Process Biochemistry 125 (2023) 61–74

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

In vitro and *in silico* bioactivities and chemical profiling of *Nepeta leucolaena* to validate its use in nutraceutical or biopharmaceutical applications

Shabnam Mustafa [a], Mamona Nazir [b], Naheed Riaz [a], Muhammad Saleem [a,*], Saba Tauseef [c], Muhammad Imran Tousif [d,*], Zaheer Abbas [e], Abdul Basit Kamran [d], Wed Mohammed Ali Alaerjani [f], Saleh S. Alarfaji [f], Shabbir Muhammad [f], Gokhan Zengin [g,*], Mohamad Fawzi Mahomoodally [h,i,j], Mohammad Ali Shariati [k,l]

[a] *Institute of Chemistry, Baghdad Campus the Islamia University of Bahawalpur, 63100 Bahawalpur, Pakistan*
[b] *Department of Chemistry, Government Sadiq College Women University Bahawalpur, 63100 Bahawalpur, Pakistan*
[c] *Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan*
[d] *Department of Chemistry, Division of Science and Technology, Education University Lahore, 54000, Pakistan*
[e] *Department of Botnay, Division of Science and Technology, Education University Lahore, 54000, Pakistan*
[f] *Department of Chemistry, College of Science, King Khalid University, Abha 61413, P.O. Box 9004, Saudi Arabia*
[g] *Department of Biology, Science Faculty, Selcuk University, Konya, Turkey*
[h] *Department of Health Sciences, Faculty of Medicine and Health Sciences, University of Mauritius, Réduit 80837, Mauritius*
[i] *Center for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College, Saveetha Insti-tute of Medical and Technical Science, Chennai, India*
[j] *Centre of Excellence for Pharmaceutical Sciences (Pharmacen), North West University, Potchefstroom 2520, South Africa*
[k] *Department of Scientific Research, K.G. Razumovsky Moscow State University of technologies and management (The First Cossack University), 73 Zemlyanoy Val, Moscow 109004, Russian Federation*
[l] *Department of Scientific Research, Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, 49 Timiryazevskaya str., Moscow 127550, Russian Federation*

ARTICLE INFO

Keywords:
Nepeta leucolaena
UHPLC-MS analysis
Total bioactive contents
Antioxidant activities
Enzyme inhibition studies
Docking studies

ABSTRACT

Nepeta leucolaena grows worldwide and is being used in folk medicine system without any scientific evidence. The present study was carried out to unveil the metabolic picture, biological potential and to valid the medicinal uses of *N. leucolaena*. The flowers and leaves of this plant were separately extracted with *n*-hexane, chloroform, ethyl, acetate and alcohol to get respective extracts. The total bioactive contents estimation disclosed that the highest value of phenolics (39.50 ± 0.53 mg GAE/g extract) and flavonoid (24.99 ± 0.53 mg GAE/g extract) in ethanolic extract of flowers and leaves, respectively. Further chloroform extract of flowers, and ethyl acetate extract of leaves also contain significant amount of phenolics. In DPPH free radical scavenging assay, only ethanolic extracts of flowers and leaves exhibited antioxidant activity with values of 49.02 ± 0.12 and 46.33 ± 0.10 mg TE/g extract, whereas, in ABTS free radical scavenging activity, in addition to ethanolic extract flowers (113.37 ± 0.07 mg TE/g extract) and ethanolic extract leaves (98.95 ± 0.57 mg TE/g extract), other extracts also showed moderate to weak activity. Highest metal reducing power was also associated with ethanolic extract of flowers and ethanolic extract of leaves (CUPRAC: 167.86 ± 3.57 and 151.76 ± 1.59 mg TE/g extract, respectively and FRAP: 108.30 ± 0.98 and 101.30 ± 0.32 mg TE/g extract, respectively), while other extracts also exhibited moderate activity. Almost equal (1.6 mmol TE/g extract) total antioxidant capacity was measured for ethanolic extract of leaves and flowers and *n*-hexane extract leaves, however, all fractions showed significant and comparable metal chelating activity with highest value was noticed for *n*-hexane extract flowers (25.99 ± 0.10 mg EDTA/g extract) and leaves (25.93 ± 0.79 mg EDTA/g extract). Similarly, all extracts showed nearly equal inhibitory activity against AChE (3.06–4.06 mg GALAE/g extract), while against BChE low polar extracts were more active with highest potential in *n*-hexane extract leaves (3.99 ± 0.20 mg GALAE/g extract) and chloroform extract of leaves (3.67 ± 0.48 mg GALAE/g extract). *n*-hexane extract of flowers displayed highest inhibitory contents against tyrosinase enzyme followed by ethyl acetate extract flowers and leaves. In anti-diabetic assays, the hexane extracts exhibited highest values against α-glucosidase (1.08 ± 0.006 and 1.05 ± 0.019 mmol ACAE/g extract, respectively) followed by ethyl acetate and chloroform extracts respectively. UHPLC-MS analysis of the

* Corresponding authors.
E-mail addresses: m.saleem@iub.ed.pk (M. Saleem), mimrantousif@gmail.com (M.I. Tousif), gokhanzengin@selcuk.edu.tr (G. Zengin).

https://doi.org/10.1016/j.procbio.2022.12.005
Received 10 October 2022; Received in revised form 29 November 2022; Accepted 5 December 2022
Available online 7 December 2022

ethanolic extract of the whole plant (Flowers and leaves together) helped to identify 139 compounds mostly of phenolic, flavonoid, steroids, terpenoids and glycoside classes of secondary metabolites. Docking studies of some of the major compounds against various enzymes substantiated the biological potential of *N. leucolaena*, and make it a potential ingredient to be used in several health promoting formulations.

1. Introduction

The Lamiaceaeous herb *Nepeta leucolaena* is found in Afghanistan, Pakistan, Kashmir, North West India, Nepal, and Southwest China. In Pakistan, it grows in Skardu, where it is known as Azumal. *N. leucolaena* is locally used to treat the gastric ulcer, constipation, abdominal pain and other stomach problems. Other *Nepeta* species are also characterized by a wide range of uses in folk medicinal system like antimicrobial, insect repellent, insecticide, larvicide, cytotoxic anticarcinogen, antioxidant, anticonvulsant, analgesic, anti-inflammatory agent, and antidepressant. These activities mark *Nepeta* plants as economically very special herbs in medicinal and agricultural fields [1]. Particularly in the Himalayan regions of India (Uttarakhand, Himachal Pradesh, Jammu and Kashmir, and Leh-Ladakh), Pakistan (Khyber Pakhtunkhwa and Azad Kashmir), Nepal (Baglund district), China, and hilly regions of Turkey and Iran. Other communities use *Nepeta* plants against chicken pox, tuberculosis, malaria, pneumonia, influenza, measles, stomach problems, eye complaints, respiratory disorders, asthma, colds, and coughs [2,3]. Literature reports revealed that methanolic leaf extract of *N. juncea*, displayed potential antioxidant, cytotoxic, antifungal and antibacterial activity [4]. A medicinal formulation based on *N. tenuifolia* is used to treat endocrine dysfunction [5]. According to the studies mentioned, *Nepeta* plants have significant potential for both the prevention and treatment of a wide range of disorders. *Nepeta* species are producing wide range of secondary metabolites such as terpenoids and phenolics [6], and thus the research has demonstrated the use of *Nepeta* species and their active ingredients in several clinical and pharmacological studies as anti-depressive, antidiabetic, analgesic, anti-inflammatory, lipid-lowering, and cardioprotective medications [6,7]. However, it is crucially required to do more extensive and thorough clinical trials before using them to treat ailments. The use of extracts from *Nepeta* species in medical treatments has significant promise in the pharmaceutical, cosmetic, and food industries. Nutrition and food science research has recently concentrated on plant products with potential biological activity, such as antioxidants. Additionally, high in fiber, free of cholesterol, and loaded with antioxidants like flavonoids and other phenolic/polyphenolic substances make *Nepeta* plants important from medicinal point of view. In addition to the aforementioned biological features, young leaves of *Nepeta* herbs are also edible uncooked and can be added to salads because of their aromatic, mint-like flavor [7].

Although several ethno medicinal studies unveiled remarkable therapeutic effects of *N. leucolaena*, however, clinical studies are warranted to confirm preclinical findings [6]. A recent study revealed that *N. leucolaena* is one of the leading herb used to manage epilepsy, memory, or as a brain/nerve tonic [1]. In Leh, Chumathang, Nubra, Zanskar, Kargil and Drass, Ladakh (Amchis) the whole plant *N. leucolaena* is used as cerebral tonic [1,8]. Therefore, diverse uses of *Nepeta* plants and their utilization as medicine prompted us to investigate the under explored herb *N. leucolaena* for its medicinal potential and bioactive secondary metabolites. In preclinical screening, different parts of the plant were evaluated through six different antioxidant assays to find its potential as antioxidant agent and against five enzymes of clinical significance as enzyme inhibitor. Computation analysis was also performed that substantiate the significance and bioactivities of *N. leucolaena*.

2. Methods and material

2.1. Collection of the plant material and development of the extracts

The aerial parts (leaves and flowers) of *N. Leucolaena* were collected from Skardu, with Coordinates 35.2394° N, 75.6135° E, Pakistan, during the plant's peak growing period i.e. first week of March 2021. The plant was identified by Dr. Zaheer Abbas, botanist at University of Education Lahore and Voucher number HHU-1874 was assigned to the plant specimen. The leaves and flowers from plant material were separated and dried under shade for 20 days. A total of 2 Kg of leaves and 1 Kg of flowers were ground into semi-powder, divided into four equal parts and were extracted with appropriate amount of ethanol, ethyl acetate, chloroform and hexane to get respective extracts of flowers (ethanol: NLF-A 41 mg, ethyl acetate: NLF-E 15 mg, chloroform: NLF-C 13 mg and *n*-hexane: NLF-H 17 mg) and leaves (ethanol: NLL-A 65 mg, ethyl acetate: NLL-E 35 mg, chloroform: NLL-C 34 mg and *n*-hexane: NLL-H 52 mg).

2.2. Estimation of total phenolic and flavonoid contents

Total bioactive contents of various extracts of *N. leucolaena* were measured through Folin–Ciocalteu (FC) reagent method with slight modification [9]. 4 mL of FC reagent (diluted 10 times) in a 25 mL volumetric flask added, 1 mL of the diluted extract solution and 10 mL of distilled water, and the mixture was shaken for 10 min, and volume was made upto 25 mL with 7% Na_2CO_3 solution. The mixture was incubated for 90 min at a temperature below 37 ^{0}C and absorbance was measured at 760 nm. The same experiment was performed with gallic acid (as reference standard). The total phenolic contents (TPC) values were calculated as mg of gallic acid equivalent per gram of the extract (mg GAE/g). To determination the total flavonoids contents (TFC), the $AlCl_3$ calorimetric method was used, whereas rutin was used as a control. Sample (0.2 mL) (1 mg/mL, 2.5 mg/mL and 5 mg/mL) were mixed with 0.30 mL of 5% sodium nitrite solution and incubated at room temperature for 30 min. After that, 0.5 mL of a 10% $AlCl_3$ solution was added to each flask, and the mixture was kept for 15 min at room temperature, followed by the addition of 10 mL of 10% NaOH solution; the reaction mixture was incubated at 25 °C for 20 min, and the absorbance was measured at 510 nm. TFC was expressed as mg RE/g extract.

2.3. Protocols for antioxidant assays

2.3.1. 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay

DPPH free radical scavenging assay was performed according to previously described methods [10]. In 1 mL of sample solution the DPPH Solution (0.267 mM 4 mL, 0.004% methanol solution) was added and kept in darkness at room temperature for 30 min. The absorbance of sample mixture was measured at 517 nm, whereas trolox was used as standard and the results were calculated as mg TE/g of the extract. The experiment was done in triplicate.

2.3.2. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay.

The dilution of ABTS solution was done with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. The preparation of $ABTS^+$ cationic radical was taken by mixing the equal amount of 7 mM ABTS solution with 2.45 mM potassium persulfate. Further 2 mL of ABTS solution and 1 mL of sample solution (1 mg/mL) were mixed thoroughly. The incubation of the mixture was done at room temperature for 30 min and

absorbance was measured at 734 nm. The ABTS radical scavenging activity was expressed as milligrams of trolox equivalents (mg TE/g of the extract). The assay was performed in triplicate [10].

2.3.2. Cupric reducing antioxidant capacity (CUPRAC) assay

Cupric reducing antioxidant capacity (CUPRAC) assay was performed by mixing the equal volume (1 mL) of each neocuproine (7.5 mM), NH_4Ac buffer (1 M, pH 7.0) and $CuCl_2$ (10 mM) and sample solution (0.5 mL, 1 mg/mL) was added in it. A blank was prepared (sample solution (0.5 mL) to premixed reaction mixture (3 mL) without $CuCl_{2)}$ and incubated at room temperature for 30 min. The absorbance of both the samples was measured at 450 nm. CUPRAC activity was expressed as milligrams of trolox equivalents per gram of the dry extract (mg TE/g of the extract) [10].

2.3.3. Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed by preparing the FRAP reagent by mixing three components in a ratio of 10:1:1 (v/v/v). The acetate buffer (3.1 g $C_2H_3NaO_20.3$ H_2O and 16 mL $C_2H_4O_2$: 0.3 M, pH 3.6); 2,4,6–tris (2–pyridyl)-*S*-triazine (TPTZ) (10 mM) in 40 mM HCl; ferric chloride (FeCl3; 20 mM). The 2 mL FRAP reagent was added in 0.1 mL of each extract (1 mg/mL). The incubation reaction mixture was done at at room temperature for 30 min and absorbance was recorded at 593 nm. FRAP activity was expressed as milligrams of trolox equivalents (mg TE/g extract) [10].

2.3.4. Phosphomolybdenum assay

The 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was mixed with 0.2 mL of each sample solution. The blank solution was prepared by mixing of 0.2 mL ethanol with 4 mL of the reagent. After incubation at room temperature for 30 min the absorbance was measured at 695 nm. The standard used was Trolox and the results were calculated as mmol TE/g extracts [10].

2.3.5. Metal chelating assay

The metal chelating assay was performed for all the extracts against ferrous ions Fe^{2+} according to established methods as reported previously [10]. Samples were prepared at different concentration (25–500 mg/mL) in deionized water. 0.5 mL of the extract in 1.6 mL deionized water and 0.05 mL of $FeCl_2$ (2 mM) were mixed and exactly after 30 s, 0.1 mL ferrozine (5 mM) was added. After incubating for 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. EDTA was used as standard and the results were calculated as mg EDTAE/g extracts.

2.4. Enzyme inhibition assays

2.4.1. Cholinesterase (Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)) inhibition assays

The reaction mixture was prepared by mixing the sample solutions (1 mg/mL; 50 μL); 125 μL DTNB [5,5–dithio-bis(2–nitrobenzoic) acid]; enzyme solution (0.265 u/mL AChE or 0.026 u/mL BChE) in Tris-HCl buffer (pH 8.0) in a 96–well microplate. This reaction mixture was incubated for 15 min at 25ºC and with the addition of substrates [acetylthiocholine iodide (15 mM ATCl) or butyrylthiocholine chloride (1.5 mM BTCl, 25 mL)] reaction initiated. Similarly, a blank sample was also prepared without enzymes solution and each sample was incubated at 25 °C and analyzed. The absorbance of the both samples were measured at 405 nm. The cholinesterase inhibitory activities were calculated as galantamine equivalents (mg GALAE/g extract) [11].

2.4.2. Tyrosinase inhibition assay

To perform the tyrosinase inhibition assay, the tyrosinase solution (40 μL, Sigma) was mixed with phosphate buffer (100 μL, pH 6.8), and sample solution (1 mg/mL; 25 μL) in a 96-well microplate. Whole mixture was incubated for 15 min at 25 °C and L-DOPA (40 μL, Sigma) was added in the mixture to initiate the reaction. The blank solution sample was also prepared. The absorbance was measured at 492 nm for both blank solution and sample solution. The results were calculated as milligrams of kojic acid equivalents per gram of the dry extract (mg KAE/g extract) [11].

2.4.3. α-Amylase inhibition assay

In a 96-well microplate the sample solution (1 mg/mL; 25 μL) and α-amylase solution (50 μL; pH 6.9; 6 mM NaCl) were mixed and incubated for 10 min at 37 ºC. further this reaction was stopped by adding 25 μL of 1.0 M HCl and iodine-potassium iodide (100 μL) solution and incubated for 10 min at 37 °C. A blank sample mixture was also made. The absorbance of sample mixture and blank solution was measured at 630 nm. The results were calculated as millimoles (mM) of acarbose equivalents per gram of the dry extract (mm ACAE/g extract) [11].

2.4.4. α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was performed as reported previously [12,13]. The glutathione (0.5 mg/mL, 50 μL), PNPG (10 mM, 50 μL) and α-glucosidase solution (0.2 u/mL, 50 mL) in phosphate buffer (pH 6.8) were mixed and incubated for 15 min at 37 °C. Further reaction was stopped by adding sodium carbonate (50 μL, 0.2 M) to the mixture. In the same way as the test extract, a blank sample was prepared and at 400 nm, the absorbance was measured. The results were computed as milligrams of acarbose equivalents per gram of dry extract (mmol ACAE/g extract) [11].

2.5. UHPLC-MS experiment

Chemical profiling of *N. leucolaena* was unveiled through ultra-high performance liquid chromatography mass spectrometry (UHPLC-MS) analysis on an Agilent 1290 Infinity UHPLC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source as reported previously [10].

2.6. Multivariate analysis

The experiments were carried out in triplicate, and an ANOVA was used to compare the differences between the extracts (Tukey's test). The link between total bioactive components and biological activity assays was established using Pearson correlation analysis. The analysis was carried out by using Graph Pad Prism (version 9.2). The degree of similarity or difference between the extracts was assessed using a PCA. The statistical analysis was done using SIMCA (version 14.0).

2.7. Docking studies

The docking procedure used for this study was same as mentioned previously [12] with slight modifications for tyrosinase (PDB ID: 3NQ1) [14]. The compounds 3D structures were optimized using ChemDraw Ultra 2016, and energy minimization was performed using an MMFF94 force field. AutoDock 4.2 was used for molecular docking of compounds [15]. In order to determine if the compounds docked effectively with enzymes, the binding free energy of each molecule and the interactions of the target enzymatic residues and compounds were examined. The galantamine, acarbose and kojic aid were used as reference compounds with their respective enzymes in order to validate the docking investigations. Galantamine was docked against AChE and BChE, whereas the inhibitor acarbose was docked against amylase and glucosidase. Kojic acid was analyzed against Tyrosinase in order to validate the docking outcomes. The Discovery Studio visualizer [16] was used to do the post-docking study.

3. Results and discussions

3.1. Total bioactive contents of N. leucolaena

The estimation of total bioactive through Folin-Ciocalteu reagent showed the highest concentration of phenolic components in ethanolic extracts of flowers (NLF-A, 39.50 ± 0.53 mg GAE/g extract) and leaves (NLL-A, 31.71 ± 0.46 mg GAE/g extract), whereas, both the chloroform extracts (NLL-C and NLF-C) were next in line with the content value as 21.57 ± 0.85 and 19.20 ± 0.58, respectively. NLL-E contains significant phenolic contents whereas; NLF-E was poor in phenolic contents even lower value than hexane extracts (Table 1). The measurement of total flavonoid contents (TFC) disclosed that the NLL-A and NLF-C afford nearly equal concentration of flavonoids i.e. 24.99 mg RE/g extract, whereas, NLL-C exhibited value as 20.56 ± 0.25 followed by NLF-A and NLL-H (Table 1). These results revealed a mixed trend of phenolic and flavonoid contents in leaves and flowers and led to conclude that overall *N. leucolaena* is rich in bioactive contents with highest phenolic contents in flowers, while highest flavonoid contents were found in leaves extracts and were consistent with the previous findings where previously published data revealed that the methanolic extract of *Nepeta juncea* Benth affords higher concentration of phenolics as compared to flavonoids contents [4], however, different extraction procedures may produce varying results. Additionally, it is noted that ethanol is typically used to make tinctures from plant extracts, and can extract a significant amount of secondary metabolites, particularly phenolics.

3.2. Antioxidant activities of N. leucolaena

The antioxidant activities of all the extracts of leaves and flowers of *N. Leucolaena* were determined comprehensively through different assays to validate its use as antioxidant agent. Antioxidants are frequently referred to as "magic substances" in the general media. Nearly all human annoyances are said to be cured by antioxidants. An antioxidant is a chemical that slows down or prevents the oxidation power of another substance when present in relatively low concentrations[17]. The antioxidant activity of all the extracts was measured through radical scavenging assays DPPH (mg TE/g extract) and ABTS (mg TE/g extract), metal reducing power assays: FRAP (mg TE/g extract) and CUPRAC (mg TE/g extract), total antioxidant activity and Ferrous ion chelation method.

Table 1
Total Bioactive Contents of *N. Leucolaena*.

Solvent	Sample Code (plant part)	Total Phenolic Contents (TPC) mg GAE/g extract*	Total Flavonoid Contents (TFC) mg RE/ g extract* *
Ethanol	NLF-A (Flower)	39.50 ± 0.53^{a}	15.56 ± 0.37^{c}
	NLL-A (Leaves)	31.71 ± 0.46^{b}	24.99 ± 0.53^{a}
Ethyl acetate	NLF-E (Flower)	8.82 ± 0.58^{e}	10.49 ± 0.13^{e}
	NLL-E (Leaves)	19.26 ± 0.24^{c}	8.91 ± 0.15 ^{f}
Chloroform	NLF-C (Flower)	19.20 ± 0.58^{c}	5.17 ± 0.06 ^{g}
	NLL-C (Leaves)	21.57 ± 0.85^{c}	20.56 ± 0.25^{b}
n-Hexane	NLF-H (Flower)	16.25 ± 1.23^{d}	0.96 ± 0.11 ^{h}
	NLL-H (Leaves)	15.90 ± 1.10^{d}	13.84 ± 0.25^{d}

NLF-A: N. leucolaenaethanolic flowers extract, NLF-H: n-hexane flowers extract, NLF-E:ethyl acetate flowers extract, NLF-C: chloroform flowers extract, NLL-A: N. leucolaena leaves ethanolic extract, NLL-H: n-hexane leaves extract, NLL-E: ethyl acetate leaves extract, NLL-C: leaves chloroform extract. *Values expressed are means S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent. Different letters indicate differences in the extracts ($p < 0.05$)

In DPPH radical scavenging assays, only the alcoholic extracts (NLF-A and NLL-A) showed significant activities (49.02 ± 0.12 and 46.33 ± 0.10 mg TE/g extracts), while other extracts were inactive (Table 2). However, against ABTS free radical, in addition to alcoholic extracts (NLF-A: 113.37 ± 0.07 and NLL-A: 98.95 ± 0.57 mg TE/g extracts), ethyl acetate and chloroform extracts of leaves and flowers also exhibited significant radical scavenging potential (Table 2).

Previous studies showed that radical scavenging activity of flower extract of *N. humilis* showed DPPH and ABTS radical inhibition with IC_{50} values of 1.29 and 0.35 mg/mL, respectively, which is higher activity than the leaf extract (IC_{50} values of 5.02 and 0.93 respectively) [18]; this report fully supported our results. Usually phenolic rich extracts show potential free radical scavenging activities; therefore, higher DPPH radical scavenging power of alcoholic extracts is attributed to their higher phenolic contents, however, in ABTS assay, in addition to phenolics, other components may also impart their role.

In metal reducing power assay, the similar pattern was observed as that of radical scavenging activity, where NLF-A showed the highest inhibition (CUPRAC: 167.86 ± 3.57 and FRAP: 108.30 ± 0.98 mg TE/g extract), while NLL-A was next in line (CUPRAC:151.76 ± 1.59 and FRAP: 101.30 ± 0.32 mg TE/g extract). All other extracts displayed moderate to weak activity with ethyl acetate extracts of leaves were second in number (Table 2). Literature search disclosed that the ethanolic extract of the whole plant of *N. trachonitica* showed greater reducing power activity than the standard compound ascorbic acid in CUPRAC assay. Similarly, in FRAP assay, both ethanolic and water extract of *N. trachonitica* showed lower ferric ions reducing activity than ascorbic acid as in case of DPPH radical scavenging activity both extracts were lower inhibition values than all the extracts [19]. The presence of steroid, terpenoids and their glycosides can be correlated with the strong activity of the extracts in CUPRAC assay.

Total antioxidant capacities (TAC) of extracts were measured through phosphomomolybdenum assay and NLF-E, NLL-E and NLL-H almost exhibited the same values (1.60 ± 0.016, 1.60 ± 0.033 and 1.64 ± 0.035 mg TE/g extract, respectively), while in case of ferrous ion chelation method, various extracts of the flowers and the leaves showed the inhibition values ranging from 25.99 to 15.74 (Table 2). In which NLF-H and NLL-H have similar highest value (25.99 ± 0.10 and 25.93 ± 0.79 mg TE/g extract).

3.3. Enzyme inhibition potential of N. leucolaena

3.3.1. Cholinesterase (Acetylcholinesterase and butyrylcholinesterase) inhibition potential

Since the *Nepeta* plants have exceptional therapeutic properties against a variety of enzyme caused ailments [6]. Therefore, in present investigation we evaluated all the extracts of *N. Leucolaena* against five key enzymes of clinical significance which includes cholinesterase (AChE and BChE), α-amylase, α-glucosidase and tyrosinase. Against AChE, the NLL-A and NLL-H showed highest and same inhibitory contents (4.06 ± 0.13 mg TE/g extract, Table 3), which is followed by NLL-E and NLL-C. This observation revealed that leaves extracts are more active against AChE. In case of BChE inhibition, alcoholic extract of flowers was almost inactive, whereas, hexane and chloroform extracts of leaves showed significant potential against BChE (Table 3). However, in case of flower extracts the NLF-E was the most active with a value of 3.49 ± 0.10 which is followed by NLF-C than *n*-hexane extract (NLF-H). Previous research on other relatives of this genus had shown excellent anti-AChE capability [20].

3.3.2. Tyrosinase inhibition potential

Hyperpigmentation of human skin and or browning of food are caused by the catalytic activity of the enzyme tyrosinase. Therefore, natural safe inhibitors are the important component to improve the

Table 2
Antioxidant activities of the extracts of N.Leucolaena.

Solvent	Sample code	Radical scavenging assays		Reducing power assays		Total antioxidant activity	Ferrous ion chelation
		DPPH (mg TE/g extract)*	ABTS (mg TE/g extract)*	CUPRAC (mg TE/g extract)*	FRAP (mg TE/g extract) *	Phosphomolybdenum (mmol TE/g)*	Metal chelating (mg EDTAE/g)* *
Ethanol	NLF-A (Flower)	49.02 ± 0.12^{a}	113.37 ± 0.07^{a}	167.86 ± 3.57^{a}	108.30 ± 0.98^{a}	1.60 ± 0.016^{a}	15.94 ± 0.37^{d}
	NLL-A (Leaves)	46.33 ± 0.10^{b}	98.95 ± 0.57^{b}	151.76 ± 1.59^{b}	101.30 ± 0.32^{b}	1.64 ± 0.035^{a}	15.74 ± 0.31^{d}
Ethyl acetate	NLF-E (Flower)	2.65 ± 0.31^{c}	19.33 ± 0.95 ^{cd}	39.06 ± 2.51 ^{g}	23.74 ± 0.52^{e}	0.65 ± 0.001 ^{s}	16.30 ± 0.19^{d}
	NLL-E (Leaves)	**na*** **	21.01 ± 0.41^{c}	66.33 ± 0.99^{c}	34.21 ± 0.28^{c}	1.28 ± 0.009^{b}	22.90 ± 1.12
Chloroform	NLF-C (Flower)	**na*** **	12.25 ± 0.80^{e}	48.47 ± 0.97^{ef}	28.20 ± 0.60^{d}	0.95 ± 0.004^{c}	19.00 ± 0.80^{c}
	NLL-C (Leaves)	**na*** **	16.32 ± 2.75^{d}	58.93 ± 0.94^{d}	34.29 ± 0.29^{c}	1.19 ± 0.054^{b}	19.31 ± 0.50^{c}
n-Hexane	NLF-H (Flower)	**na*** **	5.38 ± 0.17 ^{f}	47.67 ± 0,49 ^{f}	27.29 ± 0.08^{d}	1.23 ± 0.052^{b}	25.99 ± 0.10^{a}
	NLL-H (Leaves)	**na*** **	8.09 ± 0.47 ^{f}	54.30 ± 0.89^{de}	23.84 ± 0.61^{e}	1.60 ± 0.033^{a}	25.93 ± 0.79^{a}

*TE: Trolox equivalents. * *EDTA: Ethylenediamine tetraacetate equivalents. * **na: not active. Different superscripts indicate significant differences in the tested samples ($p < 0.05$)

Table 3
Enzyme Inhibitory activities of the extracts of *N. Leucolaena*.

Solvent	Sample Code (plant part)	AChE (mg GALAE/g extract)	BChE (mg GALAE/g extract)	Tyrosinase (mg KAE/g extract)	α-amylase (mmol ACAE/g extract)	α-glucosidase (mmol ACAE/g extract)
Ethanol	NLF-A (Flower)	3.49 ± 0.10^{ab}	0.77 ± 0.05^{c}	21.29 ± 0.75^{e}	0.47 ± 0.009^{e}	0.02 ± 0.004^{e}
	NLL-A (Leaves)	4.06 ± 0.18^{a}	2.22 ± 0.25	26.08 ± 0.69^{d}	0.48 ± 0.019^{de}	0.72 ± 0.004^{d}
Ethyl acetate	NLF-E (Flower)	3.14 ± 0.14^{b}	2.38 ± 0.06^{b}	30.65 ± 1.19^{b}	0.55 ± 0.010 ^{cd}	1.07 ± 0.004^{ab}
	NLL-E (Leaves)	3.42 ± 0.09^{b}	2.63 ± 0.23^{b}	29.49 ± 0.75^{bc}	0.64 ± 0.006^{ab}	1.04 ± 0.012^{b}
Chloroform	NLF-C (Flower)	3.63 ± 0.07^{ab}	1.94 ± 0.08^{b}	28.90 ± 0.57^{bcd}	0.61 ± 0.031^{abc}	0.94 ± 0.006^{c}
	NLL-C (Leaves)	3.06 ± 0.35^{b}	3.67 ± 0.48^{a}	26.65 ± 1.73 ^{cd}	0.57 ± 0.032^{bc}	0.96 ± 0.009^{c}
n-Hexane	NLF-H (Flower)	3.22 ± 0.13^{b}	2.44 ± 0.10^{b}	34.45 ± 0.48^{a}	0.67 ± 0.016^{a}	1.08 ± 0.006^{a}
	NLL-H (Leaves)	4.06 ± 0.13^{a}	3.99 ± 0.20^{a}	27.67 ± 0.22^{bcd}	0.67 ± 0.013^{a}	1.05 ± 0.019^{ab}

*Values are reported as mean ± SD. GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different superscripts indicate significant differences in the tested samples ($p < 0.05$)

cosmetic and food industrial business. Current search of new tyrosinase inhibitor for food and cosmetic industry is thus a very important and attractive area of research [21]. Keeping in view the current industrial need, all the extracts of *N. leucolaena* were screen for tyrosinase inhibitory activity. Hexane (NLF-H) and ethyl acetate (NLF-E) extracts of flowers showed the highest inhibition with values of 34.45 ± 0.48 and 30.65 ± 1.19 mg KAE/g extract, respectively. The NLL-E and NLL-H were second in line (29.49 ± 0.75 and 27.67 ± 0.22 mg KAE/g extract, respectively). However, other extracts also exhibited comparable inhibitory potential (Table 3). The *Nepeta* plants have previously been reported active against the enzyme tyrosinase, which supported our findings [20]. *N. binaludensis* extracts of different parts are reported to inhibit melanogenesis in murine B16F10 melanoma cells [22,23]. Since, all the extracts of *N. leucolaena* showed significant inhibitory potential against tyrosinase activity, it offers its strong candidature to be used in anti-tyrosinase formulations.

3.3.3. α-amylase and α-Glucosidase inhibition potential

Diabetes mellitus is among the most threatening diseases to human, which have strong negative impact on health and economy. Therefore, researchers are searching for new, effective and safer antidiabetic agents mostly from natural sources. As part of these efforts, various extracts of *N. Leucolaena* were evaluated for their α-amylase and α-glucosidase inhibitory potential (Table 3). The results indicated that hexane and chloroform extracts of flowers (NLF-H and NLF-C: 0.67 ± 0.016 and 0.61 ± 0.031 mmol ACAE/g extract, respectively), and hexane and ethyl acetate extract of leaves (NLL-H and NLL-E: 0.67 ± 0.013 and 0.64 ± 0.006 mmol ACAE/g extract, respectively) exhibited higher inhibitory values against tyrosinase. However, other extracts also exhibited significant inhibition with least activity was observed for ethanol extracts (NLL-A and NLF-A: 0.48 ± 0.019 and 0.47 ± 0.009 mmol ACAE/g extract, respectively, Table 3). In α-glucosidase inhibitory assay, hexane and ethyl acetate extracts (NLF-H, NLF-E and NLL-H, NLL-E) displayed higher activity with values as 1.08 ± 0.006, 1.07 ± 0.004, 1.05 ± 0.019 and 1.04 ± 0.012 mmol ACAE/g extract, respectively. NL-C and NLL-C were next in activity level (Table 3) with alcoholic extracts as weak inhibitor of α-glucosidase. These results disclosed that low polar extracts of flowers were the most active agents, followed by the leaves extracts. Since phenolic rich alcoholic extracts were least antidiabetic agents, which indicated that other metabolites like terpenoids and lipids impart significant role in antidiabetic properties of *N. Leucolaena*.

3.4. Metabolic profiling through UHPLC-MS analysis

based on its best antioxidant activity on most assays as well as considerable enzyme inhibition activity, the ethanolic extracts of leaf and flower were combined and subjected to UHPLC-MS analysis, which disclosed the presence of 139 compounds (Table 4) of steroid, megastigmane glycoside, triterpenoid, phenolic, flavonoid, and alkaloid classes of secondary metabolites. This is the first report on phytochemical profiling of *N. leucolaena*. Presence of high amount of steroids, triterpenes, phenolics, flavonoids, withanolide and alkaloids endorses the significant medicinal properties of *N. leucolaena* and authenticate the use of this plant to treat rheumatism, diabetes, and leprosy, as well as acting as antiseptics and disinfectants [6,7]. The presence of phenolic in higher concentrations, as well as flavonoids and withanolide, makes this

Table 4
UHPLC-MS analysis of ethanolic extract of whole plant.

Sr. No.	Analyte Peak Name	Retention Time	Area / Height	Tentative identified Compounds	Class of compound	Mass	M. Formula
1	188.0741	1.58	6.83	Indoleacrylic acid	Alkaloid	187.1947	$C_{11}H_9NO_2$
2	248.0929	1.63	7.33	Aspartyl-Asparagine	Peptide	247.0804	$C_8H_{13}N_3O_6$
3	367.1182	1.64	5.94	Isoglycyrol	polyphenol	366.1103	$C_{21}H_{18}O_6$
4	337.0878	1.70	4.73	3-Caffeoyl-1,5-quinolactone	Phenolic	336.0845	$C_{16}H_{16}O_8$
5	685.2285	1.73	4.29	Citbismine C	Acridones	684.2319	$C_{37}H_{36}N_2O_{11}$
6	707.1902	1.82	3.87	Kaempferol 3-[2‴,3‴,4‴-triacetyl-α-L-arabinopyranosyl-(1->6)-glucoside]	Flavonoid	706.1745	$C_{32}H_{34}O_{18}$
7	367.0863	1.82	6.61	12a-Methoxydolineone	Flavonoid	366.0740	$C_{20}H_{14}O_7$
8	522.1921 M+NH_4^+	1.86	3.29	6-Hydroxykaempferol 3,5,7,4′-tetramethyl ether 6-rhamnoside	Flavonoid	504.1632	$C_{25}H_{28}O_{11}$
9	343.1002 M+H^+	1.85	5.19	Glucocaffeic acid	Phenolic	342.0951	$C_{15}H_{18}O_9$
10	292.0814	2.00	8.29	Lycoricidine	phenanthridines.	291.0743	$C_{14}H_{13}NO_6$
11	317.0986 M+H^+	3.09	11.26	5,2′-Dihydroxy-7,5′-dimethoxyflavanone	Flavonoid	316.0947	$C_{17}H_{16}O_6$
12	279.1228	3.10	12.57	ethyl 6,7-dimethoxy-4-oxo-2,3-dihydro-1 H-naphthalene-2-carboxylate	Phenolic	278.1154	$C_{15}H_{18}O_5$
13	289.0681	3.20	14.68	5,7,2′,5′-Tetrahydroxyflavanone	Flavonoid	288.0634	$C_{15}H_{12}O_6$
14	379.0757	3.14	4.14	Anastatin A	Flavonoid	378.0740	$C_{21}H_{14}O_7$
15	375.1888	3.19	3.69	Maximaflavanone A	Flavonoid	374.1882	$C_{25}H_{26}O_3$
16	316.1149	3.32	9.95	Citpressine II	Acridones	315.1107	$C_{17}H_{17}NO_5$
17	373.1664 M+NH_4 +	3.13	9.26	Marshmine	Acridones	355.1420	$C_{20}H_{21}NO_5$
18	346.1477 M+NH_4 +	3.79	9.94	3-(4-Hydroxyphenyl)− 3-oxopropyl-α-D-glucopyranoside	Phenolic	328.1158	$C_{15}H_{20}O_8$
19	333.0928	3.86	10.14	5,2′,5′-Trihydroxy-7,8-dimethoxyflavanone	Flavonoid	332.0896	$C_{17}H_{16}O_7$
20	240.0654	3.88	4.09	Marcanine A	Alkaloid	239.0582	$C_{14}H_9NO_3$
21	416.1873 M+NH_4 +	3.88	9.30	Methyl 3,4-dihydroxy-5-prenylbenzoate 3-glucoside	Phenolic	398.1577	$C_{19}H_{26}O_9$
22	279.1226	4.06	7.98	11-Hydroxy-12-methoxydihydrokawain	Phenolic	278.1154	$C_{15}H_{18}O_5$
23	315.1622 M+NH_4 +	4.06	7.91	Phenethylamine glucuronide	Phyto	297.1212	$C_{14}H_{19}NO_6$
24	328.1168 M+Na+	4.36	7.80	N-Hydroxynorcocaine	Alkaloid	305.1263	$C_{16}H_{19}NO_5$
25	289.0681	4.26	10.35	5,7,2′,5′-Tetrahydroxyflavanone	Flavonoid	288.0634	$C_{15}H_{12}O_6$
26	372.1787 M+NH_4 +	4.35	7.38	Flemistrictin D	Chalcones	354.1467	$C_{21}H_{22}O_5$
27	303.0833	4.27	8.06	3′,5,7-Trihydroxy-3′-methoxyflavanone, Homoeriodictyol	Flavonoid	302.0790	$C_{16}H_{14}O_6$
28	324.1412	4.30	8.03	Acremoauxin A	Alkaloid	323.1369	$C_{16}H_{21}NO_6$
29	293.1148	4.37	7.44	(2 S,4 S)-Monatin	Phyto	292.1059	$C_{14}H_{16}N_2O_5$
30	460.2123 M+NH_4 +	4.35	6.23	cortisol 21-sulfate	Steroid	442.1661	$C_{21}H_{30}O_8S$
31	325.1269 M+CH_3OH+H+	4.37	10.21	trans-Grandmarin	Coumarins	292.0947	$C_{15}H_{16}O_6$
32	366.1380	4.48	5.59	2-(acetylamino)− 1,5-anhydro-2-deoxy-3-O-β-D-galactopyranosyl-D-arabino-Hex-1-enitol	glycoside	365.1322	$C_{14}H_{23}NO_{10}$
33	417.1908	4.75	6.45	Leonubiastrin	Terpenoids	416.1835	$C_{23}H_{28}O_7$
34	333.0921 M+Na+	4.73	11.65	(E)− 1-O-Cinnamoyl-β-D-glucose	Phenolic	310.1053	$C_{15}H_{18}O_7$
35	315.1585	4.88	7.00	Anisocoumarin H	Coumarins	314.1518	$C_{19}H_{22}O_4$
36	338.1276	4.83	5.53	Indole-3-acetyl-β-1-D-glucoside	Alkaloid	337.1162	$C_{16}H_{19}NO_7$
37	321.0938	4.92	9.14	Gallocatechin 4′-methyl ether	Phenolic	320.0896	$C_{16}H_{16}O_7$
38	323.0878	4.96	4.19	3,4′-Dimethoxyfurano[2,3:7,8]flavone	Flavonoid	322.0841	$C_{19}H_{14}O_5$
39	549.2671	5.04	4.16	Archangelolide	Terpenoids	548.2621	$C_{29}H_{40}O_{10}$
40	377.1177	5.06	7.67	5,3′-Dihydroxy-6,7,4′,5′-tetramethoxyflavanone	Flavonoid	376.1158	$C_{19}H_{20}O_8$
41	339.1418	5.05	5.72	1-Peroxyferolide	lactone	338.1267	$C_{17}H_{22}O_7$
42	307.1157 M+Na+	5.14	7.41	2-Phenylethyl β-D-glucopyranoside	Phenolic	284.1260	$C_{14}H_{20}O_6$
43	383.1285 M+Na+	5.22	7.70	1-(3,4-Dimethoxyphenyl)− 1,2-ethanediol 1-O-b-D-glucoside	Phenolic	360.1420	$C_{16}H_{24}O_9$
44	404.2241 M+NH4 +	5.33	5.47	(3E)− 1-Hydroxy-4-[(1 R)− 2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]− 3-buten-2-yl ?-D-glucopyranoside	Phyto	386.1941	$C_{19}H_{30}O_8$
45	293.1383	5.33	7.15	Coriandrone A	Phenolic	292.1311	$C_{16}H_{20}O_5$
46	577.2623	5.34	6.99	Ptilosaponoside B	Steroid	576.2604	$C_{27}H_{44}O_{11}S$
47	285.0728	5.38	3.96	6-Methylapigenin	Flavonoid	284.0685	$C_{16}H_{12}O_5$
48	495.2327 M+Na+	5.51	4.42	9a-Acetoxy-7b-hydroxy-3-(hydroxymethyl)− 1,1,6,8-tetramethyl-5-oxo-1a,1b,4,4a,5,7a,7b,8,9,9a-decahydro-1 H-cyclopropa[3,4]benzo[1,2-*e*]azulen-9-yl (2E)− 2-methyl-2-butenoate	Phenolic	472.2461	$C_{27}H_{36}O_7$
49	420.2185 M+NH_4 +	5.50	6.33	Methyl 7-epi-12-hydroxyjasmonate glucoside	Glycosides	402.1890	$C_{19}H_{30}O_9$
50	479.1831	5.50	6.29	Kelampayoside A	Phenolic glycosides	478.1686	$C_{20}H_{30}O_{13}$
51	367.1725	5.54	4.71	4-Acetyl-2-prenylphenol glucoside	Phenolic	366.1679	$C_{19}H_{26}O_7$
52	449.2534	5.60	5.18	Tetrahydrocortisone-3,21-Diacetate	Steroid	448.2461	$C_{25}H_{36}O_7$
53	247.0954 M+Na+	5.63	9.19	Acoramone	Phenolic	224.1049	$C_{12}H_{16}O_4$
54	281.1387	5.63	6.06	Artabsinolide A	butyrolactones	280.1311	$C_{15}H_{20}O_5$
55	391.2303	5.73	6.56	(3 S,5 R,6 S,7E,9x)− 7-Megastigmene-3,6,9-triol 9-glucoside	Megastigmane glycosides	390.2254	$C_{19}H_{34}O_8$
56	395.1071 (M+K+)s	5.63	4.57	(E)− 7-hydroxy-6-methoxy-7-(6-oxo-3,6-dihydro-2 H-pyran-2-yl)hept-4-ene-2,3-diyl diacetate	Phyto	356.1471	$C_{17}H_{24}O_8$
57	390.2524 M+NH_4 +	5.71	4.02	9-Hydroxy-7-megastigmen-3-one glucoside	Megastigmane glycosides	372.2148	$C_{19}H_{32}O_7$
58	563.2808 M+CH_3OH+H^+	5.96	7.87	Uscharidin	cardenolide glycoside	530.2516	$C_{29}H_{38}O_9$
59	451.1883 M+Na+	5.81	7.13	Taraxacolide 1-O-b-D-glucopyranoside	Terpenoids	428.2046	$C_{21}H_{32}O_9$
60	493.2763	5.82	4.78	20α-Dihydroprogesterone glucuronide	Steroid	492.2723	$C_{27}H_{40}O_8$

(*continued on next page*)

Table 4 (*continued*)

Sr. No.	Analyte Peak Name	Retention Time	Area / Height	Tentative identified Compounds	Class of compound	Mass	M. Formula
61	561.1657	5.81	4.58	3′-Deoxymaysin	Flavonoid glycoside	560.1530	$C_{27}H_{28}O_{13}$
62	319.1507 M+CH_3OH+H+	5.90	4.52	Latifolin	Phenolic	286.1205	$C_{17}H_{18}O_4$
63	419.2424	5.87	6.62	6α-Methylhydrocortisone 21-acetate	Steroid	418.2355	$C_{24}H_{34}O_6$
64	463.2679 M+CH_3OH+H+	6.10	6.70	21-Hydroxypregn-4-ene-3,20-dione hydrogen succinate	Steroid	430.2355	$C_{25}H_{34}O_6$
65	396.1769 M+Na+	5.80	4.12	Hasubanonine	Isoquinoline alkaloid	373.1889	$C_{21}H_{27}NO_5$
66	444.2180 M+NH_4 +	6.22	13.52	Abscisic acid glucose ester	Phyto	426.1890	$C_{21}H_{30}O_9$
67	479.2811	6.16	4.11	3-Geranyl-4,2′,4’,6’-tetrahydroxy-5-prenyldihydrochalcone	Phenolic	478.2719	$C_{30}H_{38}O_5$
68	375.1654	6.29	5.37	Deoxyloganin	Iridoids	374.1577	$C_{17}H_{26}O_9$
69	511.2451 M+Na+	6.30	5.72	α-Ionol O-[arabinosyl-(1->6)-glucoside]	fatty acyl glycosides	488.2621	$C_{24}H_{40}O_{10}$
70	507.3096	6.31	6.07	Pentahydroxy-1-oxowith-24-enolide	Withanolides steroids	506.2880	$C_{28}H_{42}O_8$
71	405.1649	6.38	9.70	Euchrenone a1	Flavonoid	404.1624	$C_{25}H_{24}O_5$
72	325.1633	6.34	6.82	Chamissonolide	Pseudoguaianolides	324.1573	$C_{17}H_{24}O_6$
73	521.3258	6.35	5.51	Digoxigenin monodigitoxoside	Steroid saponin	520.3036	$C_{29}H_{44}O_8$
74	369.2056	6.58	8.55	11,12,13-trihydroxy-9-octadecenoic acid	Fatty acid	330.2406	$C_{18}H_{34}O_5$
75	391.1696	6.58	7.64	Methyl (1 S)− 1-(β-D-glucopyranosyloxy)− 7-hydroxy-7-methyl-1,4a,5,6,7,7a-hexahydrocyclopenta[*c*]pyran-4-carboxylate	Iridoids	390.1526	$C_{17}H_{26}O_{10}$
76	351.1780	6.59	8.89	Cathenamine	Isoquinoline alkaloid	350.1630	$C_{21}H_{22}N_2O_3$
77	369.1878	6.59	8.61	Strictosidineaglycone	Isoquinoline alkaloid	368.1736	$C_{21}H_{24}N_2O_4$
78	414.2317 M+NH_4 +	6.67	6.39	pregnenolone sulfate	Steroid	396.1970	$C_{21}H_{32}O_5S$
79	414.2617 M+NH_4 +	6.66	5.07	Melengestrol acetate	Steroid	396.2301	$C_{25}H_{32}O_4$
80	372.2355 M+NH_4 +	6.66	5.11	Rauwolscine	Isoquinoline alkaloid	354.1943	$C_{21}H_{26}N_2O_3$
81	402.2446 M+NH_4 +	6.74	9.51	16-Methoxy-2,3-dihydro-3-hydroxytabersonine	Alkaloid	384.2049	$C_{22}H_{28}N_2O_4$
82	879.2553 M+Na+	6.76	4.14	Sesaminolglucosyl-(1->2)-[glucosyl-(1->6)]-glucoside	Lignin glycosides	856.2637	$_{38}H_{48}O_{22}$
83	455.0687 M+Na+	6.69	3.66	Kaempferol 3-p-coumarate	Flavonoid	C24H16O8	$C_{24}H_{16}O_8$
84	573.1637	6.79	5.40	2’’,4’’,6’’-Triacetylglycitin	Polyphenolic	572.1530	$C_{28}H_{28}O_{13}$
85	425.1529	6.82	6.01	8-C-Methylvelloquercetin 3,5,3′-trimethyl ether	Flavonoid	424.1522	$C_{24}H_{24}O_7$
86	416.2777 M+NH_4 +	6.84	5.45	Pregna-5,16,20-triene-3β,20-diol diacetate	Pregnanes steroid	398.2457	$C_{25}H_{34}O_4$
87	490.3135 M+CH_3OH+H+	6.78	5.09	N-docosahexaenoyl glutamic acid	N-acylamides	457.2828	$C_{27}H_{39}NO_5$
88	368.2044 M+NH_4 +	6.86	6.39	Vomilenine	Isoquinoline alkaloid	350.1630	$C_{21}H_{22}N_2O_3$
89	369.2058 M+CH_3OH+H+	6.85	8.96	Tephrowatsin B	Flavonoid	336.1725	$C_{22}H_{24}O_3$
90	421.1794 M+Na+	6.73	8.89	Ptaquiloside	Terpenoids	398.1941	$C_{20}H_{30}O_8$
91	697.4108	6.91	4.83	Momordicoside E	Steroid glycosides	696.4085	$C_{37}H_{60}O_{12}$
92	503.2982 M+CH_3OH+H+	6.99	4.95	Withanolide A	Withanolides steroids	470.2668	$C_{28}H_{38}O_6$
93	725.4213	7.04	5.14	Bismurrayafoline E	carbazoles	724.4240	$C_{48}H_{56}N_2O_4$
94	357.2145	7.07	7.51	(17Z)− 3,11-Dioxopregna-4,17(20)-dien-21-oic acid methyl ester	Pregnanes steroid	356.1988	$C_{22}H_{28}O_4$
95	537.2235	7.05	8.17	Schisantherin A	Lignan	536.2046	$C_{30}H_{32}O_9$
96	395.2034	7.06	5.53	Nigakilactone N	Terpenoids	394.1992	$C_{21}H_{30}O_7$
97	357.2295	7.08	5.89	5,7-Megastigmadien-9-ol glucoside	Megastigmane glycosides	356.2199	$C_{19}H_{32}O_6$
98	505.2964 M+CH_3OH+H+	7.13	4.40	3-Sulfodeoxycholic acid	Steroids	472.2495	$C_{24}H_{40}O_7S$
99	372.2342 M+NH_4 +	7.05	6.43	Yohimbine	Isoquinoline alkaloid	354.1943	$C_{21}H_{26}N_2O_3$
100	415.2477 M+CH_3OH+H+	7.10	5.18	3,12-Dioxochola-1,4,9(11)-trien-24-oic Acid	Steroids	382.2144	$C_{24}H_{30}O_4$
101	665.3461 (M+CH_3OH+H+)	7.28	9.21	Coagulin R 3-glucoside	Withanolides steroids	632.3197	$C_{34}H_{48}O_{11}$
102	353.1934	7.20	9.61	Geissoschizine	Isoquinoline alkaloid	352.1787	$C_{21}H_{24}N_2O_3$
103	458.2555 (M+NH_4 +)	7.25	5.71	Deacetylgedunin	limonoid	440.2199	$C_{26}H_{32}O_6$
104	474.2638 M+NH4 +	7.02	9.02	Vindoline	Isoquinoline alkaloid	456.2260	$C_{25}H_{32}N_2O_6$
105	414.2623 M+H+	7.33	7.08	N-Desalkylbuprenorphine	Isoquinoline alkaloid	413.2566	$C_{25}H_{35}NO_4$
106	618.3325	7.37	6.74	Romucosine C	Isoquinoline alkaloid	355.1420	$C_{20}H_{21}NO_5$
107	593.3827	7.36	4.77	nuatigenin 3-β-D-glucopyranoside	Pregnanes steroid	592.3611	$C_{33}H_{52}O_9$
108	431.1059	7.47	3.41	Coumestrol 3-O-glucoside	Phyto	430.0900	$C_{21}H_{18}O_{10}$
109	611.3141	7.51	8.77	Liensinine	Isoquinoline alkaloid	610.3043	$C_{37}H_{42}N_2O_6$
110	441.1257	7.54	4.88	Hallactone B	Terpenoids	440.1141	$C_{20}H_{24}O_9S$
111	487.2835 (M+H+)	7.56	7.53	15β-Hydroxynicandrin B	Withanolides steroids	486.2618	$C_{28}H_{38}O_7$
112	507.2491	7.62	6.86	Artelastofuran	Flavonoid	506.2305	$C_{30}H_{34}O_7$
113	548.3345 (M+NH_4 +)	7.66	7.79	Physapubescin	Withanolides steroids	530.2880	$C_{30}H_{42}O_8$
114	583.3009 (M+CH_3OH+H+)	7.89	6.46	Aspecioside	cardenolide glycoside	550.2778	$C_{29}H_{42}O_{10}$
115	739.4180 (M+CH_3OH+H+)	7.82	10.50	Hovenidulcioside A2	diterpene glycosides	706.3928	$C_{38}H_{58}O_{12}$
116	985.4347	7.85	3.62	Basellasaponin C	triterpene saponins	984.4202	$C_{47}H_{68}O_{22}$

(*continued on next page*)

Table 4 (*continued*)

Sr. No.	Analyte Peak Name	Retention Time	Area / Height	Tentative identified Compounds	Class of compound	Mass	M. Formula
117	592.3595 (M+NH_4+)	7.90	6.11	Cevadine	Alkaloid	591.3407	$C_{32}H_{49}NO_9$
118	489.2829	7.96	7.13	Withaphysacarpin	Withanolides steroids	488.2774	$C_{28}H_{40}O_7$
119	623.3728	7.97	6.91	3-O-Protocatechuoylceanothic acid	triterpene	622.3506	$C_{37}H_{50}O_8$
120	773.4352 (M+Na+)	8.18	5.66	Oleanolic acid 3-[glucosyl-(1->4)-xyloside]	Steroids glycosides	750.4554	$C_{41}H_{66}O_{12}$
121	770.4671 (M+NH_4+)	8.18	6.25	Polypodoside C	steroid saponin	752.4347	$C_{40}H_{64}O_{13}$
122	680.4097 (M+NH_4+)	8.06	6.60	Prosapogenin	triterpene saponins	662.3666	$C_{36}H_{54}O_{11}$
123	715.3471 (M+K+)	8.07	6.91	(S)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1->4)-a-L-rhamnopyranosyl-(1->2)-b-D-glucopyranoside]	Terpenoids	676.3670	$C_{33}H_{56}O_{14}$
124	861.4677	8.29	5.41	Cyclotricuspidoside C	triterpene saponins.	860.4770	$C_{43}H_{72}O_{17}$
125	793.3852 (M+Na+)	9.40	8.54	Spongipregnoloside C	steroid saponin	770.4089	$C_{39}H_{62}O_{15}$
126	365.1692 (M+Na+)	10.27	8.08	7-(4-Hydroxy-3-methoxyphenyl)− 5-methoxy-1-phenyl-3-heptanone	Phenolic	342.1831	$C_{21}H_{26}O_4$
127	331.1652	10.30	4.93	6-Hydroxy-2-bornanone glucoside	Terpenoids	330.1679	$C_{16}H_{26}O_7$
128	451.3806	10.37	4.51	ergosta-3β,5α,6β,25-tetrol	Steroid	450.3709	$C_{28}H_{50}O_4$
129	358.2352 (M+NH_4+)	10.55	7.03	17-Hydroxy-3-oxo-17α-pregna-1,4-diene-21-carboxylic acid, gamma-lactone	Pregnanes steroid	340.2038	$C_{22}H_{28}O_3$
130	347.292	10.84	15.30	24-Nor-5β-chol-22-ene-3α,6α-diol	Pregnanes steroid	346.2872	$C_{23}H_{38}O_2$
131	531.3802	10.93	11.97	Acinospesigenin A	triterpenoids	530.3607	$C_{32}H_{50}O_6$
132	413.2584	11.14	8.18	Boviquinone 4	Terpenoids	412.2614	$C_{26}H_{36}O_4$
133	353.1627	11.00	4.37	Palmatine	Alkaloid	352.1549	$C_{21}H_{22}NO_4$
134	419.2901	11.40	18.44	3α,12α-Dihydroxy-5β-pregnan-20-one diacetate	Pregnanes steroid	418.2719	$C_{25}H_{38}O_5$
135	694.3685 (M+NH_4+)	11.29	6.09	Cucurbitacin I 2-glucoside	Steroid	676.3459	$C_{36}H_{52}O_{12}$
136	468.3789 (M+NH_4+)	11.32	9.94	3α,7α,12α-Trihydroxy-5α-cholestan-26-oic acid	Steroid	450.3345	$C_{27}H_{46}O_5$
137	405.2134	11.30	5.19	Boesenbergin A	Phenolic	404.1988	$C_{26}H_{28}O_4$
138	561.5062	11.41	7.16	1,2-Epoxy-1,2,7,7′,8,8′,11’,12’-octahydro-psi,psi-carotene	Carotenoid	560.4957	$C_{40}H_{64}O$
139	513.3911	11.29	10.12	Methyl 3b-hydroxy-13(18)-oleanen-28-oate	Steroid	512.3866	$C_{33}H_{52}O_4$

plant more important and can be correlated to antioxidant and enzyme inhibition properties. The docking studies of some selected alkaloids, phenolics and withanolides substantiate our results of enzyme inhibition, since the docked compounds showed higher binding free energy when compared to the control.

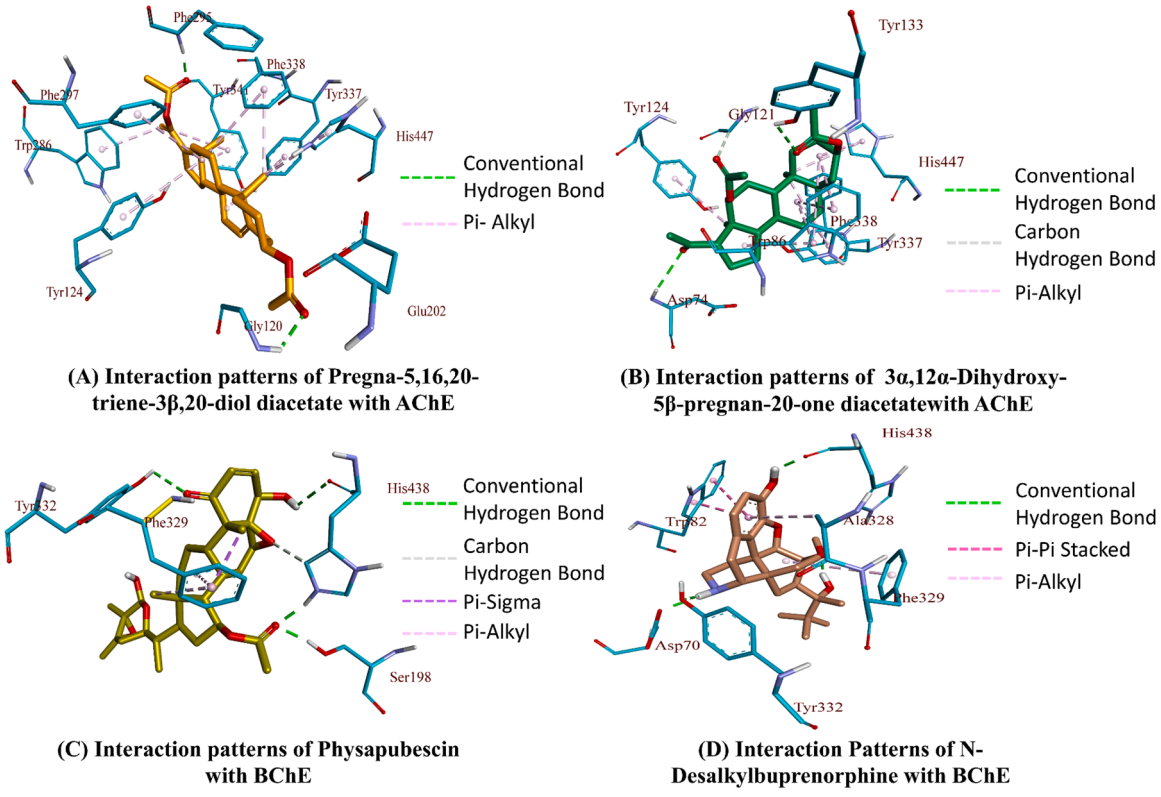


Fig. 1. Binding interactions of compounds with AChE and BChE.

3.5. Multivariate analysis

In the present study, we performed a Pearson's correlation analysis between biological activity assays. The obtained heat map is given in Fig. 4. Clearly, radical scavenging and reducing power assays correlated strongly with the total phenolic content of the tested extracts (R>0.7). However, total flavonoid content showed a moderate correlation with antioxidant parameters. These results indicated that phenolic compounds were main contributors to the antioxidant properties of *N. leucolaenta*. Consistent with our results, several researchers have reported a linear correlation between total phenolic and antioxidant parameters for some plant species [24,25]. However, the spectrophotometric results should be confirmed in future studies by the individual quantification analysis by LC-MS to understand which compound(s) might be the main players in the test systems. As can be seen from Fig. 5, the observed metal chelating ability did not correlate with the total phenolic and flavonoid content of the tested extracts. This fact could be explained by the presence of non-phenolic chelating agents such as peptides, polysaccharides or sulfides. With regard to the enzyme inhibitor analysis, we did not determine any correlation with the total content of phenols and flavonoids. Similar results were also reported from previous studies in which no correlation was observed between total bioactive components and AChE/BChE, [26], tyrosinase [27] and amylase [28]. Principal component analysis (PCA) was performed to distinguish between the tested extracts based on the results of the biological activity assays. Two components were used in the analysis, accounting for 81.3% of the total components (PC1: 66.5%; PC2: 14.8%). From Fig. 5, the extracts were divided into two groups. The tested ethanol extracts were classified into the same groups, characterized by high antioxidant potentials and total bioactive components. Other extracts, namely n-hexane, ethyl acetate and chloroform, were very closely distributed and featured significant enzyme inhibitory abilities. Based on the results, we suggested that we could select ethanol as the solvent if we wanted to make an application on antioxidant properties using *N. leucolaenta*. In addition, if we wanted to any applications regarding enzyme inhibitory properties, we could select non-polar solvents such as n-hexane, ethyl acetate or chloroform. These findings might be a scientific starting point on further applications using *N. leucolaenta.*

3.6. Post docked analysis

The docking analysis of 12 compounds revealed that most of these compounds showed good binding potential against AChE, BChE and α-glucosidase than their respective reference compound (Table 5). In case of α-amylase all docked compounds showed significant binding free energy, but no compound has binding potential greater than reference compound acarbose. These compounds showed higher inhibitory potential towards tyrosinase than the reference compound kojic acid as indicated by binding free energy (Table 5). The binding potential of the tested compounds and their respective predicted inhibition constants are presented in Table 5. The compound pregna-5,16,20-triene-3β,20-diol diacetate showed high inhibitory potential against AChE, α-amylase and tyrosinase while physapubescin exhibited best inhibitory potential against BChE, α-amylase and α-glucosidase.

The compound coagulin-R-3-glucoside which was found to bind effectively with active sites of BChE, α-amylase, α-glucosidase and tyrosinase, but failed to show significant binding potential against AChE (Table 5). The binding pose for top two highly ranked compounds against AChE, BChE, α-amylase, α-glucosidase and tyrosinase were visualized through Discovery Studio visualizer (Figs. 1–3). The interaction patterns identified for these compounds are summarized in Table 6. The binding free energy of the compounds against AChE was in range − 8.81 to − 11.84 kcal/mol excluding coagulin-R-3-glucoside. The binding interaction patterns for pregna-5,16,20-triene-3β,20-diol diacetate and 3α,12α-dihydroxy-5β-pregnan-20-one diacetate strong hydrogen bonds were mediated by these compounds towards AChE (Fig. 1 (A and B)) along with some Pi-Alkyl interactions. pregna-5,16,20-triene-3β,20-diol diacetate and 3α,12α-dihydroxy-5β-pregnan-20-one

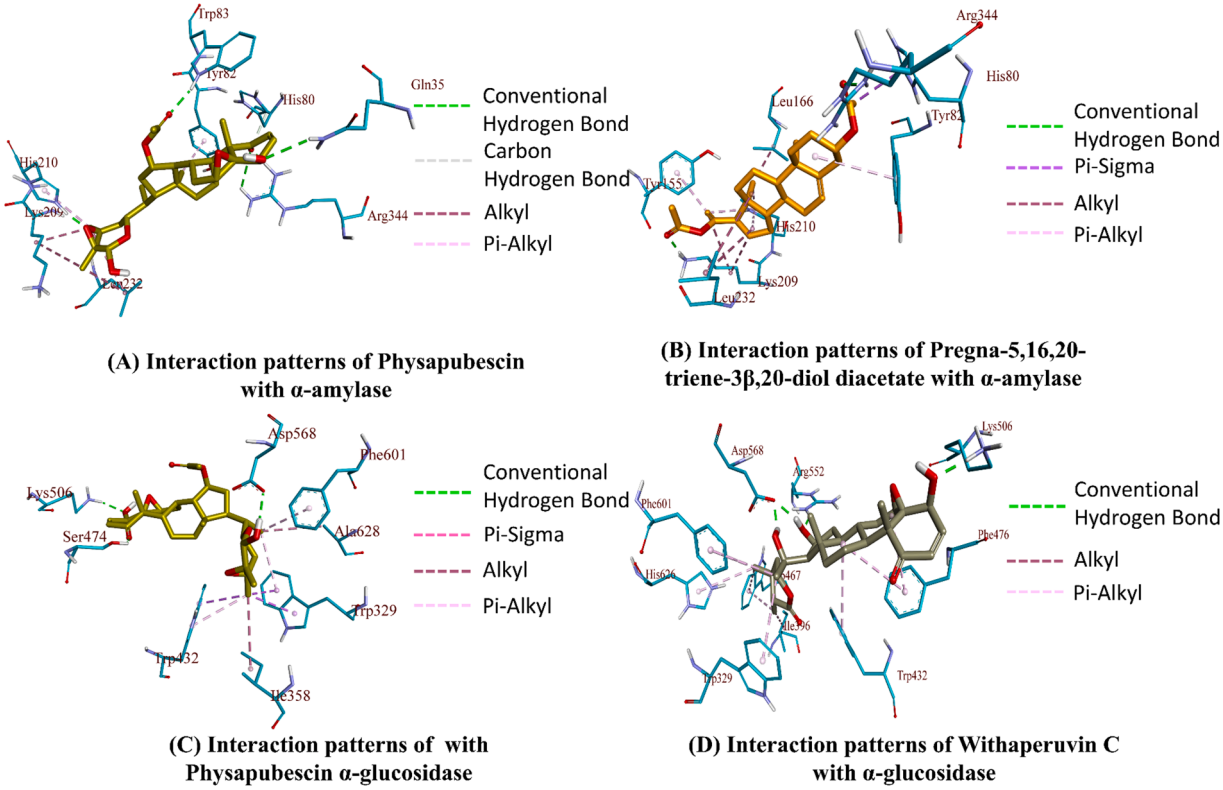
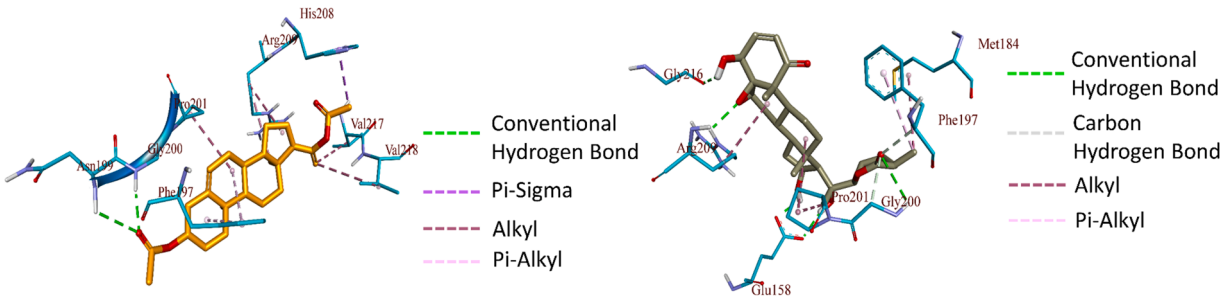


Fig. 2. Binding interactions of compounds with α-amylase and α-glucosidase.



(A) Interaction patterns of Pregna-5,16,20-triene-3β,20-diol diacetate with Tyrosinase

(B) Interaction patterns of Withaphysacarpin with Tyrosinase

Fig. 3. Binding interactions of compounds with α-amylase and α-glucosidase.

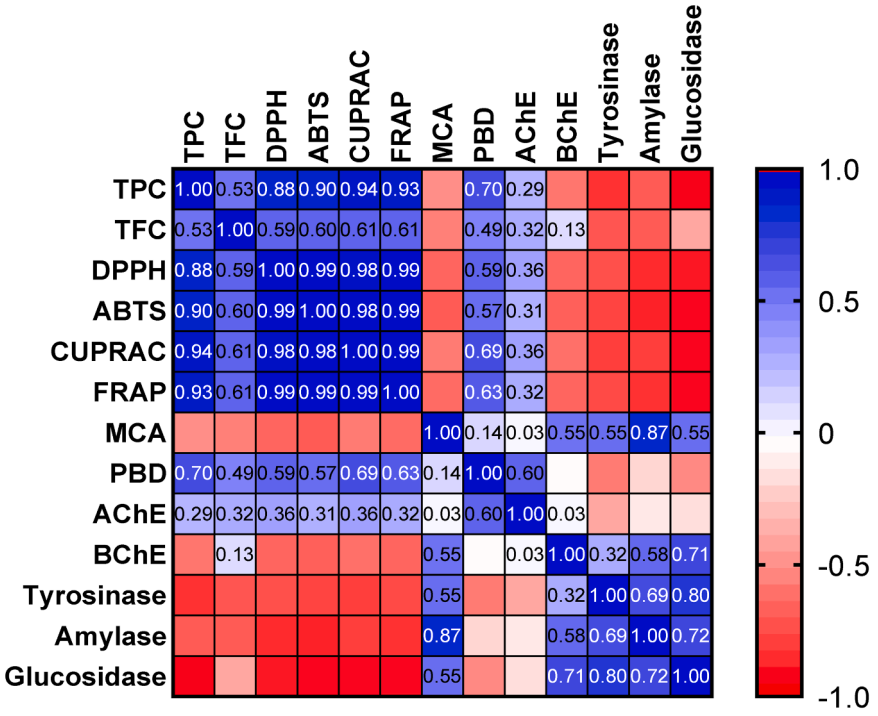


Fig. 4. Pearson correlation values between biological activity assays ($p < 0.05$).

diacetate have greater inhibitory potential than galantamine as well as rest of the compounds as predicted through docking studies.

The binding potential against BChE was found to be in range − 7.79 to − 10.82 kcal/mol. the compounds physapubescin and N-Desalkylbuprenorphine have almost similar binding potential of − 10.82 and − 10.57 kcal/mol. These compounds mediated conventional hydrogen bonds with ASP70, TYR332, HIS438 and SER198 (Fig. 1 (C and D) along with various other interactions. Pregna-5,16,20-triene-3β, 20-diol diacetate and physapubescin showed similar binding potential i.e. − 9.29 and − 9.28 kcal/mol against α-amylase while the overall binding potential of 12 compounds ranged between − 6.74 to − 9.29 kcal/mol. The physapubescin formed five hydrogen bonds with GLN35, TRP83, HIS210 and ARG344 and an additional carbon hydrogen bond with HIS80 while pregna-5,16,20-triene-3β,20-diol diacetate formed two hydrogen bonds with LYS209 and ARG344 (Fig. 2 (A and B)).

Evaluation of the compounds against α-glucosidase suggested that the binding affinity of these compounds ranges between − 6.56 to − 10.70 kcal/mol. The compounds physapubescin and withaphysacarpin bind effectively with α-glucosidase. The physapubescin mediated three conventional hydrogen bonds with SER474, LYS506, and ASP568 while withaphysacarpin mediated four hydrogen bonds with LYS506, ARG552 and ASP568. The binding interaction patterns for these compounds are given in Fig. 2 (C and D).

The binding potential of the twelve compounds against tyrosinase suggested that compounds more effectively towards inhibition tyrosinase than the reference compound kojic acid. The compound 5,7-megastigmadien-9-ol glucoside having lowest binding potential i.e. − 4.16 kcal is almost equal to binding potential of kojic acid 4.07 kcal/mol. The compound withaphysacarpin has highest binding potential − 8.30 kcal/mol in term of negative values. The compounds Pregna-5,16,20-triene-3β,20-diol diacetate and physapubescin has almost similar binding potential − 7.80 and − 7.79 kcal/mol (Table 5). The binding interaction patterns for withaphysacarpin and pregna-5,16,20-triene-3β,20-diol diacetate are provided in Fig. 3 (A and B). The docking analysis suggested that these compounds have potential to inhibit AChE, BChE, α-amylase, α-glucosidase and tyrosinase effectively.

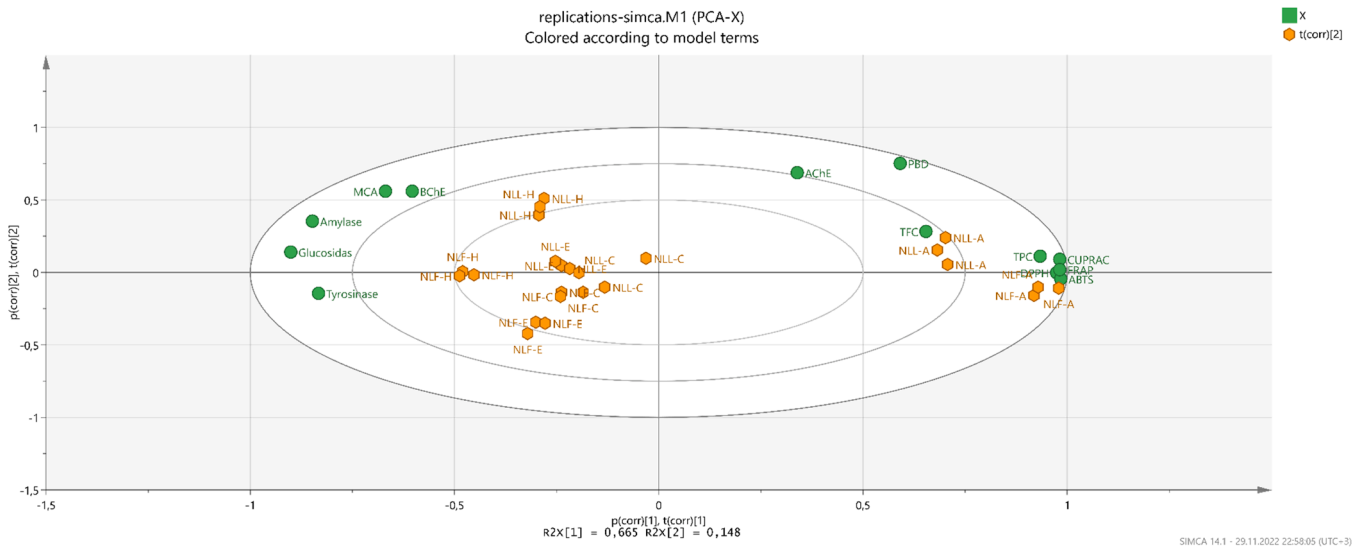


Fig. 5. Biplot presentation for Principal component analysis between tested samples and biological activity assays. TPC: Total phenolic content; TFC: Total flavonoid content; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; DPPH: 1,1-diphenyl-2-picrylhydrazyl; CUPRAC: Cupric reducing antioxidant capacity; FRAP: Ferric reducing antioxidant power; AChE: acetylcholinesterase; BChE: butyrylcholinesterase;.

Table 5
Binding free energy and inhibition constants of compounds against Acetylcholinesterase, butyrylcholinesterase, α-amylase, α-glucosidase and Tyrosinase.

Compounds	Acetylcholinesterase		butyrylcholinesterase		α-amylase		α-glucosidase		Tyrosinase	
	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants	Binding free energy (kcal/mol)	Inhibition constants
*Reference Compound	-9.27	161.01 nM	-8.10	1.15 μM	-10.42	22.86 nM	-8.57	522.01 nM	-4.07	1.04 mM
Coriandrone A	-8.81	349.82 nM	-7.79	1.95 μM	-7.37	3.98 μM	-6.56	15.47 μM	-7.67	2.40 μM
(3E)− 1-Hydroxy-4-[(1 R)− 2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl]− 3-bμten-2-yl ?-D-glucopyranoside	-9.83	62.36 nM	-10.11	39.10 nM	-7.36	4.00 μM	-6.69	12.44 μM	-5.48	96.59 μM
Physapubescin	-9.09	218.43 nM	-10.82	11.75 nM	-9.28	158.63 nM	-9.51	106.36 nM	-7.79	1.94 μM
Coagulin R 3-glucoside	-0.33	572.98 mM	-9.20	180.55 nM	-8.49	594.20 nM	-9.10	213.54 nM	-7.55	2.91 μM
3α,12α-Dihydroxy-5β-pregnan-20-one diacetate	-11.80	2.24 nM	-10.54	18.68 nM	-8.32	797.58 nM	-8.45	635.98 nM	-7.19	5.33 μM
Hasubanonine	-10.87	10.75 nM	-8.74	393.29 nM	-7.20	5.32 μM	-6.72	11.90 μM	-5.80	55.59 μM
N-Desalkylbuprenorphine	-11.00	8.58 nM	-10.57	17.89 nM	-8.95	275.96 nM	-8.01	1.34 μM	-7.63	2.57 μM
Pregna-5,16,20-triene-3β,20-diol diacetate	-11.84	2.11 nM	-10.21	32.68 nM	-9.29	154.82 nM	-8.59	505.76 nM	-7.80	1.93 μM
Rauwolscine	-10.85	11.18 nM	-9.76	70.45 nM	-8.57	525.84 nM	-8.98	263.51 nM	-6.73	11.60 μM
5,7-Megastigmadien-9-ol glucoside	-9.73	73.15 nM	-9.26	6.51 μM	-6.84	9.69 μM	-7.86	1.73 μM	-4.16	887.11 μM
RomucosineC	-8.93	283.86 nM	-9.13	204.19 nM	-6.74	11.39 μM	-7.28	4.60 μM	-5.36	117.48 μM
Withaphysacarpin	-10.35	26.06 nM	-9.85	60.48 nM	-8.80	354.57 nM	-10.70	14.34 nM	-8.30	828.02 nM

* For α-glucosidase and α-amylase, acarbose is used as reference compound. Galantamine is used reference compound for AChE and BChE. While kojic acid is used as reference for Tyrosinase.

4. Conclusions

In this work, various polarity solvent extracts of leaves and flowers of *N. leucolaena* were tested for their antioxidant and enzyme inhibition properties, and the activity potential of each extract was correlated with their bioactive contents. The ethanolic extracts of flowers and leaves were rich in phenolics and also showed potential radical scavenging and metal reducing activities, which led to conclude the antioxidant potential of *N. leucolaena* is due to its phenolic compounds. However, in phsphomolybdenum and metal chelating assays, other extracts also displayed significant activity. It is therefore, concluded that overall *N. leucolaena* is an important antioxidant plant and thus can be potential ingredient in nutraceutical and functional food formulation. On the other hand, all the extracts exhibited significant enzyme inhibition activities against five different enzymes of clinical importance; this fact substantiate the above deduction that *N. leucolaena* is medicinally an important plant. A comparison of our results with literature also led to conclude that solvents and their affinity with plant extract and extraction techniques affect the biological activities and chemical profiling. After observation of these results and metabolic pathways, it is concluded that ethanol extract could be the best choice for the extraction of bioactive plant components. Since, NLF-A and NLL-A have noteworthy total antioxidant potential, on the other hand, NLL-E has minimum antioxidant potential; this difference between activity potential was attributed to the total bioactive contents in these extracts. Altogether a mixed trend of activities was observed for flower and leaves extracts, therefore, keeping in view these factors and due to laboratory and financial limitations, only ethanolic extract of the whole plant

Table 6
Details of Interaction Patterns after Post-Dock Analysis of Docked Complex for Selected Compounds.

Ligands	Bond Category	*Bond type*	*Bond Distance*	*Interactions*			
				Residue Name and Groups	*From Chemistry*	*Residue Name and Groups*	*To Chemistry*
Interaction Patterns for Acetylcholinesterase							
Pregna-5,16,20-triene-3β,20-diol diacetate	Hydrogen Bond	Conventional Hydrogen Bond	2.15647	A:GLY120:HN	H-Donor	:LIG0:O	H-Acceptor
			1.84516	A:PHE295:HN		:LIG0:O	
	Hydrophobic	π-Alkyl	5.40739	A:TYR124	π-Orbitals	:LIG0	Alkyl
			4.13697	A:TRP286		:LIG0:C	
			5.19048	A:PHE297		:LIG0	
			5.20021	A:TYR337		:LIG0	
			4.44747	A:TYR337		:LIG0:C	
			4.31873	A:PHE338		:LIG0:C	
			5.14042	A:PHE338		:LIG0	
			3.89265	A:TYR341		:LIG0	
			5.11471	A:TYR341		:LIG0:C	
			4.52115	A:HIS447		:LIG0:C	
3α,12α-Dihydroxy-5β-pregnan-20-one diacetate	Hydrogen Bond	Conventional Hydrogen Bond	2.76432	A:ASP74:HN	H-Donor	:LIG0:O	H-Acceptor
			2.86757	A:TYR133:HH		:LIG0:O	
	Hydrogen Bond	Carbon Hydrogen Bond	3.02563	A:GLY121:CA	H-Donor	:LIG0:O	H-Acceptor
	Hydrophobic	π-Alkyl	4.11712	A:TRP86	π-Orbitals	:LIG0	Alkyl
			4.53853	A:TRP86		:LIG0	
			5.44077	A:TRP86		:LIG0	
			4.57391	A:TRP86		:LIG0	
			4.54395	A:TRP86		:LIG0	
			5.06854	A:TYR124		:LIG0:C	
			4.69541	A:TYR337		:LIG0	
			5.46572	A:TYR337		:LIG0:C	
			5.33258	A:PHE338		:LIG0:C	
			4.89092	A:HIS447		:LIG0	
			5.33546	A:HIS447		:LIG0:C	
Interaction Patterns for butyrylcholinesterase							
Physapubescin	Hydrogen Bond	Conventional Hydrogen	1.74891	A:SER198:HG	H-Donor	A:LIG1:O	H-Acceptor
			3.09335	A:TYR332:HH		A:LIG1:O	
			1.87527	A:HIS438:HE2		A:LIG1:O	
			2.51683	A:LIG1:H		A:HIS438:O	
	Hydrogen Bond	Carbon Hydrogen Bond	3.15211	A:HIS438:CD2	H-Donor	A:LIG1:O	H-Acceptor
	Hydrophobic	π-σ	3.88054	A:LIG1:C	C-H	A:PHE329	π-Orbitals
	Hydrophobic	Pi-Alkyl	5.2193	A:PHE329	π-Orbitals	A:LIG1	Alkyl
			5.30378	A:PHE329		A:LIG1:C	
N-Desalkylbuprenorphine	Hydrogen Bond	Conventional Hydrogen Bond	1.97205	:LIG0:H	H-Donor	A:ASP70:OD1	H-Acceptor
			2.07277	:LIG0:H		A:TYR332:OH	
			1.82367	:LIG0:H		A:HIS438:O	
			1.64675	:LIG0:H		:LIG0:O	
	Hydrophobic	π-π Stacked	3.74187	A:TRP82	π-Orbitals	:LIG0	π-Orbitals
			4.83302	A:TRP82		:LIG0	
	Hydrophobic	π-Alkyl	5.45468	A:PHE329	π-Orbitals	:LIG0	Alkyl
			5.01146	:LIG0		A:ALA328	
Interaction Patterns for α-amylase							
Physapubescin	Hydrogen Bond	Conventional Hydrogen Bond	2.71755	A:GLN35:HE21	H-Donor	A:LIG1:O	H-Acceptor
			2.14119	A:TRP83:HE1		A:LIG1:O	
			1.63239	A:HIS210:HE2		A:LIG1:O	
			1.68903	A:ARG344:HH11		A:LIG1:O	
			2.91781	A:ARG344:HH21		A:LIG1:O	
	Hydrogen Bond	Carbon Hydrogen Bond	2.82374	A:HIS80:CD2	H-Donor	A:LIG1:O	H-Acceptor
	Hydrophobic	π-Alkyl	4.71209	A:TYR82	π-Orbitals	A:LIG1:C	Alkyl
			5.39573	A:HIS147		A:CA480:CA	
			4.25011	A:HIS210		A:LIG1:C	
	Hydrophobic	Alkyl	5.20489	A:LIG1:C	Alkyl	A:LYS209	Alkyl
			3.78254	A:LIG1:C		A:LEU232	
			4.25949	A:LIG1:C		A:LYS209	
			5.03635	A:LIG1:C		A:LEU232	
Pregna-5,16,20-triene-3β,20-diol diacetate	Hydrogen Bond	Conventional Hydrogen Bond	1.93978	A:LYS209:HZ1	H-Donor	:LIG0:O	H-Acceptor
			2.2055	A:ARG344:HH11		:LIG0:O	
	Hydrophobic	π-Alkyl	4.93509	A:TYR82	π-Orbitals	:LIG0	Alkyl
			4.13384	A:TYR155		:LIG0:C	
			4.52637	A:HIS210		:LIG0	
			4.35959	A:HIS210		:LIG0:C	
			4.4445	A:HIS210		:LIG0:C	
	Hydrophobic	Alkyl	5.37238	A:LYS209	Alkyl	:LIG0	Alkyl
			5.13289	A:LEU232		:LIG0	
			4.7404	:LIG0		A:LEU232	

(*continued on next page*)

Table 6 (*continued*)

Ligands	Bond Category	*Bond type*	*Bond Distance*	*Interactions*			
				Residue Name and Groups	*From Chemistry*	*Residue Name and Groups*	*To Chemistry*
			3.99089	:LIG0:C		A:LEU166	
			5.16045	:LIG0:C		A:LYS209	
	Hydrophobic	π-σ	3.5739	:LIG0:C	C-H	A:HIS80	π-Orbitals
Interaction Patterns for α-glucosidase							
Physapubescin	Hydrogen	Conventional Hydrogen	2.15497	A:SER474:HG	H-Donor	A:LIG1:O	H-Acceptor
	Bond	Bond	2.20415	A:LYS506:HZ2		A:LIG1:O	
			2.08828	A:LIG1:H		A:ASP568:OD2	
	Hydrophobic	π-Alkyl	4.33554	A:TRP329	π-Orbitals	A:LIG1:C	π-Orbitals
			4.24853	A:TRP432		A:LIG1:C	
			4.33482	A:PHE601		A:LIG1:C	
	Hydrophobic	Alkyl	4.46194	A:ALA628	Alky	A:LIG1:C	Alkyl
			4.72246	A:LIG1:C		A:ILE358	
	Hydrophobic	π-σ	3.77247	A:LIG1:C	C-H	A:TRP329	π-Orbitals
			3.33212	A:LIG1:C		A:TRP329	
			3.82683	A:LIG1:C		A:TRP432	
Withaphysacarpin	Hydrogen	Conventional Hydrogen	1.94402	A:LYS506:HZ2	H-Donor	:LIG0:O	H-Acceptor
	Bond	Bond	1.90997	A:ARG552:HH11		:LIG0:O	
			1.71128	:LIG0:H		A:ASP568:OD1	
			1.88137	:LIG0:H		A:ASP568:OD1	
	Hydrophobic	π-Alkyl	4.33201	A:TRP329	π-Orbitals	:LIG0:C	Alkyl
			5.35325	A:TRP432		:LIG0	
			5.05788	A:TRP467		:LIG0:C	
			5.23707	A:TRP467		:LIG0:C	
			5.40456	A:PHE476		:LIG0	
			4.36111	A:PHE476		:LIG0	
			5.1653	A:PHE601		:LIG0:C	
			4.31339	A:PHE601		:LIG0:C	
			4.75601	A:HIS626		:LIG0:C	
	Hydrophobic	Alkyl	4.86719	:LIG0:C	Alkyl	A:ILE396	Alkyl
Interaction Patterns for Tyrosinase							
Pregna-5,16,20-triene-3β,20-diol diacetate	Hydrogen	Conventional Hydrogen	3.00963	A:ASN199:HN	H-Donor	:LIG0:O	H-Acceptor
	Bond	Bond	2.18741	A:GLY200:HN		:LIG0:O	
	Hydrophobic	Alkyl	4.61611	A:PRO201	Alkyl	:LIG0	Alkyl
			4.39383	A:ARG209		:LIG0	
			4.85744	:LIG0:C		A:ARG209	
			4.84923	:LIG0:C		A:VAL217	
			4.9496	:LIG0:C		A:VAL218	
	Hydrophobic	π-Alkyl	4.85606	A:PHE197	π-Orbitals	:LIG0	Alkyl
			4.87009	A:PHE197		:LIG0	
	Hydrophobic	π-σ	3.76778	:LIG0:C	C-H	A:HIS208	π-Orbitals
Withaphysacarpin	Hydrogen	Conventional Hydrogen	3.01913	A:GLY200:HN	H-Donor	:LIG0:O	H-Acceptor
	Bond	Bond	2.42587	A:ARG209:HE		:LIG0:O	
			2.32159	:LIG0:H		A:GLY216:O	
			2.07851	:LIG0:H		A:GLU158:OE2	
			1.99065	:LIG0:H		A:GLU158:OE1	
	Hydrogen	Carbon Hydrogen Bond	2.95024	A:PHE197:CA	H-Donor	:LIG0:O	H-Acceptor
	Bond		2.9348	A:GLY200:CA		:LIG0:O	
	Hydrophobic	Alkyl	4.95731	A:PRO201	Alkyl	:LIG0	Alkyl
			5.1789	A:ARG209		:LIG0	
			3.72014	:LIG0:C		A:PRO201	
			4.72093	:LIG0:C		A:MET184	
	Hydrophobic	π-Alkyl	5.01881	A:PHE197	π-Orbitals	:LIG0:C	Alkyl

material was analyzed for its secondary metabolic profile, which disclosed 139 compounds belonging to important classes of secondary metabolites. However, the above discussed activities and metabolic profile led to conclude that overall, *N. leucolaena* extracts are rich in bioactive small molecules and thus displayed have biochemical and functional foods activity. Still, in the future, these results can be used to reveal the medicinal and harmful effects of this plant. However, to validate preclinical findings, clinical investigations are required.

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.

Data availability

Data will be made available on request.

Acknowledgement

The authors from King Khalid University of Saudi Arabia extend their appreciation to Deanship of Scientific Research for financial support through project RGP.1/318/43. The authors would like to acknowledge technical support for sample analysis from Research Center for Advanced Materials Science (RCAMS) at King Khalid University, Abha, Saudi Arabia.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.procbio.2022.12.005.

References

[1] K. Singh, B. Kumar, J. Sharma, S. Gairola, Medicinal plants used to manage CNS and memory-related problems by indigenous communities of Jammu & Kashmir (UT) and Ladakh (UT), India, Plant Arch. 20 (2) (2020) 1959–1974.

[2] A. Sharma, R. Cooper, G. Bhardwaj, D.S. Cannoo, The genus Nepeta: traditional uses, phytochemicals and pharmacological properties, J. Ethnopharmacol. 268 (2021), 113679.

[3] M.A. Salim, S. Ranjitkar, R. Hart, T. Khan, S. Ali, C. Kiran, A. Parveen, Z. Batool, S. Bano, J. Xu, Regional trade of medicinal plants has facilitated the retention of traditional knowledge: case study in Gilgit-Baltistan Pakistan, J. Ethnobiol. Ethnomed. 15 (1) (2019) 1–33.

[4] M. Sharifi-Rad, F. Epifano, S. Fiorito, J.M. Álvarez-Suarez, Phytochemical analysis and biological investigation of Nepeta juncea Benth. different extracts, Plants 9 (5) (2020) 646.

[5] S.A. Mohajeri, N. Hedayati, M. Bemani-Naeini, Available saffron formulations and product patents, Saffron (2020) 493–515.

[6] I. Süntar, S.M. Nabavi, D. Barreca, N. Fischer, T. Efferth, Pharmacological and chemical features of Nepeta L. genus: its importance as a therapeutic agent, Phytother. Res. 32 (2) (2018) 185–198.

[7] B. Salehi, M. Valussi, A.K. Jugran, M. Martorell, K. Ramírez-Alarcón, Z. Z. Stojanović-Radić, H. Antolak, D. Kręgiel, K.S. Mileski, M. Sharifi-Rad, Nepeta species: From farm to food applications and phytotherapy, Trends Food Sci. Technol. 80 (2018) 104–122.

[8] T. Srivastava, O. Gupta, Medicinal plants used by amchies in Ladakh, Cultiv. Util. Aromat. Plants N. Delhi (1982) 103–106.

[9] M.I. Tousif, M. Nazir, M. Saleem, S. Tauseef, R. Uddin, M. Altaf, G. Zengin, G. Ak, R.B. Ozturk, M.F. Mahomoodally, Exploring the industrial importance of a miracle herb Withania somnifera (L.) Dunal: Authentication through chemical profiling, in vitro studies and computational analyses, Process Biochem (2022).

[10] M. Saleem, N. Shazmeen, M. Nazir, N. Riaz, G. Zengin, H.M. Ataullah, F. Nisar, M. Mukhtar, M.I. Tousif, Investigation on the phytochemical composition, antioxidant and enzyme inhibition potential of polygonum plebeium r. br: a comprehensive approach to disclose new nutraceutical and functional food ingredients, Chem. Biodivers. 18 (12) (2021), e2100706.

[11] M. Zubair, M. Nazir, M. Saleem, N. Raiz, S. Touseef, S. Khan, G. Zengin, M. Ehsan Mazhar, M. Imran, Tousif, chemodiversity, biological activities and molecular docking studies of leptadenia pyrotechnica (forssk.) decne: a comprehensive approach to validate its medicinal use, Chem. Biodivers. (2022), e202100884.

[12] M.I. Tousif, M. Nazir, M. Saleem, S. Tauseef, R. Uddin, M. Altaf, G. Zengin, G. Ak, R.B. Ozturk, M.F. Mahomoodally, Exploring the industrial importance of a miracle herb Withania somnifera (L.) Dunal: authentication through chemical profiling, in vitro studies and computational analyses, Process Biochem 121 (2022) 514–528.

[13] N. Shazmeen, M. Nazir, N. Riaz, M. Saleem, M.I. Tousif, S. Tauseef, R. Uddin, M. Mukhtar, G. Zengin, A.J.F.B. Mollica, In vitro antioxidant and enzyme inhibitory studies, computational analysis and chemodiversity of an emergency food plant Caralluma edulis (Edgew.) Benth. ex Hook. f: a multifunctional approach to provide new ingredients for nutraceuticals and functional foods, Food Biosci. 50 (2022), 102097.

[14] J.L. Sussman, D. Lin, J. Jiang, N.O. Manning, J. Prilusky, O. Ritter, E.E. Abola, Protein data bank (PDB): database of three-dimensional structural information of biological macromolecules, Acta Crystallogr, D. ACTA CRYSTALLOGR D. 54 (6) (1998) 1078–1084.

[15] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A. J. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (16) (2009) 2785–2791.

[16] D.S. Biovia, Discovery studio visualizer, San Diego, CA, USA 936 (2017).

[17] A. Bast, G.R. Haenen, Pharmaceutical compounds with antioxidant activity. Antioxidants and Cardiovascular Disease, Springer,, 2000, pp. 71–83.

[18] A. Gökbulut, G. Yilmaz, Nepeta humilis Bentham: first evaluation of phenolic profile and radical scavenging potential, J. Res. Pharm. 24 (2020) 901–907.

[19] E.A. Trends GuideKöksal, H. Tohma, Ö. Kılıç, Y. Alan, A. Aras, İ. Gülçin, E. Bursal, Assessment of antimicrobial and antioxidant activities of Nepeta trachonitica: analysis of its phenolic compounds using HPLC-MS/MS, Sci. Pharm. 85 (2) (2017) 24.

[20] G. Zengin, M.F. Mahomoodally, A. Aktumsek, J. Jekő, Z. Cziáky, M.J. Rodrigues, L. Custodio, R. Polat, U. Cakilcioglu, A. Ayna, Chemical profiling and biological evaluation of Nepeta baytopii extracts and essential oil: an endemic plant from Turkey, Plants 10 (6) (2021) 1176.

[21] S. Zolghadri, A. Bahrami, M.T. Hassan Khan, J. Munoz-Munoz, F. Garcia-Molina, F. Garcia-Canovas, A.A. Saboury, A comprehensive review on tyrosinase inhibitors, J. Enzym. Inhib. Med. Chem. 34 (1) (2019) 279–309.

[22] Z. Tayarani-Najaran, M. Akaberi, M. Vatani, S.A. Emami, Evaluation of antioxidant and anti-melanogenic activities of different extracts from aerial parts of Nepeta binaludensis Jamzad in murine melanoma B16F10 cells, Iran. J. Basic Med. Sci. 19 (6) (2016) 662.

[23] M. Otręba, J. Rok, E. Buszman, D. Wrzesniok, Regulacja melanogenezy: rola cAMP i MITF (Regulation of melanogenesis: the role of cAMP and MITF), Post. Hig. Med. Dosw 66 (2012) 33–40.

[24] J. Pazos, P. Zema, G.B. Corbino, J. Gabilondo, R. Borioni, L.S. Malec, Growing location and root maturity impact on the phenolic compounds, antioxidant activity and nutritional profile of different sweet potato genotypes, Food Chem: Mol. Sci. 5 (2022), 100125.

[25] J. Wei, S. Li, T. Su, J. Zhao, Y. Jiang, Y.A. Zubarev, Y. Bi, Phenolic compositions and antioxidant activities of Hippophae tibetana and H. rhamnoides ssp. sinensis berries produced in Qinghai-Tibet Plateau, Food Chem.: X 15 (2022), 100397.

[26] B. Kirkan, C. Sarikurkcu, M.S. Ozer, M. Cengiz, N. Atılgan, O. Ceylan, B. Tepe, Phenolic profile, antioxidant and enzyme inhibitory potential of Onosma tauricum var. tauricum, Ind. Crops Prod. 125 (2018) 549–555.

[27] A.I. Uba, G. Zengin, D. Montesano, U. Cakilcioglu, S. Selvi, M.D. Ulusan, G. Caprioli, G. Sagratini, S. Angeloni, S. Jugreet, Antioxidant and enzyme inhibitory properties, and HPLC–MS/MS profiles of different extracts of Arabis carduchorum Boiss.: an endemic plant to Turkey, Appl. Sci. 12 (13) (2022) 6561.

[28] C. Sarikurkcu, G. Zengin, Polyphenol profile and biological activity comparisons of different parts of Astragalus macrocephalus subsp. finitimus from Turkey, Biology 9 (8) (2020) 231.