Biomedical Compound Fingerprinting and Antioxidant Effect of Nyctanthes Arbortristis

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**Abstract:** Metabolite profiling has become a key technology for analyzing complex chemical mixtures and identifying compounds in biological samples. Among the various techniques, gas chromatography-mass spectrometry (GC-MS) stands out as a rapid and precise method widely utilized in diagnostics, functional genomics, and screening applications. After solvent extraction and derivatization, GC-MS can simultaneously characterize hundreds of metabolites from diverse chemical categories in a single analysis. This technique can efficiently detect various metabolites, including sugars, acids, polyols, and a range of phenolic and cyclic compounds. In this study, GC-MS-based metabolic profiling was performed on the medicinal plant *Nyctanthes arbortristis*. The methanolic leaf extract demonstrated a significant antioxidant effect, showing up to 92% inhibition in assays such as DPPH, nitric oxide scavenging, and free radical reduction, when compared to standard antioxidant drugs. The culinary and medicinal uses of *Nyctanthes arbortristis* further highlight its potent pharmacological potential in treating various diseases.

**Keywords:** Novel drug design, GC-MS, phytocemicals, *Nyctanthes arbortristis,* antioxidant, Health and well being.

# Introduction

Chromatographic techniques for the detection and identification of metabolites in plant materials have advanced considerably in recent years, mainly due to improvements in analysis speed, sensitivity, and separation efficiency (Aparna et al., 2021). These methods can be classified into targeted and non-targeted approaches depending on the research objectives (Ganapathy, 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021; Lisec et al., 2006). Gas chromatography (GC) is particularly renowned for its sensitivity and reliable ability to separate and detect complex mixtures. With the advent of high-resolution, high-throughput technologies, referred to as metabolomics, large-scale GC-MS profiling has become feasible, allowing for the identification of a wide range of metabolites across multiple experiments (Lisec et al., 2006). One of the most advantageous systems in this regard is GC coupled with time-of-flight mass spectrometry (GC-TOF-MS), which offers rapid scanning, high sensitivity, and precise mass measurements compared to traditional systems such as quadrupole GC-MS (GC-QMS) or ion trap GC-MS (GC-ITMS). Consequently, GC-TOF-MS has emerged as the standard in many metabolomics laboratories (Ganapathy; Merchant et al., 2022; Pandiyan et al., 2022).For GC-MS-based metabolite profiling, sample preparation generally involves solvent extraction, concentration to dryness, and subsequent derivatization, often following a two-step process (Chokkattu et al., 2022; Marya et al., 2022; Ramamurthy et al., 2022). In the initial step, methoximation (or methoxyamination) is carried out by reacting sample components with O-methoxylamine hydrochloride in pyridine. This stabilizes enolic aldehydes and ketones, converting them into oximes or alkyl oximes (Quanbeck et al., 2012; Hall et al., 2002). The second step involves the derivatization of metabolites with silylating reagents, which is crucial for converting non-volatile compounds into a form suitable for GC-MS analysis, enabling the detection of a wide range of metabolites, including those with polar characteristics and high boiling points (Jain & Verma, 2022; Sreevarun et al., 2023; Wadhwani et al., 2022). The resulting detectable compounds include sugars (mono-, di-, and trisaccharides), sugar alcohols/acids, amino acids, fatty acids, phosphorylated intermediates, and various plant secondary metabolites, such as phenolics, terpenoids, steroids, and alkaloids (Mushtaq et al., 2014).

# Materials and methods

## Sample collection and extraction

The leaves of *Nyctanthes arbortristis* were harvested from Kalvarayan Hills (Latitude: 8.495920838311422, Longitude: 78.13086270300187). After collection, the samples were thoroughly washed with fresh water and subsequently extracted using methanol (weight/volume). The methanolic extract was then dried and ground into a powder. This dried extract was utilized for biological screening.



**Figure.1:** Nyctanthes arbortristis leaves

## Gas-Chromatography and Mass Spectrometry phytochemical fingerprinting

The methanolic leaf extracts of Nyctanthes arbortristis were derivatized using BF3-methanol (10% boron trifluoride in methanol), following a modified procedure from Arbona et al. (2009). A 5 mg sample of the extract was placed in a 5-mL “V” vial, and 0.2 mL of the derivatization reagent was added with a syringe. The vials were heated at 60°C on a block heater for 10 minutes. After cooling, the samples were transferred to a separatory funnel with 2 mL of hexane. The samples were washed twice with a saturated sodium chloride (NaCl) solution and dried with anhydrous sodium sulfate (Na₂SO₄). The solvents were then evaporated using steam in a water bath. The residues were resuspended in 1 mL of hexane and transferred to a 1.5-mL vial for injection into the GC-MS system.The Eragrostis teff extracts were analyzed using GC-MS with a DB-5 column (Agilent, USA), 30 meters in length, with an internal diameter of 0.25 mm and a film thickness of 0.25 mm. The GC injector was set at 220°C, and the oven temperature was initially held at 40°C for 3 minutes, followed by an increase from 40°C to 250°C at 5°C/min, and held at 250°C for 2 minutes. The transfer line temperature was set to 250°C, with helium as the carrier gas at a flow rate of 1 mL/min. The mass spectrometer (MS) source operated in electron impact (EI) mode at 70 eV, scanning the m/z range from 40 to 500. A NIST library search was performed to identify the compounds.

## Antioxidant Assay

## DPPH Assay

To evaluate the DPPH radical scavenging activity, 1.0 mg of *Nyctanthes arbortristis* extract was mixed with 0.25 mL of a 0.5 mM DPPH solution in ethanol. After allowing the mixture to stand at room temperature for 20 minutes, the absorbance was measured at 517 nm. The scavenging activity was calculated as the percentage reduction in absorbance at 517 nm compared to the control (Kedare & Singh, 2011).

## Nitric Oxide Scavenging Activity

The nitric oxide scavenging ability was assessed based on its reaction with sodium nitroprusside, which produces nitric ions in the presence of oxygen molecules. The assay was carried out following the method of Naresh et al. (2015) with minor modifications. A 1.0 mg sample of *Nyctanthes arbortristis* extract was added to 3 mL of a reaction mixture containing 10 mM sodium nitroprusside in phosphate-buffered saline. The mixture was incubated at 25°C for 150 minutes. Every 30 minutes, 0.5 mL of the incubated solution was removed and mixed with 0.5 mL of Griess reagent. Absorbance was recorded at 546 nm. Quercetin was used as a reference antioxidant.

## Ferrous Ion Chelating Activity

Ferrous ion chelation was determined according to the method outlined by Dinis et al. (1994). A 1 mg sample of the extract was added to 0.05 mL of a 2 mM FeCl₂ solution. The reaction was initiated by adding 0.2 mL of 5 mM ferrozine, and the mixture was shaken vigorously and left at room temperature for 10 minutes. The absorbance of the solution was measured at 562 nm. A control sample, containing only FeCl₂ and ferrozine, was also prepared. The percentage of inhibition of the Fe²⁺-ferrozine complex was calculated using the following formula:

% Inhibition = [(A₀ – A₁) / A₀] × 100

Where A₀ represents the absorbance of the control, and A₁ is the absorbance in the presence of the sample or standard.

## Reducing Power Assay

The reductive capacity of *Nyctanthes* arbortristis extract was assessed using the method described by Güder & Korkmaz (2012). In this assay, 1 mg of the extract was dissolved in 1 mL of distilled water and mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide (K₃Fe(CN)₆). The reaction mixture was incubated at 50°C for 20 minutes. After incubation, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 1000g for 10 minutes. The upper layer (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl₃. The absorbance was measured at 700 nm to determine the reductive ability of the extract.

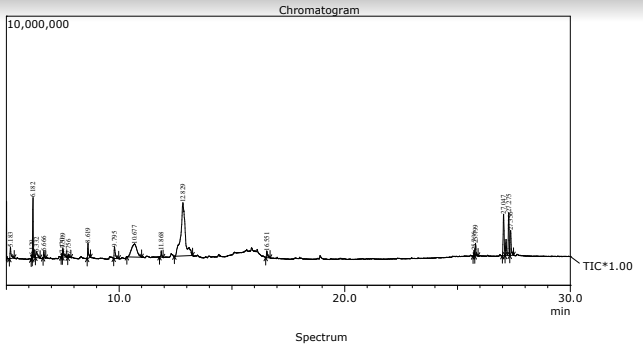
## Statistical analysis

The experiments were done in triplicate assay to obtain standard error mean ± values. One-Way ANOVA was performed to validate the p value of significance where p>0.5 was considered significant. SPSS package was used for One way ANOVA.

# Results

## Phytochemical analysis by GC-M

The GC-MS analysis of *Nyctanthes arbortristis* extracts revealed the presence of various phytochemical components, with fifteen distinct compounds identified. These fifteen compounds were represented by peaks in the chromatogram (Figure 17), corresponding to fifteen allelochemicals(Rafi et al., 2024). Upon comparing the mass spectra of these compounds with the NIST library, the allelochemicals were characterized and listed in Table 8. Some of these identified allelochemicals include Citral, Trans-α-Bergamotene, 3-Cyclohexanol, 1-methyl-4-(1-methylethenyl)-acetate, Cyclopentyl-1-propyne, β-Bisabolene, Cyclohexene, 1-methyl-4-(1-methylethylidene)-, and γ-Terpinene, among others (Table 1, Figure 2).



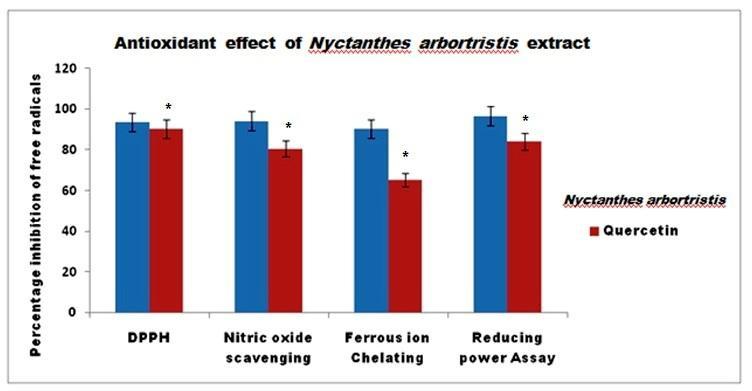
**Figure.2:** GC-MS spectrum of *Nyctanthes arbortristis* extracts

**Table.1:** Phytochemical profile of *Nyctanthes arbortristis* extracts

| No | RT | Name of compound | MF | CAS |
| --- | --- | --- | --- | --- |
| 1 | 4.264 | 3-cyclohexanol,1-methyl-4(1-methylethenyl)-, acetate | C12h20O | 10198-23-9 |
| 2 | 3.789 | Cyclopentyl-1-propyne | C8H12 | 116279-08-4 |
| 3 | 4.604 | 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)- | C10H18O | 20126-76-5 |
| 4 | 4.976 | Cyclohexene,1-metyl-4-(1-methylethylidene- | C10H16 | 586-62-9 |
| 5 | 5.768 | Bicyclo [2,2,1] heptan-2-one,1,7,7-trimethyl- | C10H16O | 464-48-2 |
| 6 | 5.470 | Y-Terpinene | C10H16 | 99-85-4 |
| 7 | 6.331 | Terpineol | C10H18O |  |
| 8 | 6.880 | (1R,5S,6R)-2,7,7-Trimethyl bicyclo[3,1,1]hept-2-en-6-yl acetate | C12H18O2 | 67999-48-8 |
| 9 | 7.327 | Citral | C10H16O | 5392-40-5 |
| 10 | 7.099 | 2,6-Octadien-1-ol,3,7-dimethyl-, | C10H18O | 106-25-2 |
| 11 | 8.524 | 2,6-Octadien-1-ol,3,7-dimethyl-acetate- | C12H20O2 | 141-12-8 |
| 12 | 8.770 | 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate | C12H20O2 | 25905-14-0 |
| 13 | 9.441 | Bicyclo[7.2.0)undec-4-ene,4,11,11-trimethyl-8-methylene- | C15H24 | 118-65-0 |
| 14 | 9.566 | Trans-a-Bergamotene | C15H24 | 13474-59-4 |
| 15 | 10.66 | B-Bisabolene | C15H24 | 495-61-4 |

## Antioxidant effect

The *Nyctanthes arbortristis* extract demonstrated significant in-vitro antioxidant activity, effectively scavenging free radicals and reducing agents. The extract exhibited a 93.57% scavenging effect against DPPH radicals, a 94.27% scavenging effect against nitric oxide, and a 90.47% chelation of ferrous ions (Tuluwengjiang et al., 2024). Additionally, a 96.68% reduction in radical activity was observed in the reducing power assay. Quercetin was used as a positive control antioxidant standard for comparison.(Figure.3).



**Figure.3:** Antioxidant effect of *Nyctanthes arbortristis* extract

# Discussion

This study investigates the phytochemical composition and antioxidant properties of the Nyctanthes arbortristis leaf extract. Phytochemical analysis revealed the presence of alkaloids, terpenoids, and phenolic compounds. In total, 15 allelochemicals were identified, including compounds such as Citral, Trans-α-Bergamotene, 3-Cyclohexanol, 1-methyl-4-(1-methylethenyl)-acetate, Cyclopentyl-1-propyne, and β-Bisabolene, as detailed in Table 8. The antioxidant activity of the leaf extract was assessed through DPPH radical scavenging and reducing power assays, both of which showed results comparable to standard antioxidant agents. The observed strong antioxidant effects can likely be attributed to the presence of phenolic compounds, terpenoids, and alkaloids (Subramanian & Harikrishnan, 2023).Previous research also supports the presence of common plant constituents in N. arbortristis leaves, such as alkaloids, steroids, tannins, flavonoids, reducing sugars, saponins, and terpenoids. Moreover, studies have suggested that the leaves may exhibit anti-asthmatic properties, potentially due to the presence of β-sitosterol (Marone et al., 1997). Additional bioactive compounds found in the leaves include iridoid glucosides, the primary alkaloid nyctanthin, arbortristosides A, B, and C, nyctanthic acid, tannic acid, D-mannitol, methyl salicylate, volatile oils, carotene, terpenoids, and cardiac glucosides (Rathore et al., 2007). These compounds align with the traditional medicinal use of the plant for treating conditions related to bronchoconstriction and its antioxidant benefits.

# Conclusion

*Nyctanthes* arbortristis extract has various phytochemicals in terms of alkaloids, terpenoids, fatty acids and phenols as their phytochemical profile which is quantified through GC-MS analaysis. At the same time Nyctanthes arbor-tristis extract showed strong antioxidant efficacy through preliminary experimental results. Further research on this will lead to the isolation of drug candidates for advanced biomedical research.

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