction by polar solvents like water, a phenomenon confirmed by Khoddam et al. [26]. Additionally, ethanolic extracts excel in extracting methylated or glycosylated flavonoids and other unmeasured polyphenols, enhancing antioxidant activity [30, 31]. These observations highlight the role of leaves as primary sites of plant defense and protection. As photosynthetic organs, they accumulate significant amounts of phenolic compounds, which are essential for protection against UV radiation, herbivores, and pathogens [32]. Stems, while less rich in secondary metabolites, contain notable amounts of polyphenols crucial for structural and defensive functions [33].

Extraction efficiency varies significantly depending on the solvent used, underscoring the importance of compound polarity. Ethanol, with its polar-apolar nature, extracts a wide range of polyphenols, including less polar ones, whereas water, being highly polar, favors hydrosoluble compounds such as tannins. These distinctions reveal a direct correlation between metabolite polarity, their distribution in plant organs, and their affinity for specific solvents [34].

The DPPH radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl) is one of the most commonly used in vitro methods to evaluate the antioxidant activity of plant extracts [35, 36]. The antioxidant activity of *M. spicata* extracts, as measured by IC_{50}. The ethanolic extract of the leaves exhibits significantly higher antioxidant activity compared to the aqueous extract, suggesting that the leaves of *M. spicata* are particularly rich in bioactive compounds soluble in ethanol. In contrast, an opposite trend is observed for the stems, where the aqueous extract

demonstrates greater antioxidant activity than the ethanolic extract (Fig. 4). This difference could be attributed to a specific chemical composition of the stems, which favors the extraction of active metabolites in polar solvents such as water. These findings highlight the critical influence of both the plant organ and the extraction solvent on the antioxidant efficacy of *M. spicata* extracts. The antioxidant activity is strongly correlated with the total concentrations of polyphenols, flavonoids, and condensed tannins, with total polyphenols emerging as the primary contributors to this activity. The findings confirm ethanol's efficiency in extracting bioactive metabolites with strong antioxidant capacities. The same conclusions were observed by Tourabi et al. [37] regarding the different extracts of *M. longifolia* L. Previous studies, such as those of Gulluce et al. [38] on *M. piperita* and Dorman et al. [39] on *M. × piperita*, corroborate these results, emphasizing the key role of flavonoids, particularly quercetin and luteolin, in overall antioxidant activity.

Furthermore, synergistic interactions between various phenolic metabolites play a crucial role in the biological efficacy of the extracts [40, 36]. Rice-Evans et al. [33] demonstrated that this synergy is particularly pronounced in extracts with balanced proportions of flavonoids and tannins, such as the aqueous leaf extracts.

These findings suggest that optimizing extraction conditions can maximize the biological activity of the extracts, paving the way for diverse applications in therapeutic, cosmetic, and food industries.

5. Conclusion

The present study quantified the secondary metabolites and evaluated the antioxidant activity of aqueous and ethanolic extracts from the leaves and stems of *M. spicata* collected in Tiaret region, Algeria. The obtained results highlight the higher content of leaves of polyphenols and tannins compared to the stems, regardless of the solvent used. Flavonoids were also more

abundant in the leaves than in the stems, particularly in ethanolic extracts, with a less pronounced difference observed in aqueous extracts. The assessment of antioxidant activity, based on IC_{50} values obtained from the DPPH assay, revealed significant antioxidant capacities in *M. spicata* extracts, particularly those derived from the leaves. These results underscore the critical role of phenolic compounds in the neutralization of free radicals. These findings confirm the high antioxidant potential of plants from the *Mentha* genus and reinforce the value of *M. spicata* as a rich natural source of bioactive compounds. The study underscores its potential for applications in therapeutic, food, and cosmetic industries, emphasizing the importance of targeted extraction strategies to maximize its benefits.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

Additional information

No additional information is available for this paper.

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