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# Essential Oil Composition, Antioxidant Properties and Phytochemical Constituents of the Aerial Parts of *Solenostemon monostachyus*

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## ABSTRACT

**Background and Objective:** Plants are a valuable source of secondary metabolites, providing a vast assortment of chemical compounds beneficial for human use. This study aimed to investigate the essential oil obtained from the aerial parts of *Solenostemon monostachyus*, as well as evaluate its antioxidant potential and phytochemical composition. **Materials and Methods:** The essential oil was obtained through hydrodistillation using a Clevenger-type apparatus, while the aqueous extract was prepared via infusion. **Results:** Analysis of the essential oil using Gas Chromatography-Mass Spectrometry (GC-MS) identified twenty-two compounds, with limonene (55.40%) and neral (10.39%) as the predominant constituents, followed by trans-verbenol (6.43%) and decanal (3.25%). Phytochemical screening of the aqueous extract revealed various bioactive compounds, including terpenes, alkaloids, sterols, tannins, reducing sugars, flavonoids, saponins and anthraquinones. High-Performance Liquid Chromatography (HPLC) detected sixteen key compounds, notably tannins, caffeic acid, rutin, ferulic acid, morin and apigenin. The antioxidant activity of *Solenostemon monostachyus* was assessed across a concentration range from 20-0.08 mg/mL, demonstrating the presence of strong antioxidant compounds. Both aqueous and hydroalcoholic extracts of *Solenostemon monostachyus* exhibited significant antioxidant effects. **Conclusion:** This study revealed that the aerial parts of *Solenostemon monostachyus* are rich in phytochemicals and essential oils, exhibiting significant antioxidant and antimicrobial properties. Toxicity tests indicated that the plant is safe for use as an herbal remedy for various ailments. The findings support the traditional use of *Solenostemon monostachyus* leaves, as both the essential oil and aqueous extracts demonstrated noteworthy antimicrobial and antioxidant activities.

## KEYWORDS

*Solenostemon monostachyus*, DPPH, antioxidant, antimicrobial, essential oil, phytochemicals



## INTRODUCTION

For centuries, medicinal plants have been essential for human health, offering treatments, preventatives and remedies[1]. Throughout history, traditional medicine across various cultures has depended on plants,

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many of which possess therapeutic properties recognized by modern Western medicine. Currently, medicinal plants remain vital sources for new drug discovery and development, playing a significant role in healthcare[2].

Plants provide a diverse range of secondary metabolites, comprising numerous chemical compounds beneficial for human applications. Within biological systems, oxidative stress can originate from various oxidation processes, such as oxygen reduction resulting in the production of Reactive Oxygen Species (ROS) or free radicals[3]. Antioxidants also referred to as scavengers of free radicals, play a crucial role in neutralizing these reactive species. This action helps safeguard cellular components such as lipids, carbohydrates, DNA and proteins from oxidation[4].

*Solenostemon monostachyus*, a perennial aromatic plant belonging to the Lamiaceae family and commonly found in West and Central Africa, exemplifies nature's wealth of medicinal resources. In Nigeria, its aerial parts have long been employed traditionally for treating malaria and are noted for their diverse pharmacological activities, which include anti-inflammatory, pain-relieving, anti-sickling, diuretic, antimicrobial, antihypertensive and antiulcer effects[5].

*Solenostemon monostachyus*, commonly referred to as "monkey's potato" is a perennial aromatic plant belonging to the Lamiaceae family. It is native to West and Central Africa and thrives in diverse habitats such as rocky savannahs and human-altered environments. This plant holds deep cultural and medicinal significance, particularly in Nigeria, where infusions made from its aerial parts have been integral to traditional medicine for centuries and used extensively to treat a wide range of health conditions.

The aerial parts of *S. monostachyus* have attracted considerable interest for their medicinal properties, encompassing anti-inflammatory, pain-relieving, antisickling, diuretic, antimicrobial, antihypertensive and antiulcer effects. Moreover, this plant demonstrates notable antioxidant capabilities, effectively scavenging Reactive Oxygen Species (ROS) and reducing ferric ions under laboratory conditions[6-11].

Recently, there has been an increasing focus on investigating the phytochemical makeup and antioxidant attributes of medicinal plants such as *S. monostachyus*. Chromatographic methods like Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) have become essential for analyzing the chemical components of plant extracts. These techniques facilitate the detection and measurement of active compounds found in herbal formulations, offering a valuable understanding of their potential therapeutic benefits[12-16].

Moreover, the antioxidant activities of plant extracts have drawn considerable attention from researchers and healthcare practitioners[17-19].

Oxidative stress, which results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, is linked to the development of several chronic illnesses such as cardiovascular diseases, neurodegenerative disorders and cancer. Therefore, there is increasing interest in exploring natural antioxidants sourced from plants as potential therapeutic agents[20].

In light of this context, the current research seeks to explore the chromatographic composition and antioxidant properties of the aerial part extract derived from *Solenostemon monostachyus*. By employing chromatographic techniques, we aim to characterize the chemical components present in the extract. Additionally, antioxidant assays will assess its capability to counteract free radicals and alleviate oxidative stress[21]. Through detailed analysis of the phytochemical makeup and antioxidant capacity of *S. monostachyus*, this study aimed to enhance the existing knowledge on medicinal plants and their potential therapeutic uses[22].

Moreover, *Solenostemon monostachyus* exhibits notable antioxidant capabilities, effectively neutralizing hydroxyl and hydrogen peroxide radicals and demonstrating *in vitro* ferric ion reduction activity. The leaves and roots of this plant are traditionally used for diverse medicinal applications, including the treatment of rheumatism, menstrual pain, infertility, snake bites, fever, eye ailments, foot infections, headaches, convulsions and skin infections. Additionally, it is employed in managing conditions such as onchocerciasis, hyperlipidemia and diabetes[23].

Phytochemical analysis of *S. monostachyus* indicates a variety of secondary metabolites, including polyphenols, flavonoids, coumarins, tannins, saponins, alkaloids, phytates, reducing sugars, carbohydrates and anthraquinones. Furthermore, the essential oil extracted from the leaves of *S. monostachyus* contains compounds such as β-pinene, oct-1-en-3-ol, β-caryophyllene, octan-3-ol and (E, E)-α-farnesene[24]. This study aimed to investigate the essential oil extracted from the aerial parts of *Solenostemon monostachyus*, as well as to evaluate its antioxidant capacity and phytochemical profile.

## MATERIALS AND METHODS

**Study area and sites:** This research took place in Benin City, Edo State, Nigeria, which is positioned at a Latitude of 6.34°N and a Longitude of 5.63°E. The city's elevation is 88 m above sea level. With a population of over 2,125,058, Benin City is the largest urban area in Edo State. This study spanned from September, 2021 to July, 2023.

**Chemicals and reagents:** All chemicals and reagents used in this study were of analytical grade and obtained from Sigma Aldrich (Germany), unless specified otherwise.

**Collection and identification of plant sample:** The 20 kg of plant material was gathered from the Idu Industrial Area in Abuja, Nigeria. It underwent identification and authentication at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, where the voucher specimen (NIPRD/H/6817) was deposited[24].

**Preparation of plant extracts:** The aerial parts of *S. monostachyus* were initially crushed using mortar and pestle shown in Fig. 1. The crushed sample was soaked in 70% ethanol for 24 hrs, followed by an overnight settling period. Subsequently, the mixture was filtered using a Buchner filter and the filtrate was dried using a water bath. The resulting yield was quantified and the dried extract was stored in an airtight container.

For the second extraction, the aerial parts of *S. monostachyus* were cut with scissors and hydrodistillation was performed to extract the oil for antimicrobial analysis. The resulting hot water extract underwent filtration and was dried using a water bath, then stored until required.

The third extraction method involved an infusion process. The dried aerial parts of the plant, which had been air-dried for 3 weeks, were crushed. Then, 25 g of the crushed plant material was placed in a jar and 500 mL of boiled water was added. After steeping for 24 hrs, the mixture was filtered and the filtrate was concentrated using a water bath. This infusion process was repeated twice to obtain sufficient extract for further analysis[25].

**Phytochemical analysis:** Phytochemical screening of *Solenostemon monostachyus* was conducted to identify the presence or absence of a range of plant compounds[26].

**High-Performance Liquid Chromatography (HPLC) analysis:** The HPLC system used in this study included a Shimadzu Ultra-Fast LC-20AB prominence with an SIL-20AC autosampler, a DGU-20A3 degasser, an SPD-M20A UV diode array detector (UV-DAD) covering wavelengths from 190-800 nm, a

Fig. 1: *Solenostemon monostachyus* in its natural habitat

CTO-20AC column oven, a CBM-20Alite system controller and Shimadzu's Windows LC solution software (Shimadzu Corporation, Kyoto, Japan). A VP-ODS column with dimensions of 150×4.6 mm and a particle size of 5 µm was employed.

The chromatographic conditions were maintained with a mobile phase consisting of solvent A (0.2% v/v formic acid) and solvent B (acetonitrile), operating isocratically at a flow rate of 0.6 mL/min. A 5 µL injection from a 20 mg/mL sample solution (HYP) was used and detection occurred at a wavelength of 254 nm using UV detection. Reference standards including rutin, quercetin, caffeic acid, ferulic acid and apigenin (Fluka, Germany) at a concentration of 50 µg/mL in methanol were separately analyzed under identical chromatographic conditions as the sample (HYP). The HPLC conditions were set to maintain solvent B at 20%, with a column oven temperature of 40°C. Each analysis had a total runtime of 40 min[27].

**Essential oil extraction:** The procedure began by chopping 500 g of fresh bulbs into small pieces, which were then subjected to hydrodistillation for 4 hrs using a Clevenger-type apparatus. The colorless oil obtained from the distillation process was dehydrated with anhydrous sodium sulfate. Afterward, the oil was filtered through a 0.22-micron filter and stored in sealed vials at 4°C in a dark place until further analysis[28].

**Gas chromatography-mass spectral analysis:** The essential oil analysis was conducted using GC-MS following the methodology described by Okhale *et al.*[29]. A Shimadzu QP-2010 GC equipped with a QP-2010 mass selective detector (MSD) was utilized for the analysis. The MSD operated in Electron Ionization (EI) mode with an electron energy of 70 eV, scanning a range of 45-400 atomic mass units (amu) at a rate of 3.99 scans per second. Instrument control and data analysis were managed by the Shimadzu GCMS solution data system. An HP-5MS fused silica capillary column was employed, featuring a (5% phenyl)-polymethylsiloxane stationary phase with dimensions of 30 m in length, 0.25 mm internal diameter and 0.25 µm film thickness. Helium was used as the carrier gas at a flow rate of 1.61 mL/min. The GC oven temperature program included an initial isothermal period at 60°C, followed by a temperature increase to 180°C at a rate of 10°C per min, held at 180°C for 2 min and then a further increase to 280°C at a rate of 15°C per min, with a final hold at 280°C for 4 min. The injection port temperature was set at 250°C. Sample ionization occurred in EI mode at 70 eV, with injector and detector temperatures at 250 and 280°C, respectively. The sample, diluted 1/100 in hexane (v/v), was injected in a volume of 1.0 µL using an autosampler in split mode, with a split ratio of 10:90[30].

**Antioxidant assay:** This study adapted the methodology outlined by Muraina *et al.*[31] with specific modifications. Each extract was initially diluted twofold with distilled water to achieve a concentration of 20 mg/mL, then dispensed into wells of a 96-well micro-dilution plate. Following this,

50 µL of a 0.2 mg/mL solution of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well. The plate was then incubated at 37°C for one week. Post-incubation, wells were inspected for bluish coloration or precipitate, which would indicate antioxidant activity. Distilled water served as the negative control and ascorbic acid was used as the standard reference. The Minimum Inhibitory Concentration (MIC) was identified as the lowest sample concentration that showed antioxidant activity, indicated by the disappearance of bluish coloration. This experiment was conducted in triplicate to ensure the accuracy and reproducibility of the results.

**Thin layer chromatography analysis of antioxidant:** The antioxidant properties of the extracts were assessed using Thin-Layer Chromatography (TLC) combined with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Each extract (100 µL) was applied to a 10×10 $cm^2$ TLC plate. The plate was developed in a solvent system of hexane and ethyl acetate (3:2) to separate the extract constituents. After drying, the TLC plate was observed under visible and UV light at 254 and 366 nm, respectively, to detect separation spots and calculate their retention factor (RF) values. A 0.05% DPPH solution in methanol was then sprayed on the TLC plate, followed by a 10 min incubation at room temperature. Active antioxidant components in *Solenostemon monostachyus* appeared as yellowish-white spots due to DPPH bleaching by the resolved bands on the TLC plate. The antioxidant potency of the constituents was visually compared to the bleached color intensity of positive standards, categorizing them as strong or weak antioxidants. Ascorbic acid and gallic acid were used as positive controls, while a blank TLC plate served as the negative control[32].

**Statistical analysis:** Statistical analysis was conducted using the BMDP software package, specifically the BMDP 2R program for stepwise multiple regression. Results were reported as the mean of triplicate analyses[33,34].

## RESULTS AND DISCUSSION

Qualitative phytochemical analysis of *Solenostemon monostachyus* aqueous extracts revealed the presence of secondary metabolites including saponins, tannins, flavonoids, reducing sugars, anthraquinones, alkaloids, terpenes and sterols shown in Table 1.

Phytochemical analysis of *Solenostemon monostachyus* identified several compounds, including essential oil constituents like β-pinene, oct-1-en-3-ol, β-caryophyllene, octan-3-ol and (E,E)-α-farnesene[35]. Alkaloids, which contain basic nitrogen atoms, are naturally occurring compounds found in various organisms such as bacteria, fungi, plants and animals. Known for their pharmacological properties, many alkaloids are used for medicinal and recreational purposes, though some can be toxic and have a bitter taste[36]. Flavonoids,

Table 1: Qualitative phytochemical analysis of the hydrosol of *Solenostemon monostachyus*

| Secondary metabolites | Test | Observation | Inference |
|---|---|---|---|
| Saponins | Froth test | Persistent honeycomb | +ve |
| | Fehling's test | Persistent honeycomb | +ve |
| Alkaloids | Mayer's test | Creamy precipitate | +ve |
| | Dragendorff's | Orange precipitate | +ve |
| | Hager's | Yellow precipitate | +ve |
| | 10% tannic acid | No precipitate | +ve |
| | Wagner's | No precipitate | +ve |
| Tannins | Ferric chloride | Greenish ppt | +ve |
| Flavonoids | Sodium hydroxide | Yellow solution | +ve |
| | Ferric chloride | Greenish precipitate | +ve |
| Reducing sugar | Fehling's test for reducing sugar | Brick red color | +ve |
| | Fehling's test for combined reducing sugar | Blue color | +ve |
| Terpenes | Liebermann-burchard test | A reddish brown-violet color at the interface | +ve |
| Sterols | Salkowski's test | A reddish-brown coloration at the interface | +ve |
| Carbohydrate | Molish reagent | Purple ring | +ve |
| Anthraquinones | Potassium hydroxide | Pink color | +ve |

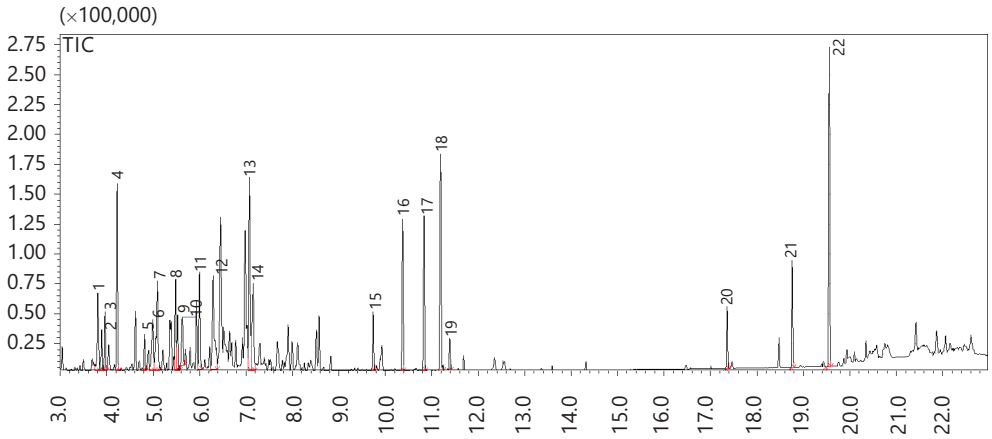


Fig. 2: GC-MS chromatogram of *Solenostemon monostachyus* essential oil

which originate from 2-phenylchromen-4-one, are celebrated for their antioxidant capabilities and are prevalent in plants. They play multiple roles, such as contributing to flower pigmentation and defending plants against microbial and insect threats. Flavonoids are known as nature's biological response modifiers because they can influence the body's reactions to allergens, viruses and carcinogens[37]. Flavonoids exhibit a range of activities, including anti-allergic, anti-inflammatory, antimicrobial and anticancer effects, as well as strong antioxidative and free radical scavenging abilities. Tannins, known for their astringent taste, also possess antioxidant properties but can harm the digestive tract's mucosal lining if consumed in large amounts. Nonetheless, tannins have shown potential antiviral, antibacterial and antiparasitic properties[38]. Saponins are glycosides of steroids or triterpenes, commonly present in different plant parts, characterized by their bitter taste and capacity to hemolyze red blood cells. Medically, saponins are used as expectorants, emetics and remedies for ailments such as excessive salivation, epilepsy and migraines. In Ayurvedic medicine, they are applied to manage skin conditions such as eczema and psoriasis and are also considered beneficial for cholesterol management[39]. Digitalis-type saponins enhance cardiac muscle strength and hinder cancerous tumor growth while sparing healthy cells, acting akin to the plant's defense system against microbes. The antioxidant capabilities of *Solenostemon monostachyus* were assessed through the MTT assay and a 96-well microdilution method[38,40]. The extract concentrations ranged from 20-0.01 mg/mL, while those of the reference ascorbic acid ranged from 0.2-0.0001 mg/mL. The presence of antioxidant activity, indicated by the formation of bluish formazan, was observed from the highest dilution of 20 mg/mL down to the lowest at 0.08 mg/mL, across 2-fold dilutions. This suggests the presence of antioxidant compounds within these concentrations. Similarly, ascorbic acid exhibited antioxidant activity ranging from concentrations of 0.2-0.013 mg/mL. These results indicate that the antioxidant properties of *S. monostachyus* could potentially be beneficial for food preservation and mitigating oxidative damage associated with various diseases[41].

The antioxidant potential of *Solenostemon monostachyus* was further assessed using the MTT assay and a 96-well microdilution method. Results indicated antioxidant activity across various concentrations, comparable to the standard ascorbic acid. This suggests the plant's potential for use in food preservation and mitigating oxidative stress-related diseases.

The essential oil was analyzed on GC-MS (Shimadzu, Japan) using a capillary column (HP 5-MS) attached to a mass selective detector. The chromatogram showed 22 chemical components as shown in Fig. 2.

Table 2 provides a summary of the chemical constituents of *Solenostemon monostachyus* essential oil, listing 22 identified compounds along with their retention index (RI) values and percentage compositions. The major components include 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (12.04%), γ-Muurolene (9.57%)

Table 2: Chemical constituents of *Solenostemon monostachyus* essential oil

| Name | RI | Composition (%) |
|---|---|---|
| 1-Ethyl-2-methylbenzene | 973 | 3.73 |
| | | 1-Ethyl-2-methylbenzene is a natural product found in *Gossypium hirsutum, Lavandula angustifolia* and other organisms |
| 1,2,3-Trimethylbenzene | 998 | 1.65 |
| | | 1,2,3-Trimethylbenzene is a natural product found in *Ferulago nodosa, Vitis vinifera* and other organisms |
| 1-Octen-3-ol | 1009 | 2.00 |
| | | 1-Octen-3-OL is a natural product found in *Nepeta nepetella, Origanum syriacum* and other organisms |
| Mesitylene | 1013 | 6.75 |
| | | Mesitylene is a natural product found in *Lepidium meyenii* and *Ferulago nodosa* |
| o-Cymene | 1025 | 5.39 |
| | | O-Cymene is a natural product found in *Piper nigrum, Curcuma aromatica* and other organisms |
| 1-Methyl-3-propylbenzene | 1050 | 3.05 |
| 1-Methylindane | 1085 | 2.16 |
| 3,7-Dimethyldecane | 1126 | 2.62 |
| Isodurene | 1131 | 3.86 |
| Caryophyllene oxide | 1580 | 6.60 |
| Sabinol | 1140 | 1.38 |
| | | Sabinol is a natural product found in *Artemisia kaschgarica* |
| Dodecane | 1200 | 8.59 |
| | | Dodecane is a natural product found in *Erucaria microcarpa, Synechocystis* |
| α-Cubebene | 1338 | 2.08 |
| β-Caryophyllene | 1415 | 6.18 |
| α-Caryophyllene | 1448 | 6.14 |
| γ-Muurolene | 1478 | 9.57 |
| α-Farnesene | 1498 | 1.18 |
| Hexadecanoic acid | 1983 | 2.26 |
| 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 2118 | 12.04 |
| Phytol | 2123 | 3.99 |

RI: Retention index

and Dodecane (8.59%). Other notable compounds include Mesitylene (6.75%), Caryophyllene oxide (6.60%) and β-Caryophyllene (6.18%). Each compound is identified with its natural occurrence in various plants, highlighting the complex nature of the essential oil's composition and its potential bioactivity. The Table helps in understanding the predominant chemicals within the oil, which may contribute to its biological properties.

**High-Performance Liquid Chromatography analysis (HPLC):** The HPLC analysis revealed a total of nine peaks in the spectrum, corresponding to the highest peak at retention times of 3.538 min, as depicted in Fig. 3.

Caffeic acid and rutin were notably detected at retention times of 4.661 and 6.700 min, respectively. The chromatogram of *Solenostemon monostachyus* extracts indicated the presence of gallic acid, caffeic acid, ferulic acid, tannin, apigenin and rutin. These compounds are widely recognized as bioactive phenolic acids and flavonoids known for their strong antioxidant properties and other beneficial effects[42,43].

In conclusion, *Solenostemon monostachyus* exhibits significant pharmacological potential due to its rich phytochemical profile, including saponins, tannins, flavonoids, alkaloids, terpenes, sterols, reducing sugars, anthraquinones and essential oils. The antioxidant and antimicrobial activities observed in this study support its traditional use in folk medicine for treating various ailments. Further research into its specific mechanisms of action and clinical applications could provide valuable insights into its therapeutic benefits. This comprehensive investigation underscores the importance of *Solenostemon monostachyus* as a valuable source of natural bioactive compounds with potential applications in medicine and beyond.

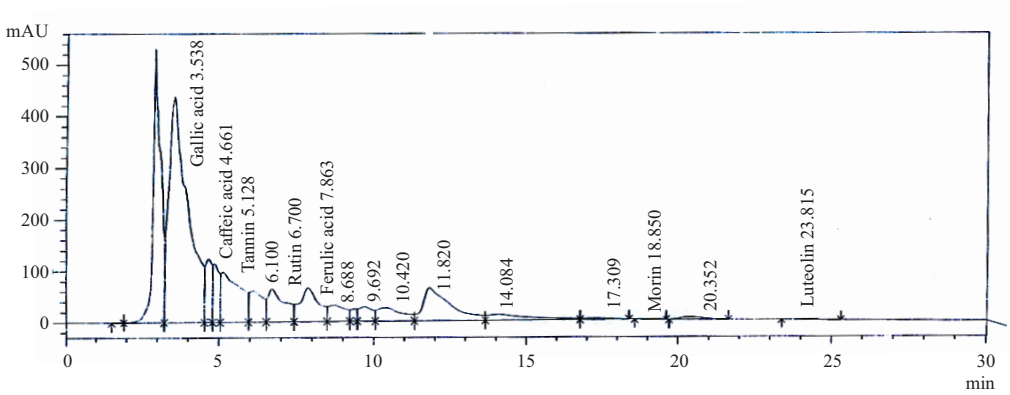


Fig. 3: HPLC profile of the hydrosol of *Solenostemon monostachyus*

## CONCLUSION

This study reveals that the aerial components of *Solenostemon monostachyus* contain abundant phytochemicals and essential oil, displaying antioxidant and antimicrobial characteristics. Furthermore, toxicity assessments confirm its suitability as an herbal treatment for diverse ailments. The results indicate that both the essential oil and water extracts of *Solenostemon monostachyus* exhibit antimicrobial and antioxidant properties, validating its traditional application in folk medicine.

## SIGNIFICANCE STATEMENT

The investigation into the aerial parts of *Solenostemon monostachyus* revealed a notable presence of essential oil and diverse phytochemicals, indicating its potential as a valuable natural resource. The essential oil predominantly contains limonene and neral, while the aqueous extract showcases a variety of phytochemicals, emphasizing the plant's pharmacological relevance. The significant antioxidant activity observed at various dilution points to the presence of potent compounds that can combat oxidative stress-related conditions. This study offers important insights into the antioxidant capacity and phytochemical profile of *S. monostachyus*, suggesting its promising medicinal properties and potential pharmaceutical applications.

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