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**ARAŞTIRMA MAKALESİ** **RESEARCH PAPER**

# Antibacterial, Antioxidant, Antidiabetic Potentials and Chemical Composition of *Nicotiana glauca* Graham Leaf Extract

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**Abstract:** The purpose of this study is to investigate the chemical composition and potentiality of *Nicotiana glauca* G. leaf extract taken from the Turkish Republic of Northern Cyprus (TRNC), such as antibacterial, antioxidant, and antidiabetic compounds. In February 2020, a leaf of *N. glauca* was gathered in the Kyrenia district of Northern Cyprus. Methanol was used to extract the leaf sample. The content of essential oils in the extract was determined using gas chromatography and a headspace system test. Several methods were used to investigate the extract's antibacterial, antioxidant activities, and antidiabetic potential. On all microorganisms examined, the leaf methanol extract of *N. glauca* had no antibacterial activity. Total phenolic and flavonoid content, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, ferric reducing antioxidant power, metal chelating, and phosphomolybdenum assays were all active with the methanol extract. The extract showed no antidiabetic efficacy. Furthermore, the essential oil was not found in the extract's chemical composition. According to the findings, the presence of antioxidant components in *N. glauca* leaf extract can protect against the detrimental effects of free radicals. Its application in Northern Cyprus becomes even more crucial as a result of this potential feature.

**Keywords:** Bioactive compounds, methanol extract, *Nicotiana glauca*, Northern Cyprus.

# *Nicotiana glauca* Graham Yaprak Ekstraktının Antibakteriyel, Antioksidan, Antidiyabetik Potansiyelleri ve Kimyasal Bileşimi

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**Öz:** Bu çalışmanın amacı, Kuzey Kıbrıs Türk Cumhuriyeti'nden (KKTC) alınan *Nicotiana glauca* G. yaprak ekstraktının kimyasal bileşimini ve antibakteriyel, antioksidan ve antidiyabetik bileşikler gibi potansiyelini araştırmaktır. Şubat 2020'de Kuzey Kıbrıs'ın Girne ilçesinde *N. glauca* yaprağı toplandı. Yaprak örneğini ekstrakte etmek için metanol kullanıldı. Ekstrakttaki uçucu yağların içeriği, gaz kromatografisi ve headspace sistem testi kullanılarak belirlendi. Ekstraktın antibakteriyel, antioksidan aktivitelerini ve antidiyabetik potansiyelini araştırmak için çeşitli yöntemler kullanıldı. İncelenen tüm mikroorganizmalar üzerinde, *N. glauca*'nın yaprak metanol ekstraktının antibakteriyel aktivitesi yoktu. Toplam fenolik ve flavonoid içeriği, 1,1-difenil-2-pikrilhidrazil radikal süpürme aktivitesi, ferrik indirgeyici antioksidan gücü, metal şelatlama ve fosfomolibden deneylerinin tümü metanol ekstraktı ile aktifti. Ekstrakt antidiyabetik etkinlik göstermedi. Ayrıca, ekstraktın kimyasal bileşiminde uçucu yağ bulunmadı. Elde edilen bulgulara göre, *N. glauca* yaprak ekstraktında antioksidan bileşenlerin bulunması, serbest radikallerin zararlı etkilerine karşı koruma sağlayabiliyor. Bu potansiyel özelliği nedeniyle Kuzey Kıbrıs'ta uygulanması daha da önem kazanmaktadır.

**Anahtar Kelimeler:** Biyoaktif bileşikler, Kuzey Kıbrıs, metanol ekstraktı, *Nicotiana glauca*.

## INTRODUCTION

*Nicotiana glauca* is a species of Nicotiana. *N. glauca* Graham, often known as tree tobacco, is a garden ornamental and evergreen perennial plant in the Solanaceae family. South and North America, Western, Southern, and Northern Europe, Western United States, Australia, Southern, and Northern Africa, and Western Asia are among the plant's habitats (Abdel Rahman et al., 2011; Alharthi et al., 2021; Bogdanovic et al., 2006; DiTomaso et al., 2013; Kasiotis et al., 2020; Ollerton et al.,

2012; Rinez et al., 2012; Saliha et al., 2018; Trifa et al., 2020;). In Northern Cyprus, it is known as 'Doktor Ağacı' and 'İngiliz Yaprağı' among the locals. It's a 2-4 m tall sparsely branched tree or forested shrub with vertical growth. It has glabrous, oval-shaped leaves with a cylindrical tip that is 8 cm long on the stem. Calyx has 5 teeth, is 1 cm in length, and is shaped like a tube. The greenish-yellow corolla is 3-4 cm long and tubular. The fruit is egg-shaped and 1 cm in length. Yellow flowers are in bloom all year. This plant produces a great number of tiny seeds, which are easily disseminated by the wind and water. Wasteland, roadsides, stream beds, around ancient homes, ditch banks, near farmed areas, and sandy or gravelly soils along river banks are all common places to find it (Alharthi et al., 2021; Bogdanovic et al., 2006; DiTomaso et al., 2013; Rinez et al., 2012; Saliha et al., 2018; Trifa et al., 2020;).

Antimicrobial, anti-inflammatory, cytotoxic, and allelopathic activities have been observed in *N. glauca* extracts. They are often utilized as medications by traditional healers due to these qualities (Abdel Rahman et al., 2011; Rinez et al., 2012; Trifa et al., 2020). This plant's leaf is useful in the healing of abscesses and wounds in humans. The leaf has a thin waxy layer on it. To cure skin lesions in Northern Cyprus, this layer is removed from the leaf and the remaining leaf is placed on the inflamed wound. Furthermore, *N. glauca* extract containing the insecticidal anabasine (nicotine-like alkaloid) is utilized to reduce crop insect diseases (Saliha et al., 2018). However, there are several drawbacks to this plant. This plant's toxin, anabasine, is extremely dangerous to people and animals (Alharthi et al., 2021; DiTomaso et al., 2013; Rinez et al., 2012; Trifa et al., 2020;). Anabasine can induce fetal abnormalities in animals even if the mother eats small amounts of this plant during early pregnancy (DiTomaso et al., 2013; Trifa et al., 2020). Furthermore, it is a reservoir plant for Tobacco Mosaic Virus (TMV) and Tomato Infectious Chlorosis Crinivrus (TICV), both of which infect a wide range of plant species that have been studied in this plant as a host from around the world (Alharthi et al., 2021).

The study is unique and worthwhile because there have been no investigations on the *N. glauca* in Northern Cyprus or Turkey. The goal of the investigation is to explore the antibacterial, antioxidant, and antidiabetic effects of *N. glauca* methanolic leaf extract.

## MATERIAL AND METHOD

***Collection and preparation of plant material*:** In February 2020, a leaf of *N. glauca* was gathered in the Kyrenia district of Northern Cyprus. A paper towel was used to clean the leaf that had been collected. The sample's total weight of wet was 250 grams. Afterward, it was divided into little pieces with a hand and dried for 6 hours in a 50°C oven. The sample weighed a total of 10 grams dry. For subsequent investigation, the dried materials were pulverized and stored in a +4°C refrigerator.

***Extraction*:** Methanol (1:10 [w/v]) was used to extract the leaf of *N. glauca* for 72 hours at room temperature under shaking conditions. For extraction, 10 g of *N. glauca* leaf was utilized. Wattman No. 4 paper was then used to filter the extract. For chemical and biological investigations, the extract was kept at +4°C.

***Antibacterial activity*:** The antibacterial activity of the extract was tested on Mueller Hinton Agar (MHA) using the standard method which followed the Kirby-Bauer disc diffusion method, as per the Clinical Laboratory Standard Institute (CLSI) standards (Owusu et al., 2021; CLSI, 2012). The overnight bacterial cultures' turbidity was arranged to the McFarland standard reference range of 0.5. A pipette was used to transfer 10 µl of each microbial suspension to MHA, which was then spread evenly on the surface with a wooden cotton applicator stick. The antimicrobial blank discs which were sterile and had been treated with 20 µl of the extract were kept apart. Petri plates were incubated at 37˚C for 12-24 hours after inoculation. Following, the zones of inhibition surrounding the discs were assessed. Positive controls included Erythromycin (E; 15 µg/disc) for *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 25922; Methicillin (M; 5 µg/disc) for *Staphylococcus aureus* ATCC 25923; Penicillin (P; 10 units/disc) for *Bacillus cereus* ATCC 11778 and Polymyxin B (PB, 300 unit/disc) for *Pseudomonas aeruginosa* ATCC 27853. As a negative control, methanol was employed.

***Total phenolic (TPC) and flavonoid content (TFC):*** Folin-colorimetric Ciocalteu's method was used to determine total phenolic content as (mg GAE)/g (mg of gallic acid equivalents) (Stanković, 2011). Sharma and Vig's technique was used to measure the total flavonoid content as (mg RE)/g (mg of routine equivalent) (Sharma & Vig, 2013).

***Headspace analyses of gas chromatography:*** A 3 g powdered leaf sample was tested using gas chromatography (GC) and a headspace technique to determine the essential oil content in the extract. Following optimization methods, the following headspace conditions were determined: Incubation time (min): 30; incubation temperature (°C); 80; sample volume (µL): 1; syringe temperature (°C): 80. In the analysis, an HP-5 MS capillary column which includes a mass selective detector 7890B GC-5977MSD (Agilent, Santa Clara, USA) was performed. The following gas chromatographic circumstances were used: initial column temperature of 50°C for 2 minutes, subsequently raised to 150°C at 10°C/min, and hold for 5 minutes. Following that, the temperature was steadily increased to 240°C under the

same conditions and maintained for 5 minutes. Samples were automatically injected at a 50:1 split ratio (Sevindik, 2020). The electronic NIST14 library was used to identify the components (2014 Version). The apparatus software computed the percentage quantities (%) of every constituent in the sample from the area of the total peaks.

***Analyses of antioxidant activity***

***Scavenging capacity of DPPH˙ radical:*** The free radical scavenger method relies on the 1,1-diphenyl-2-picrylhydrazyl (DPPH˙) reagent solution becoming colorless according to the electron or proton-transfer capability of the samples. 100 µL of the extract was mixed with a solution of 3.9 mL of DPPH˙ reagent (0.025 g/L in methanol) prepared in methanol (0.1 mM) for this analysis. This combination was kept at room temperature in the dark for 30 minutes to supply the chemical reaction. Afterward, the combination's absorbance was measured spectrophotometrically at 517 nm (Biochrom, Libra S60, B, England) against a methanol blank. The scavenging activity of DPPH˙ was evaluated in Trolox equivalent (mg TE/g) (Blois, 1958; Ucan Turkmen & Mercimek Takci, 2018).

***FRAP (Ferric reducing antioxidant power):*** The ability of antioxidant compounds in the extract to reduce $Fe^{3+}$ to $Fe^{2+}$ was investigated in this assay (Oyaizu, 1986). The Prussian blue color's absorbance produced by the addition of $FeCl_3$ to the reaction mixture was measured. 1 mL of extract was combined with 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$ and 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6). This reaction mixture was incubated for 20 minutes at 50°C. To stop this reaction, 10% TCA (Trichloroacetic acid) was added and the mixture was centrifuged for 10 minutes at 2500 rpm. To 2.5 mL of supernatant, an equal volume of distilled water and 0.5 mL $FeCl_3$ (0.1%) were added. At 700 nm, the reaction mixture's absorbance was measured (Biochrom, Libra S60, B, England). Trolox equivalent (µg TE/g) was used to measure the extract's reducing capability.

***Activity of metal-chelating:*** Dinis et al., (1994) established a method for determining the extract's $Fe^{2+}$ chelating activity. The competition of metal-binding chemicals in the extract with ferrozine (a forceful iron-chelating agent) is the basis for this technique (Dinis et al., 1994). The production of the red $Fe^{2+}$/ferrozine complex is avoided by compounds with a high metal ion binding ability. 1 mL of extract was mixed with 3.7 mL distilled water and 100 µL of 2 mM $FeCl_2$. After 30 minutes of incubation at room temperature, 200 µL of 5 mM ferrozine solution was added to the reaction and stirred for 10 minutes. At 562 nm, the reaction mixture's absorbance was measured (Biochrom, Libra S60, B, England). The following equation was used to calculate chelating activity as a percentage of inhibition (%).

$$\% \text{ chelating activity} = (1-(A_{sample}/A_{control})) \times 100$$

***Phosphomolybdenum method:*** By using the phosphomolybdenum technique, the total antioxidant capacity was measured spectrophotometrically (Zengin et al., 2014). 300 µL extract was quickly combined with 3 mL of a reactive solution containing 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid. The absorbance was measured at 695 nm after 90 minutes of incubation at 95°C (Biochrom, Libra S60, B, England). Total antioxidant capacity was evaluated in Trolox equivalent (µg/TE g). At least three times, each spectrophotometric analysis was performed.

***Antidiabetic potential:*** The mixture which is in the test tube containing 1 mL extract, 1 mL 20 mM sodium phosphate buffer (pH:6.9), and 1 mL starch solution (1% w/v) was incubated at 37°C for 5 min for the α-amylase assay. After that, 1 mL of α-amylase solution was added to this mixture to begin the reaction. After 30 minutes, 1 mL of color reagent composed of 96 mM 3,5-dinitrosalicylic acid solution, 2 M NaOH, and 5.31 M sodium potassium tartrate solution was used to terminate the reaction. Its absorbance was measured at 540 nm (Biochrom, Libra S60, B, England) after the mixture was boiled for 5 minutes (Başyiğit et al., 2020).

The mixture which is in the volumetric flask containing 10 µL of extract and 40 µL of α-glucosidase enzyme solution was incubated at 37°C for 5 minutes for the α-glucosidase assay. The flask was then filled with 950 µL of 0.7 mM 4-nitrophenyl-α-D-glucopyranoside solution containing 50 mM phosphate buffer and 100 mM NaCl. The reaction was terminated by adding 1000 µL of 0.5 M Tris after 15 min of incubation at 37°C. At 400 nm, the absorbance was measured (Güngör Bilen, 2004). (Biochrom, Libra S60, B, England). All antidiabetic potential tests were repeated three times.

## RESULTS

As shown in Table 1., *N. glauca* leaf extract and negative control did not exhibit antibacterial activity against all tested microorganisms in this study. *B. cereus* ATCC 11778, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, and *P. aeruginosa* ATCC 27853 displayed antibacterial activity against commercial penicillin, erythromycin, and polymyxin B, respectively. Conversely, methicillin had no antibacterial effect against *S. aureus* ATCC 25923. Table 2. displayed the results of TPC, TFC, antioxidant activity, and antidiabetic potential of *N. glauca* leaf extract. The TPC and TFC values were determined to be 0.241±0.009 mg GAE/g and 0.1923±0.002 mg RE/g, respectively. The extract's DPPH˙ radical scavenging activity was compared to the standard antioxidant activity of Trolox equivalent (mg TE/g). The extract's DPPH˙ was found to be 27.8±0.003% (0.019±0.003 mg TE/g). The

ferric reduction capacity, metal chelating activity, and phosphomolybdenum assay results were 7.2±0.905 µg TE/g, 60.8±1.70%, and 66.50±0.827 µg TE/g, respectively. According to the findings of this study, the presence of antioxidant components in the extract could be effective in combating the detrimental effects of free radicals. The extract did not significantly affect the enzymatic activities. Table 3. showed the result of the extract's gas chromatography headspace assay in terms of retention time (min) and percentage (%). Using the NIST14 mass spectra library, 20 chemicals were detected in Gas chromatography-mass spectrometry (GC/MS). Ethylene oxide (44.15%), acetaldehyde (37.91%), dimethyl sulfide (5.19%), pentanal (3.50%), 1-propanal (2.19%), and propanal, 2-methyl (1.68%) were the most active components. Our findings suggested that the extract's chemical composition did not contain any essential oil.

**Table 1.** Diameter of the inhibition zone (mm) of *N. glauca* leaf extract.

| | ***N. glauca* extract** | **Positive Control** | **Negative Control** |
|---|---|---|---|
| *B. cereus* ATCC 11778 | - | 40 mm (Penicillin) | - |
| *S. aureus* ATCC 25923 | - | - (Methicillin) | - |
| *S. typhimurium* ATCC 14028 | - | 16 mm (Erythromycin) | - |
| *P. aeruginosa* ATCC 27853 | - | 20 mm (Polymyxin B) | - |
| *E. coli* ATCC 25922 | - | 16 mm (Erythromycin) | - |

(-) represents a no-inhibition zone against microorganisms.

**Table 2.** The total phenolic, total flavonoid content, antioxidant activity, and antidiabetic potential of *N. glauca* methanol leaf extract.

| | ***N. glauca* methanol leaf extract** |
|---|---|
| Total phenolic content (mg GAE/g) | 0.241±0.009 |
| Total flavonoid content (mg RE/g) | 0.1923±0.002 |
| DPPH (% / mg TE/g) | 27.8±0.003 / 0.019±0.003 |
| Ferric reducing capacity (µg TE/g) | 7.2±0.905 |
| Metal ($Fe^{2+}$) chelating activity (%) | 60.8±1.70 |
| Phosphomolybdenum (Total antioxidant capacity) (µg TE/g) | 66.50±0.827 |
| α-amylase activity | ND |
| α-glucosidase activity | ND |

Values are mean ± Standard deviation (SD) of three replicate analyses. ND: Not detected.

**Table 3.** The retention time (min) and percentage (%) of the chemical composition of *N. glauca* methanol leaf extract.

| **Compound** | ***N. glauca* methanol leaf extract** | |
|---|---|---|
| | **Retention time (min)** | **%** |
| Ethylene oxide | 2.359/2.475 | 44.15/37.91 |
| Carbon dioxide | 2.359/2.475 | 44.15/37.91 |
| Acetaldehyde | 2.475 | 37.91 |
| Dimethyl sulfide | 3.470 | 5.19 |
| Propanal | 3.757 | 0.89 |
| 3-Pentanamine | 3.757 | 0.89 |
| N, 1- Dimethylhexylamine | 3.757 | 0.89 |
| Propanal, 2-methyl | 3.978 | 1.68 |
| 1-Propanol | 4.877 | 2.19 |
| 2- Propanone, 1-hydroxy-, oxime | 4.877 | 2.19 |
| Butanal, 3-methyl- | 5.142/6.007 | 3.50/0.40 |
| Pentanal | 5.142 | 3.50 |
| Furan, 2-ethyl | 5.614 | 0.78 |
| 1, 4-Hexadiene, 5-methyl- | 5.614 | 0.78 |
| 2- Pentanone | 5.889 | 0.70 |
| 1- Octadecanamine, N-methyl- | 6.007 | 0.40 |
| Hexanal | 7.493 | 1.31 |
| 1-Penten-3-ol | 8.614 | 0.49 |
| Acetic acid | 12.739 | 0.60 |
| Benzaldehyde | 14.059 | 0.22 |

## DISCUSSION

The antibacterial activity of the *N. glauca* leaf methanol extract was evaluated on two Gram-positive and three Gram-negative bacterial strains. Antibacterial activity was not found in the extract against all of the microorganisms tested.

Due to the solvent difference used, Alghamdi, (2021) found the antibacterial activity of ethyl acetate leaf extracts of *N. glauca* against *E. coli* (16.3±0.71 mm) and *S. aureus* (11±0.23 mm), respectively. Furthermore, floral extracts had antibacterial activity against *E. coli* (6.7±0.65 mm) and *S. aureus* (15.8±0.52 mm). The MIC's of the *N. glauca* ethyl acetate extract against *E. coli* and *S. aureus* were found to be 1.5 and 2 mg/ml, respectively. Contrary to our results, Alghamdi determined that the leaf extract was effective against *E.coli* and *S. aureus*. On the other hand, root and stem extracts had no inhibition activity against both bacteria, similar to our findings (Alghamdi, 2021).

Aldesuquy et al., (2018) indicated that the aqueous shoot extract of *N. glauca* showed antimicrobial activity against *B. subtilis* (14.50±0.50 mm), *Klebsiella pneumonia* (13.00±0.66 mm), *Streptococcus pyogenes* (11.00±0.12 mm), *Candida albicans* (11.00±0.35 mm), *Erwinia carotovora* (8.50±0.50 mm) and *E. coli* (7.50±0.36 mm), respectively. But, the extract was no inhibitory effect against *S. aureus.* Considering the use of different plant parts such as shoot, they found that the aqueous extract was efficient against *E. coli*, which is contrary to our findings. However, like in our study, they also revealed that the extract was ineffective against *S. aureus* (Aldesuquy et al., 2018).

The antibacterial activity of methanol and hexane fresh aerial parts extracts of *N. glauca* was tested against both Gram-positive and Gram-negative bacteria known to cause infectious disease in humans. The methanol extract (6 mg/mL) of *N. glauca* displayed an antibacterial effect against *B. cereus* (15 mm), *S. aureus* 72 (5 mm), *S. aureus* 132 (11 mm), and *S. aureus* 224 (22 mm). On the contrary, the methanol extract at 6 mg/mL had no inhibitory impact on *Staphylococcus epidermidis*, *E. coli*, *Yersinia enterocolitica* ss. *Enterocolitica* ATCC 23715, and *Salmonella enterica* ATCC 25566. Similarly, with methanol extract, any inhibitory effect did not observe for hexane extract towards *K. oxytoca*, and *S. enterica* ATCC 25566 (Abdel Rahman et al., 2011). Abdel Rahman et al., (2011) reported that the methanol extract had an antibacterial impact on *B. cereus* and *S. aureus*, which is in contrast to our findings. However, it lacked any antibacterial effect against *E. coli*, confirming our findings.

Extracts showed antibacterial efficacy against *E. coli*, *S. aureus*, and *B. cereus* in studies by some researchers (Abdel Rahman et al., 2011; Aldesuquy et al., 2018; Alghamdi, 2021). In our study, however, the methanolic extract showed no inhibitory zone against *B. cereus*, *E.coli*, and *S. aureus*. The antibacterial activity results of *N. glauca* extract against *S. aureus* and *E. coli*

were akin to their findings of them (Abdel Rahman et al., 2011; Aldesuquy et al., 2018).

Today, it is widely known that phenolics contribute the most to plants' antioxidant activity. Thus, determining the extract's phenolic concentration is crucial. *N. glauca* methanol leaf extract had TPC and TFC values of 0.241±0.009 mg GAE/g and 0.1923±0.002 mg RE/g, respectively. The TPC and TFC results from many investigations with various *N. glauca* extracts are presented below.

TPC (mg GAE/g) and TFC (mg QE/g) values of DCM (dichloromethane), AE (ethyl acetate), and n-BuOH (n-butanol) extracts of *N. glauca* leaves were determined as 133.80±0.06–1.18±0.005, 351.55±0.07-105.97±0.04 and 284.98±0.08-164.44±0.07, respectively (Trifa et. al., 2020).

In another study, TPC and flavonoid values of *N. glauca* shoot aqueous extract were 24.11±0.10 and 7.28±0.11 mg/g d wt, respectively (Aldesuquy et al., 2018).

The total phenolic content (TPC) and total flavonoid content (TFC) of *N. glauca* (stem) active fraction were reported as 108.18 and 20.8 µg/mL, respectively (Tabana et al., 2015).

The total phenolic compounds and flavonoid contents of different parts (root, stem, leaves, and flowers) of four *N. glauca* which have different localities and habitats (sandplain, sand dune, salt marsh, and rocky ridge) were notified by Hassan et al., (2014). The phytochemicals (TPC and TFC) screening related to different parts of the *N. glauca* plant where the highest amounts were found in leaves and flowers extracts. The highest content of phenolic compounds (5.31±1.09 mg/g dry weight) and flavonoids content (291.39±2.55 mg/g dry weight) was recorded in the leaves of *N. glauca* growing in salt marsh and sand dune, respectively (Hassan et al., 2014).

Najah et al., (2015) revealed the flavonoid content of *N. glauca* leaves and flowers extracts in water, ethanol, ethyl acetate, chloroform, and hexane. The presence of flavonoid compounds was not encountered in the other extracts except the aqueous extract for the plant. According to the flavonoid and phenolic results of the literature studies, other researchers detected flavonoid and phenolic compounds using various polar solvents. This researcher was the only one who used methanol similar to us (Abdel Rahman et al., 2011).

DPPH$^{\cdot}$ of *N. glauca* methanol leaf extract was determined as 27.8±0.003%-0.019±0.003 mg TE/g. Moreover, ferric reducing capacity, metal chelating activity, and phosphomolybdenum assay findings were found to be 7.2±0.905 µg TE/g, 60.8±1.70%, and 66.50±0.827 µg TE/g, respectively.

Various *in vitro* methods related to screening of antioxidant capacity were performed in previous studies. DPPH, ABTS, DMSO alkaline, Phenantronile, FRAP and CUPRAC assay of *N. glauca* leaves DCM (dichloromethane), AE (ethyl acetate), and n-BuOH (n-butanol) extracts were determined by Trifa et al. (2020). A lower $IC_{50}$ value has indicated higher scavenging activity. For DPPH, $IC_{50}$ values of DCM, AE, and n-BuOH extracts were 47.17±0.67, 9.31±0.92, and 7.40±0.41 µg/mL, respectively. For ABTS, $IC_{50}$ values of DCM, AE, and n-BuOH extracts were found to be 17.51±1.23, 5.39±0.64, and 12.04±0.43 µg/mL, respectively. For DMSO alkaline, $IC_{50}$ values were 3.33±0.22, 2.32±0.14, and 3.04±0.16 µg/mL for DCM, AE, and n-BuOH extracts, respectively. For Phenantroline, $IC_{50}$ values of DCM, AE, and n-BuOH extracts were 96.33±1.30, 46.33±0.77, and 81.98±2.16 µg/mL, respectively. For FRAP, $IC_{50}$ values of DCM, AE, and n-BuOH extracts were found to be 115.83±1.83, 19.06±0.50, and 35.71±3.50 µg/mL, respectively. For CUPRAC, $IC_{50}$ values were 44.26±0.80, 13.78±0.26, and 21.24±1.30 µg/mL for DCM, AE, and n-BuOH extracts, respectively. According to the antioxidant results of extracts, the highest activity for DPPH analysis was observed in the n-BuOH extract. However, in other antioxidant assays, AE extract showed good activity (Trifa et al., 2020).

The antioxidant activity of *N. glauca* aqueous extract was determined by DPPH$^{\cdot}$ radical scavenging activity. The $IC_{50}$ value of the extract was 0.12 mg/mL (Aldesuquy et al., 2018).

Three techniques were used to test the antioxidant activity of the *N. glauca* (stem) active fraction. $IC_{50}$ values of DPPH, FRAP, and ABTS were found at 86.65, 528.61, and 67.58 µg/mL, respectively (Tabana et al., 2015).

The values of the non-enzymatic antioxidant activity of *N. glauca* were determined by the formation of the green phospho-molybdenum complex. The highest values of root, stem, leaves, and flowers were determined as 3.54±0.17, 4.92±0.21, 9.04±1.07, and 9.95±0.58 mg ascorbic acid equivalent/g dry weight, respectively (Hassan et al., 2014). In previous studies, the antioxidant activity of the extracts prepared by using different solvents from methanol was tested. Similar and different techniques were used to detect antioxidant activity in literature. Their findings are in agreement with our results.

Such bioactive properties (antioxidant activity and phytochemical content) support our idea that extracts prepared by various methods of *N. glauca* harvested from different areas can be a helpful source of medical and economic importance.

The antidiabetic potential of *N. glauca* leaf methanol extract has never been investigated before. The methanol extract of *N. glauca* leaf did not show any antidiabetic potential. For this reason, the authors could not

make a comparison regarding this result with the same species.

Similarly, the hypoglycemic potential of *Nicotiana tabacum* acetone, ethanol, and water leaf extracts was tested by *in vitro* enzymatic methods. They observed that aqueous extract was the best inhibitory potential on α-amylase ($IC_{50}$ value 5.70 mg/mL) and the most effective inhibitor for α-glucosidase was acetone extract with an $IC_{50}$ value of 4.50 mg/mL (Kazeem et al., 2014).

Unlike our results, it has been determined in the literature that extracts made with different strains of *Nicotiana* have antidiabetic potential.

Moreover, any essential oil was not demonstrated in *N. glauca* methanol leaf extract. Therefore, the authors discussed its chemical substances with the previous studies.

In one of the studies, a total of 29 compounds were determined using GC-MS analysis. The volatile oil of extract of *N. glauca* was determined to be affluent in terms of oxygenated sesquiterpenes such as *β*-bisabolol (9.02%) and carboxylic acids and esters such as ethyl linoleate (34.79%) and hexadecanoic acid (10.38%). In addition, 9,17-octadecadienal was detected as 10.83% (Massadeh et al., 2022).

The predominant ingredient in *N. glauca* leaves extract (58.49%) was eugenol. Eugenol was followed by nonadecane, eugenly acetate, tridecane, 3-methyl, and heptadecane, 8-methyl was 6.38%, 5.57%, 5.19%, and 4.19% respectively (Cherif et al., 2019).

In another study, they found various polyphenols and aromatic compounds including bicyclo heptanes, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, scopoletin, D-alpha-tocopherol, campesterol, stigmasterol and beta-sitosterol in the stem of *N. glauca* active fraction (Tabana et al., 2015).

In a prior work, in hexane extract of *N. glauca* leaves, 9 components were detected: hexacosenol, nonacosane, triacontane, octacosenol, nonacosonal, hentriacontane, dotriacontane, tritriacontane, and total hydrocarbon (Mortimer et al., 2012).

Literature findings indicated variations in GC-MS metabolomics of *N. glauca* extracts. Our GC/MS results to determine the main components of *N. glauca* showed major differences with published data. These differences might be explained by analytical procedures, cultivation and growth conditions, geographical variations, and genetic factors.

## CONCLUSION

In conclusion, the findings of the bioactive study revealed that *N. glauca* contains a high amount of phytochemical strong components, including a fraction that is useful as an antioxidant. We show the total phenolic and flavonoid components, as well as the antioxidants that are responsible for its activity. The scavenging activities of hydroxyl condensation, DNA fragmentation, and chromatin group in the present assay revealed a substantial link between the presence of phenolic compounds and antioxidant capacity. The relevance of its consumption as a natural resource and the traditional therapeutic use of *N. glauca* is highlighted by our findings relating to the antioxidant activity of the extract.

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