



Contents lists available at ScienceDirect

Arabian Journal of Chemistry

journal homepage: www.sciencedirect.com



Original article

## A comprehensive assessment of phytochemicals from *Phyla nodiflora* (L.) Greene as a potential enzyme inhibitor, and their biological potential: An *in-silico*, *in-vivo*, and *in-vitro* approach



Ahmed Alafnan<sup>a</sup>, Muhammad Faisal Nadeem<sup>b</sup>, Syed Faraz Ahmad<sup>c</sup>, Muhammad Sarfraz<sup>d</sup>, Abdulwahab Aalamri<sup>a,e</sup>, Nasrin E Khalifa<sup>f,g</sup>, Alasmari Saeed Abdullah<sup>h</sup>, Ali Murtaza<sup>i</sup>, Muhammad Danish<sup>j</sup>, Irshad Ahmad<sup>j</sup>, Riaz Hussain<sup>k</sup>, Marcello Locatelli<sup>l</sup>, Umair Khurshid<sup>j</sup>, Nafees Ahemad<sup>m</sup>, Hammad Saleem<sup>b,\*</sup>

<sup>a</sup> Department of Pharmacology and Toxicology, College of Pharmacy, University of Hail, Hail 55476, Saudi Arabia

<sup>b</sup> Institute of Pharmaceutical Sciences (IPS), University of Veterinary & Animal Sciences (UVAS), Lahore, Pakistan

<sup>c</sup> Wah Medical College, Wah Cantt, Pakistan

<sup>d</sup> College of Pharmacy, Al Ain University, Al Ain, United Arab Emirates

<sup>e</sup> Medical and Diagnostic Research Center, University of Hail, Hail 55476, Saudi Arabia

<sup>f</sup> Department of Pharmaceutics, College of Pharmacy, University of Hail, Hail 55476, Saudi Arabia

<sup>g</sup> Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum, Sudan

<sup>h</sup> MHC Clinics, Security Forces Hospital, Riyadh, Saudi Arabia

<sup>i</sup> Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

<sup>j</sup> Department of Pharmaceutical Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

<sup>k</sup> University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, 63100, Pakistan

<sup>l</sup> Department of Pharmacy, University 'G. D'Annunzio' of Chieti-Pescara, 66100, Chieti, Italy

<sup>m</sup> School of Pharmacy, Monash University Malaysia, Malaysia

## ARTICLE INFO

## Article history:

Received 14 March 2023

Accepted 31 August 2023

Available online 9 September 2023

## Keywords:

*Phyla nodiflora*

Phytochemicals

Antioxidant

Enzyme inhibition

Toxicity

HPLC

## ABSTRACT

This work explored *Phyla nodiflora* (L.) Greene as a potential source of the bioactive medicinal agent. In this aspect, methanol (PN-M) and dichloromethane (PN-D) extracts were prepared from the whole plant and evaluated for phytochemical composition (total bioactive contents, UHPLC-MS analysis, and HPLC-PDA polyphenolic quantification), biological (antioxidant and enzyme inhibition) potential and *in-vivo* toxicity. The PN-M was found to contain higher phenolic (26.08 mg GAE/g extract) and flavonoid (50.25 mg QE/g extract) contents which might correlate to the higher radical scavenging (DPPH: 52.94 mg TE/g extract; ABTS: 72.11 mg TE/g extract) and reducing power (FRAP: 71.96 mg TE/g extract; CUPRAC: 142.65 mg TE/g extract) antioxidant potential, as well as AChE (4.33 mg GALAE/g extract), tyrosinase (125.36 mg KAE/g extract), and amylase (1.86 mmol ACAE/g extract) inhibition activity of this extract. In contrast, the PN-D extract was found to be most active for phosphomolybdenum (1.30 mg TE/g extract) and metal chelation (54.84 mg EDTAE/g extract) assays in addition to BChE (4.70 mg GALAE/g extract) and glucosidase (0.62 mmol ACAE/g extract) enzyme inhibition activity. The PN-M extract on UHPLC-MS analysis revealed the tentative identification of 24 different secondary metabolites, most of which belonged to the flavonoid, glycoside, and terpenoid classes of phytochemicals. The polyphenolic composition of the extracts was appraised by HPLC-PDA. Seven phenolic compounds were identified in the extracts. PN-M was found to be rich in catechin (0.25 µg/extract) and 3-OH benzoic acid (0.64 µg/extract), while PN-D contained epicatechin (0.30 µg/extract), 3-OH-4-MeO benzaldehyde (0.21 µg/extract), and 2,3-Di-MeO benzoic acid (0.97 µg/extract) in higher amounts. The methanol extract was found to be non-toxic even at higher doses. Furthermore, the relationship between the phytochemicals and the tested enzymes was highlighted by molecular docking studies. In sum, this research showed that the studied

\* Corresponding author.

E-mail address: [hammad.saleem@uvas.edu.pk](mailto:hammad.saleem@uvas.edu.pk) (H. Saleem).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.arabjc.2023.105233>

1878-5352/© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

extracts were effective as enzyme inhibitors and antioxidants, suggesting it would be worth investigating in more depth for further advanced studies to explore its pharmacological properties.

© 2023 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Plant species have been used for medicine for as long as humans have existed. Different civilizations have provided documentary evidence of the medicinal use of plants (Phillipson 2001, Suroowan, Llorent-Martínez et al. 2023). The therapeutic effects of plants are due to the presence of different organic and inorganic salts (Kurmukov 2013, Acquaviva, Di Simone et al. 2023). After years of struggle, the use of plants was established as medicine against various ailments (Petrovska 2012). Primary and secondary metabolites produced by the plants are the sources for different drugs to be used medicinally (Balick and Cox 1996). Humans have been using folk medicines for thousands of years extracted from plants, which participated in the conception of modern medicines (Hassan 2015, Acquaviva, Di Simone et al. 2023). Drugs are obtained from plants by employing different botanical, biological, phytochemical, and molecular techniques (Balunas and Kinghorn 2005).

The medicinal plant "*P. nodiflora*" is a predominant member of the Verbenaceae family. Its common habitat is in Central and South America, Pakistan, Sri Lanka, and India. *P. nodiflora* grows best on moist soil. It is present in soil rich in moisture (Sharma and Singh 2013, Al-Snai 2019, Rahman, Javaid et al. 2021). Two flavones, luteolin-7-*O*-glucoside and 6-hydroxyluteolin-7-*O*-apioside, are mainly present in the flowers of *P. nodiflora* and the leaves of *P. nodiflora* were also found to contain different flavone glycosides (Barnabas, Gunasingh et al. 1980, Jabeen, Jillani et al. 2016). Acetoside and demethoxycentaureidin are the two compounds yielded from the alcoholic extract of *P. nodiflora* (Khalil, Lahloub et al. 1995). The isolation of stigmaterol, beta-sitosterol, and triterpenoid lippacin was reported from the methanolic extract of *P. nodiflora* (Sharma and Singh 2013). The aerial parts also reported numerous steroidal constituents (Siddiqui, Ahmed et al. 2009). Methanol and dichloromethane extract of *P. nodiflora* leaves are reported to contain the compounds halleridone and halleron (Ravikanth, Ramesh et al. 2000). A skin whitening agent, eupafolin, was yielded from dried aerial parts of the *P. nodiflora* (Yen, Wang et al. 2012). The methanol extract of *P. nodiflora* yields hispidulin, nodifloretin, and eupafolin (Ko, Chiang et al. 2014). The ethanolic extract of *P. nodiflora* contains a diverse group of chemical compounds, including betatifolin, nodifloridin, jaceosidin, lippiflorin, and nepetin (Regupathi and Chitra 2015). The ethanolic extract of *P. nodiflora* has also been reported as the main source of the ecteoside (Khalil, Lahloub et al. 1995). Likewise, stigmaterol, eugenol,  $\alpha$ -copaene,  $\beta$ -bisabolene, and  $\gamma$ -sitosterol are among the various constituents isolated from the plant's methanolic extract (Ko, Chiang et al. 2014). Similarly, steam distillation of *P. nodiflora* has reported the presence of linalool, methyl salicylate, cymen-8-ol, and  $\beta$  carboline (Elakovich and Stevens 1985). *P. nodiflora* have been previously reported for a number of biological effects (Al-Snai 2019, Paua 2022), including antimicrobial (Gopal, Balkrishna et al. 1996, Patel Janki, Shah Kinjal et al. 2005, Pirezada, Iqbal et al. 2005, Durairaj, Vaiyapuri et al. 2007, Malathi, Cholarajan et al. 2011, Sharma and Singh 2013), antitumor (Vanajothi, Sudha et al. 2012, Cheong and Teoh 2014), hepatoprotective (Durairaj, Vaiyapuri et al. 2008, Arumanayagam and Arunmani 2015), antioxidant (Ashokkumar, Thamilselvan et al. 2008, Durairaj, Vaiyapuri et al. 2008), diuretic (Shukla, Patel et al. 2009, Balamurugan and Ignacimuthu 2011), antidiabetic, hypolipidemic (Balamurugan,

Duraipandiyan et al. 2011, Balamurugan and Ignacimuthu 2011), neuropharmacological (Thirupathy, Tulshkar et al. 2011, AO and SL 2012), anti-urolithiasis (Dodoala, Diviti et al. 2010), anti-inflammatory (Ahmed, Selim et al. 2004, Balakrishnan, Janakarajan et al. 2010, Al-Snafi and Faris 2013), melanogenesis inhibition (Yen, Wang et al. 2012, Ko, Chiang et al. 2014), antihypertensive (Gadhvi, Mishra et al. 2012), skin whitening effect (Yen, Wang et al. 2012), central inhibitory (AO and SL 2012), apoptotic (Cheong and Teoh 2014), and antidandruff (Regupathi and Chitra 2015) activities.

Hence, the estimation of phytochemical contents both quantitatively and qualitatively, along with the assessment of antioxidant and enzyme inhibition potential, as well as the *in-vivo* toxicological studies of methanol and dichloromethane extracts of *P. nodiflora*, were the main objectives of this paper. The phytochemical profile was evaluated by ascertaining preliminary phytochemical testing, total phenolic and flavonoid contents, UHPLC-MS analysis, and HPLC-PDA polyphenolic quantification. The antioxidant potential was assessed via different assays, including radical scavenging (DPPH and ABTS), reducing power (FRAP and CUPRAC), total antioxidant activity (phosphomolybdenum), and metal chelation assays. Likewise, the inhibitory potential of both extracts was tested against the five therapeutically relevant enzymes, including AChE and BChE (involved in neurological problems), amylase, glucosidase (involved in diabetes), and tyrosinase (skin problems). Furthermore, *in-silico* studies against the tested enzymes were also conducted. The *in-vivo* toxicity was tested on the chicks for 21 days. This work will provide evidence for the pharmacological effectiveness of these species by identifying the best methods for isolating their bioactive components.

## 2. Material and methods

### 2.1. Plant collection and extraction

The whole plant material was collected from the peripheries of Bahawalpur City, Pakistan (GPS co-ordinates: 29°23'19.7"N 71°42'07.0"E). The plant was identified and authenticated as *P. nodiflora* by Mr. Hafiz Waris, Taxonomist at Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur. For future reference, a voucher specimen of the plant material (PN-WP-01-14-131) was also deposited at the herbarium of the Islamia University of Bahawalpur's Faculty of Pharmacy and Alternative Medicines. The plant material was subjected to shade drying for 15 days. The dried plant material was grounded into a fine powder with the help of a grinding mill. The powdered plant material was extracted with dichloromethane and methanol successively. The filtrate collected was concentrated on a Rotary evaporator at 35 °C under reduced pressure.

### 2.2. Phytochemical composition

#### 2.2.1. Total phenolic and total flavonoid contents

The total bioactive contents, including total phenolic and flavonoid contents, were estimated using well-established Folin-Ciocalteu and aluminium chloride assays, respectively. (Saleem, Zengin et al. 2019). The standard for total phenolic content was gallic acid, with results expressed as mg GAE/g (gallic acid equivalents), while quercetin was used as a standard for total flavonoid

content, and the results were expressed as mg QE/g (quercetin equivalent). The detailed protocols for these assays are presented in the [supplementary material](#) section.

### 2.2.2. UHPLC-MS analysis

UHPLC-MS analysis is a technique that is mostly used for the tentative identification of polar compounds in plant extracts. Therefore, the methanol extract (being polar one) was subjected to the UHPLC-MS analysis (negative ionization mode) for the tentative identification of possible secondary metabolites. The identification of the phytochemicals was made by using METLIN database library (Saleem, Zengin et al. 2019). The detailed instrumentation for this analysis is presented in the [supplementary material](#) section.

### 2.2.3. HPLC-PDA quantification of different phytochemicals

HPLC-PDA analysis (of both the methanol and dichloromethane extracts of *P. nodiflora*) for 22 different phytochemicals (including 4-hydroxybenzoic acid, 3-dimethoxybenzoic acid, quercetin, gallic acid, benzoic acid, catechin, epicatechin, harpagoside, naringin, vanillic acid, carvacrol, 3-hydroxybenzoic acid, naringenin, 3-hydroxy-4-methoxy benzaldehyde, *t*-cinnamic acid, *p*-coumaric acid, chlorogenic acid, rutin, syringic acid, sinapinic acid, *t*-ferulic acid, and *o*-coumaric acid) was done and quantified using previously reported method (Locatelli, Zengin et al. 2017). The gradient elution program used for HPLC analyses is given in [Table S1](#) of the [supplementary material](#) section. Likewise, the standards used, retention times, and maximum wavelengths used for quantitative analyses are shown in [Table S2](#) of the [supplementary material](#) section. The chemical standards chromatogram for the 22 standards used is depicted in [Figure S1](#) of the [supplementary material](#) section. The detailed instrumentation for this analysis is presented in the [supplementary material](#) section.

## 2.3. Biological assays

### 2.3.1. Antioxidant assays

The metal chelating, phosphomolybdenum, FRAP, CUPRAC, ABTS, and DPPH activities were made as per previously described methods (Saleem, Zengin et al. 2019). The antioxidant activities were reported as Trolox equivalents, whereas EDTA was used for the metal-chelating assay. The detailed protocols for these assays are presented in the [supplementary material](#) section.

### 2.3.2. Enzyme inhibition activities

The possible inhibitory effects of the extracts against cholinesterases, tyrosinase,  $\alpha$ -amylase, and  $\alpha$ -glucosidase were assessed using standard bio-assays (Saleem, Zengin et al. 2019). Galantamine was used as a standard for AChE, and BChE inhibition activity was expressed as (mg GALAE/g extract). In contrast, the inhibition activities for  $\alpha$ -glucosidase and  $\alpha$ -amylase were reported as millimoles of acarbose equivalent (mmol ACAE/g extract), whereas tyrosinase inhibition was noted as milligrams of kojic acid equivalent (mg KAE/g extract). The detailed protocols for these assays are presented in the [supplementary material](#) section.

## 2.4. Computation methods

### 2.4.1. Structures preparation and docking studies

Sybyl-X1.3/SKETCH (Jain 2003) was used to create a three-dimensional conformation of inhibitors 6-hydroxyluteolin 5-rhamnoside (HLR), luteolin 7-rhamnosyl (1- $\rightarrow$ 6) galactoside (LRG), and torosafavone D (TFD) ([Fig. 3](#)). To get biologically active conformation of compounds under study, all three inhibitors were subjected to energy optimization using the Tripos force field with

Gasteiger Hückel atomic charges (Powell 1978). The co-crystal structures of acetylcholinesterase (ACHE), butyrylcholinesterase (BChE), and tyrosinase (TYR) were obtained from the RCSB Protein Data Bank under the following PDB entries; (PDB ID's: 4EY7 (Cheung, Rudolph et al. 2012), 6QAE (Meden, Knez et al. 2019) and 2Y9X (Ismaya, Rozeboom et al. 2011), respectively). Before docking investigations, all protein structures were further processed using the structure preparation tools contained in the SYBYL-X 1.3 biopolymer module (Ghersli and Sanchez 2011). The energy was minimized using the Powell algorithm with a convergence gradient of 0.5 kcal (mol)<sup>-1</sup> for 1000 cycles, with missing hydrogens added, charges applied, and atom types assigned according to the AMBER 7 FF99 force field (Onufriev, Bashford et al. 2004). Finally, the energy-optimized bioactive conformation of selected compounds was docked into the active site of ACHE, BChE, and TYR enzymes using the Surflex-Dock module of the SYBYL-X 1.3 software package, following the same protocol and parameters as those published in our prior work (Chohan, Chen et al. 2016, Chohan, Qian et al. 2016). The experimentally determined active conformation of donepezil in 4EY7, N1, N2-dimethylpropane-1,2-diamine in 6QAE, and tropolone in 2Y9X was used as initial conformation to define the potential binding pocket for protomol (an idealized active site) generation (Jain 2007). The top twenty docked conformations were saved and investigated for their binding modes into the active site of their respective target. The Hammerhead scoring method was used to rate these potential ligand poses (Jain 1996, Jain 2003).

## 2.5. In-vivo toxicological studies

The *in-vivo* toxicological parameters were studied on the broiler chicks. The animal's selection, grouping, toxicity parameters, hematological and biochemical analysis, and relative and absolute weight of body organs were calculated as reported in our previously published data (Saleem, Zengin et al. 2019).

## 2.6. Statistical analysis

All the biological experiments were done in triplet, and the estimation of results was made as mean value  $\pm$  standard deviation (SD). One-way ANOVA test was used to find the mean difference, followed by data analysis using SPSS v.17.0 software.

## 3. Results and discussion

### 3.1. Antioxidant potential

The involvement of oxidative stress in disease development and progression is becoming more evident. Increasing endogenous antioxidant defenses or supplementing the body with exogenous antioxidants is a viable approach to combating ROS-induced oxidative damage (Pizzino, Irrera et al. 2017, Lan, Liu et al. 2021). The plant kingdom provides a rich supply of beneficial substances to one's health, particularly natural antioxidants. (Zengin, Mahomoodally et al. 2022). In the current study, PN-M and PN-D extracts of the *P. nodiflora* plant were tested for a panoply of antioxidant assays, including radical scavenging (DPPH and ABTS), reducing power (FRAP and CUPRAC), total antioxidant activity (phosphomolybdenum) and metal chelation activities, and the results are presented in [Table 1](#). A similar pattern to total bioactive contents was noted, and the methanol extract was found to have a higher antioxidant potential for radical scavenging (DPPH: 52.94 mg TE/g extract; ABTS: 72.11 mg TE/g extract) and reducing power (FRAP: 71.96 mg TE/g extract; CUPRAC: 142.65 mg TE/g extract) assays. This higher antioxidant activity of methanol

**Table 1**  
Total bioactive contents and antioxidant properties of *P. nodiflora* methanol and dichloromethane extracts.

Extracts	Total bioactive contents		Antioxidant activities					
			Radical Scavenging activity		Reducing power		Total antioxidant capacity (TAC)	Ferrous chelating
	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (mgTE/g extract)	ABTS (mgTE/g extract)	FRAP (mgTE/g extract)	CUPRAC (mgTE/g extract)	PBD (mgTE/g extract)	Metal Chelating (mgEDTA/g)
PN-M	26.08 ± 1.32	50.25 ± 0.15	52.94 ± 0.47	72.11 ± 2.49	71.96 ± 1.67	142.65 ± 1.41	1.17 ± 0.03	32.18 ± 0.53
PN-D	18.85 ± 0.38	31.80 ± 0.84	3.44 ± 0.58	12.86 ± 0.86	35.66 ± 0.67	86.61 ± 1.01	1.30 ± 0.09	54.84 ± 0.43

PN-M: *P. nodiflora* methanol extract; PN-D: *P. nodiflora* dichloromethane extract.

GAE: gallic acid equivalent; QE: quercetin equivalent; TE: trolox equivalent; EDTAE: EDTA equivalent. Values expressed are means ± S.D. of three parallel measurements.

extract might be linked to the higher amount of phenolic and flavonoid contents for this extract. These findings agree with some of the previous research data, which have provided a positive relationship between bioactive contents and antioxidant potential (Chang, Ye et al. 2022, Chen, Ran et al. 2022, Li, Shi et al. 2022, Zengin, Fernández-Ochoa et al. 2023). However, in the case of phosphomolybdenum and MCA assay, different results were obtained, and the dichloromethane extract was found to contain higher activity. This higher activity could be linked to the non-phenolic compounds present in this extract. These findings correlate with the previous studies, which also presented the same trend, and the methanolic extract of *P. nodiflora* has higher antioxidant potential for tested reducing power, scavenging free radical, and scavenging superoxide anion radical activities and other antioxidant assays (Ashokkumar, Thamilselvan et al. 2008, Durairaj, Vaiyapuri et al. 2008). Meticulous correlation values between these assays in plant extracts have been published by various researchers, consistent with our findings. However, we noticed a low correlation value for the PBD assay, which could be explained by additional reducing chemicals in the extracts (peptides, sugars, etc.). The poor correlation value obtained between total phenolics and the metal chelating assay can be explained by the activity of non-phenolic chelators present in the examined extracts, as demonstrated in the previous study (References) (Zengin, Ak et al. 2022).

### 3.2. Enzyme inhibition

Drug discovery relies heavily on the use of enzyme inhibitors. The malfunction, overexpression, or hyperactivation of enzymes is the root cause of many diseases, thanks to advances in molecular biology. Enzyme inhibitors can be used to treat such hyperactivation or overexpression of enzymes. These efforts have resulted in the clinical use of various enzyme inhibitors, some of which are of natural origin. (Zengin, Mahomoodally et al. 2022).

Several clinically relevant enzymes involved in major pathologies, including neurodegenerative disorders (AChE and BChE), skin diseases (tyrosinase), and diabetes (amylase and glucosidase), were tested for enzyme inhibition potential using *P. nodiflora* methanol and dichloromethane extracts. The outcomes of these

tests are summarised in Table 2. The PN-M extract was most active against AChE and tyrosinase enzymes with an inhibition potential of 4.33 mg GALAE/g extract and 125.36 mg KAE/g extract, respectively. Likewise, the dichloromethane extract was noted to show higher inhibition values against BChE (4.70 mg GALAE/g extract) and amylase (1.97 mmol ACAE/g extracts) enzymes. However, both extracts presented weak inhibition potential against the glucosidase enzyme.

Mice have been shown to benefit from extracts of *P. nodiflora* in chloroform and ethanol as anxiolytics and anticonvulsants (Thirupathy, Tulshkar et al. 2011). *P. nodiflora* methanol extract has previously shown anti-diabetic action in rats induced with Streptozotocin. A significant increase in insulin levels and body weight resulted in decreased blood glucose and glycosylated hemoglobin levels (Balamurugan and Ignacimuthu 2011). Eupafolin, an active flavonoid derived from *P. nodiflora*, exhibited skin-whitening effects in B16F10 mice via a melanin suppression (Ko, Chiang et al. 2014). Likewise, another study has reported the tyrosinase inhibitory potential of *P. nodiflora* in the dose-dependent matter (Yen, Wang et al. 2012).

### 3.3. Phytochemical composition

Natural products play a key role in drug development; therefore, screening plants to identify and isolate active ingredients is a necessary step to developing effective drugs against a wide range of diseases (Bibi Sadeer, Sinan et al. 2022). Therefore, total flavonoid and phenolic contents were estimated, with results depicted in Table 1. The tested extracts contained considerable quantities of total phenolic and flavonoid contents. However, the methanol extract was higher in polyphenolic contents (TPC: 26.08 mg GAE/g extract; TFC: 50.25 mg QE/g extract) than dichloromethane extracts. The higher amount of phenolic and flavonoids in methanol extract might be attributed to the polar nature of the methanol solvent. Our findings are in line with the previous studies that had reported higher bioactive contents for polar extracts (Zekri, Zerkani et al. 2021, Wairata, Fadlan et al. 2022). Current studies made use of colorimetric methods to quantify phenolic compounds. Although, the effectiveness of these methods is quite controversial in producing accurate results (Zengin, Ak et al. 2022). Thus, a punc-

**Table 2**  
Enzyme inhibition results of *P. nodiflora* methanol and dichloromethane extracts.

Extracts	Neurodegenerative diseases		Skin problems	Diabetes	
	AChE inhibition (mg GALAE/g extract)	BChE inhibition (mg GALAE/g extract)	Tyrosinase (mg KAE/g extract)	Glucosidase (mmol ACAE/g extract)	Amylase (mmol ACAE/g extract)
PN-M	4.33 ± 0.05	2.11 ± 0.06	125.36 ± 1.65	0.60 ± 0.02	1.86 ± 0.03
PN-D	3.48 ± 0.53	4.70 ± 0.14	116.04 ± 1.12	0.62 ± 0.01	1.97 ± 0.01

PN-M: *P. nodiflora* methanol extract; PN-D: *P. nodiflora* dichloromethane extract.

GALAE: galantamine equivalent; KAE: kojic acid equivalent; ACAE: acarbose equivalent. All values expressed are means ± S.D. of three parallel measurements.

tual UHPLC-MS and HPLC-PDA analysis was used to reveal the quantitative and qualitative composition of the phenolic compounds.

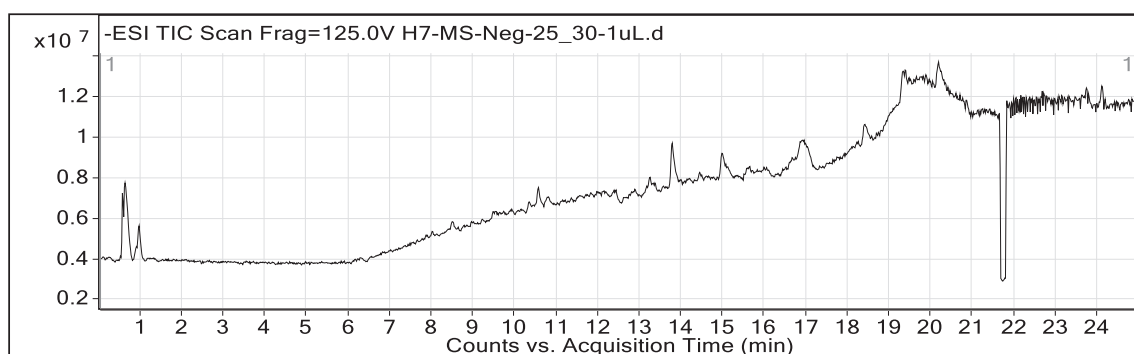
As mentioned above, to have further insight into the composition of the secondary metabolites, the methanol extract was subjected to UHPLC-MS analysis in negative ionization mode which resulted in the tentative identification of 24 different phytochemicals. The list of these identified compounds is presented in Table 3, while the total ion chromatogram (TIC) is shown in Fig. 1. It can be noted from Table 2 that the maximum number of phytochemicals identified belong to the flavonoid group of secondary metabolites. The identified flavonoids were 6-methoxytaxifolin, luteolin 7-

rhamnosyl (1->6) galactoside, 6-hydroxyluteolin 5-rhamnoside, luteolin 7-rhamnosyl (1->6) galactoside, maysin, acacetin 7-(6''-methylglucuronide), demethyltorosaflavone D, grossamide, and torosaflavone D. Likewise, periandrin V, betavulgaroside X, spinacoside D, spinacoside C, betavulgaroside VI, and medicoside H were belonging to terpene derivatives. Similarly, three glycosides, including sarmentosin epoxide, citrusin F, and calendulaglycoside E, were also identified. One phenolic (formononetin 7-O-glucoside-6''-O-malonate), and saponin (durupcoside B) derivative was also identified. The presence of these phytochemical classes in the PN-M extract agrees with some previous studies that reported the isolation/identification of different

**Table 3**  
UHPLC-MS tentative secondary metabolites identification of methanol extract of *P. nodiflora* (negative ionization mode).

S.no	RT (min)	BP (m/z)	Compounds tentatively identified	Mol. Formula	Comp class	Mol. mass	AUC
1	0.63	333.06	6-Methoxytaxifolin	C <sub>16</sub> H <sub>14</sub> O <sub>8</sub>	Flavonoid	334.06	180,964
2	0.762	290.09	Sarmentosin epoxide	C <sub>11</sub> H <sub>17</sub> NO <sub>8</sub>	C. glycoside	291.09	1,010,800
3	8.462	593.15	Luteolin 7-rhamnosyl(1->6)galactoside	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	Flavonoid	594.15	665,330
4	8.504	225.12	12-hydroxyjasmonic acid	C <sub>12</sub> H <sub>18</sub> O <sub>4</sub>	Acid	226.12	559,385
5	8.512	447.10	6-Hydroxyluteolin 5-rhamnoside	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	Flavonoid	448.10	1,264,764
6	8.66	519.18	Citrusin F	C <sub>22</sub> H <sub>32</sub> O <sub>14</sub>	Phenolic Glycoside	520.18	257,059
7	8.979	577.16	Isovitexin 7-O-rhamnoside	C <sub>27</sub> H <sub>30</sub> O <sub>14</sub>	Flavonoid	578.16	1,665,617
8	8.987	593.15	Luteolin 7-rhamnosyl(1->6)galactoside	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	Flavonoid	594.15	665,330
10	9.504	575.14	Maysin	C <sub>27</sub> H <sub>28</sub> O <sub>14</sub>	Flavonoid	576.14	379,636
11	9.505	577.16	Isovitexin 7-O-rhamnoside	C <sub>27</sub> H <sub>30</sub> O <sub>14</sub>	Flavonoid	578.16	561,198
12	9.604	515.12	Formononetin 7-O-glucoside-6''-O-malonate	C <sub>25</sub> H <sub>24</sub> O <sub>12</sub>	Phenol	516.12	225,692
13	9.939	473.11	Acacetin 7-(6''-methylglucuronide)	C <sub>23</sub> H <sub>22</sub> O <sub>11</sub>	Flavonoid	474.11	536,694
14	10.77	355.05	Demethyltorosaflavone D	C <sub>18</sub> H <sub>12</sub> O <sub>8</sub>	Flavonoid	356.05	536,952
15	11.37	777.41	Periandrin V	C <sub>41</sub> H <sub>62</sub> O <sub>14</sub>	Triterpene	778.41	335,538
16	11.39	909.45	Betavulgaroside X	C <sub>46</sub> H <sub>70</sub> O <sub>18</sub>	Diterpenoid	910.45	296,898
17	11.85	793.44	Calendulaglycoside E	C <sub>42</sub> H <sub>66</sub> O <sub>14</sub>	Glycoside	794.44	599,057
18	11.99	623.24	Grossamide	C <sub>36</sub> H <sub>36</sub> N <sub>2</sub> O <sub>8</sub>	Flavonoid	624.24	793,947
19	12.03	763.39	Spinacoside D	C <sub>40</sub> H <sub>60</sub> O <sub>14</sub>	Oleane triterpenoid	764.39	321,379
20	12.08	925.45	Spinacoside C	C <sub>46</sub> H <sub>70</sub> O <sub>19</sub>	Oleane triterpenoid	926.45	773,840
21	12.081	971.45	Betavulgaroside VI	C <sub>47</sub> H <sub>72</sub> O <sub>21</sub>	Diterpenoid	972.45	421,626
22	12.36	369.06	Torosaflavone D	C <sub>19</sub> H <sub>14</sub> O <sub>8</sub>	Flavonoid	370.06	311,323
23	12.43	941.48	Medicoside H	C <sub>47</sub> H <sub>74</sub> O <sub>19</sub>	Triterpene glycoside	942.48	356,757
24	13.37	925.48	Durupcoside B	C <sub>47</sub> H <sub>74</sub> O <sub>18</sub>	Saponin	926.48	3,474,724

RT: retention time; B.P: base peak; Mol. Formula; AUC: area under curve.



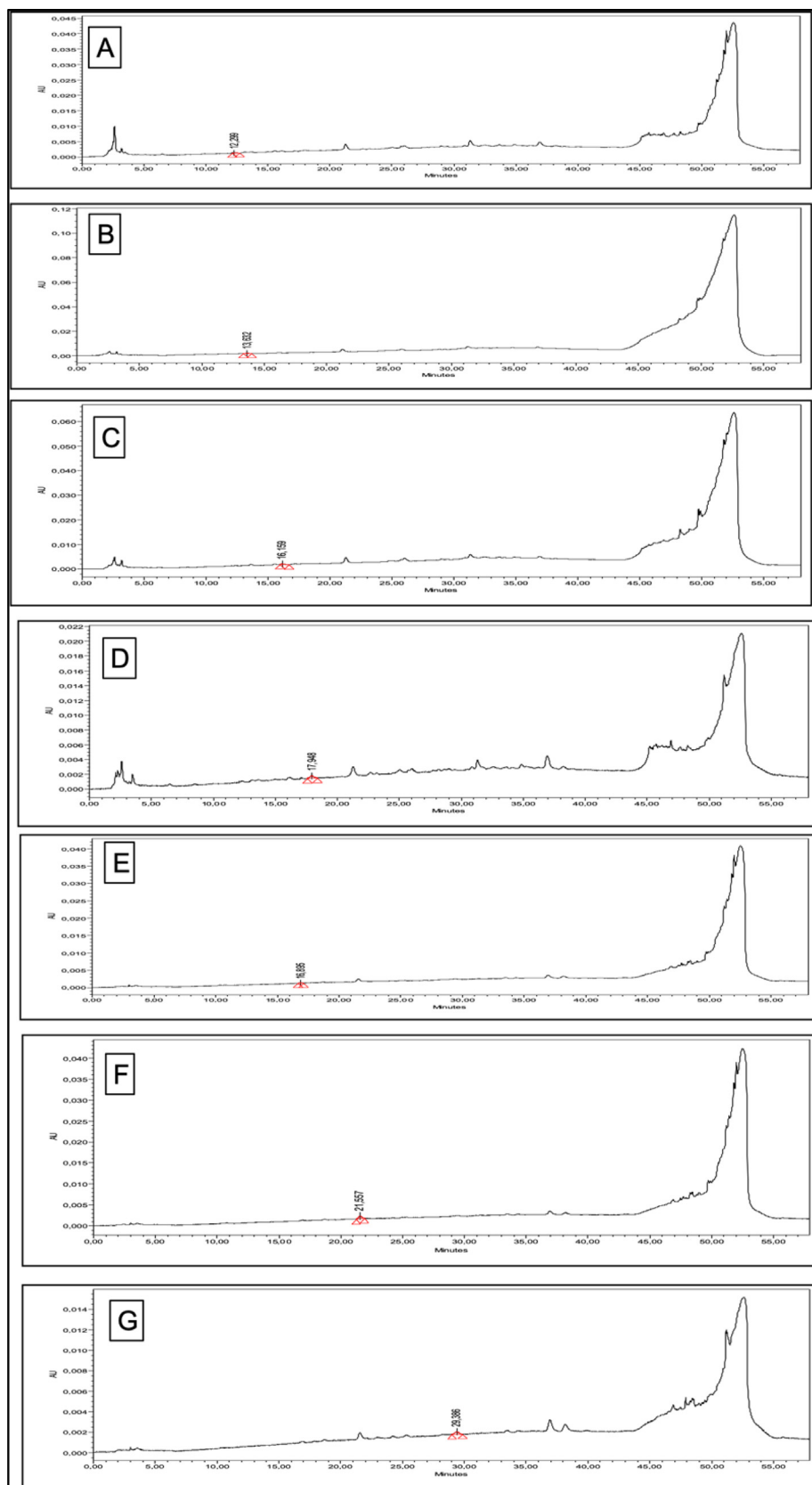
**Fig. 1.** Total ion chromatogram (TIC) of UHPLC-MS analysis *P. nodiflora* methanol extract.

**Table 4**  
HPLC-PDA polyphenolic quantification of *P. nodiflora* methanol and dichloromethane extracts.

Tested extracts	Polyphenolics quantified						
	Catechin	<i>p</i> -OH benzoic acid	Vanillic acid	Epicatechin	3-OH benzoic acid	3-OH-4-MeO benzaldehyde	2,3-di-MeO benzoic acid
PN-M	0.25 ± 0.02	BLD	BLD	nd	0.64 ± 0.05	nd	nd
PN-D	nd	nd	nd	0.30 ± 0.02	nd	0.21 ± 0.01	0.97 ± 0.08

PN-M: *P. nodiflora* methanol extract; PN-D: *P. nodiflora* dichloromethane extract.

nd: not detected; BLD: below limit of detection (<0.1 µg/mL); Chlorogenic acid, *p*-coumaric acid, rutin, sinapinic acid, *t*-ferullic acid, *o*-coumaric acid, quercetin, harpagoside, *t*-cinnamic, carvacrol, benzoic acid, naringin, syringic acid, vanillic acid, and epicatechin were not detected in any of the tested extracts.



**Fig. 2.** HPLC chromatograms of the quantified compounds from *P. nodiflora* extracts. (A: catechin 278 nm; B: *p*-OH benzoic acid 256 nm; C: vanillic acid 260 nm; D: 3-OH benzoic acid 295 nm; E: epicatechin 278 nm; F: 3-OH 4-MeO benzaldehyde 275 nm; G: 2,3-di-MeO benzoic acid 299 nm).

flavonoids, glucosides, and triterpenoid derivatives (Barnabas, Gunasingh et al. 1980, Khalil, Lahloub et al. 1995, Ko, Chiang et al. 2014, Al-Snai 2019).

Likewise, the polyphenolic composition of the extracts was appraised by HPLC-PDA quantification for the 22 different phenolic standards, and the results of the quantified compounds are presented in Table 4, while their HPLC chromatograms are depicted in Fig. 2. It was noted that seven phenolic compounds were quantified in both extracts. PN-M was found to be rich in catechin (0.25 µg/extract) and 3-OH benzoic acid (0.64 µg/extract). In comparison, PN-D contained epicatechin (0.30 µg/extract), 3-OH-4-MeO benzaldehyde (0.21 µg/extract), and 2,3-Di-MeO benzoic acid (0.97 µg/extract) in higher amounts.

### 3.4. Molecular docking studies

Molecular docking studies in the ligand-binding cavity of enzymes AChE, BChE, and TYR were done to explain the binding

behavior of three phytoconstituents, HLR, LRG, and TFD (Fig. 3). A greater understanding of the structures of AChE, BChE, and TYR may aid in identifying important molecular interactions involved in forming ligand–protein complexes. In order to investigate the changes in interaction patterns that contribute to different docking scores, optimal docking conformations for each compound are saved and displayed graphically. As shown in Fig. 2, the examined ligands occupy the same binding site of AChE, BChE, and TYR as observed in their co-crystallized complexes (4EY7, 6QAE, and 2Y9X) with their respective inhibitors.

A series of molecular docking simulations were performed to study the pattern of molecular interactions contributing to the variance in the conformations of the analyzed ligands to the target protein. Based on the cumulative score (cScore) (Table 5), the best probable poses of ligands in the active site of proteins were chosen. The docking scores (cScore) of selected inhibitors bonded to the AChE complex are 8.78, 7.94, and 7.20 for ligands HLR, LRG, and TFD, respectively. These results indicate that the compound HLR

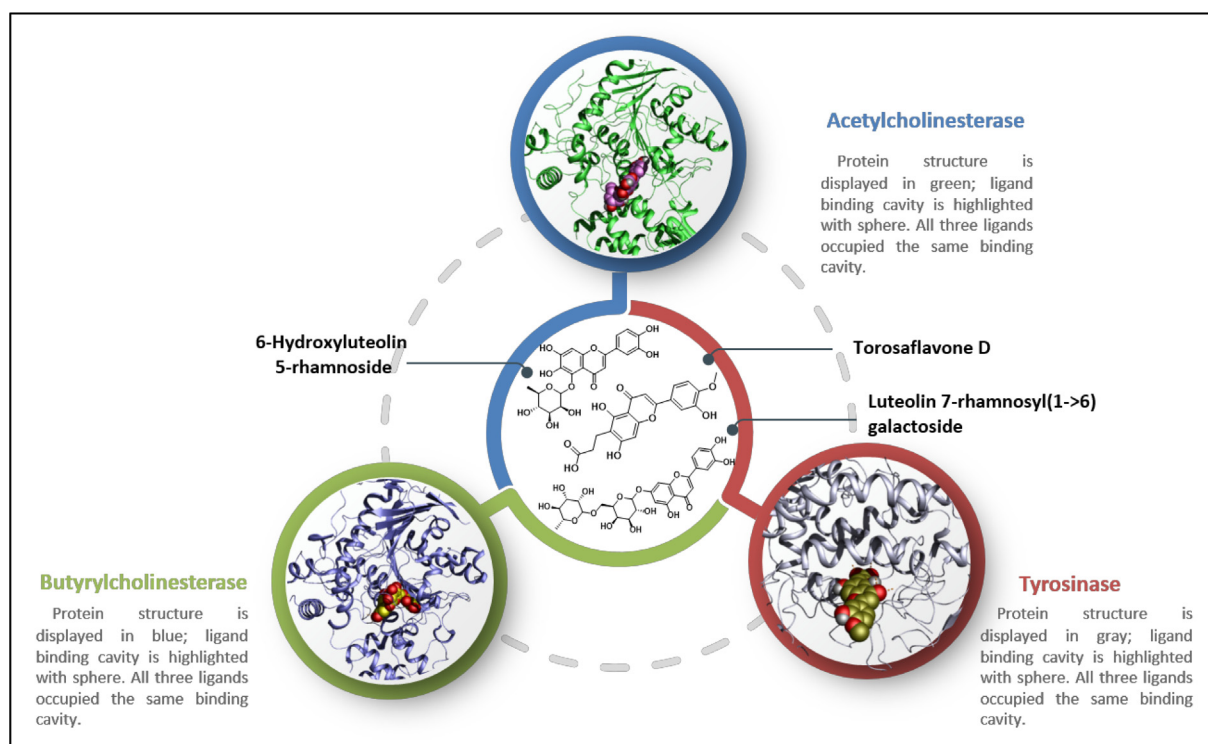


Fig. 3. Chemical structures of selected ligands HLR, LRG, and TFD for molecular docking analysis. 3D-conformation of selected hits in their corresponding molecular targets.

Table 5

Surflex scores of docked ligands HLR, LRG, and TFD in the binding site of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYR) enzymes.

Protein	Ligand	Cscore <sup>a</sup>	Crash score <sup>b</sup>	Polar score <sup>c</sup>	D score <sup>d</sup>	PMF score <sup>e</sup>	G score <sup>f</sup>	Chem score <sup>g</sup>	Amino acid interaction
AChE	HLR	8.78	-3.20	3.71	-133.143	-86.23	-187.93	-35.13	D72, Y121, S286, Y334, H440
	LRG	7.94	-2.17	5.37	-170.15	-97.31	-206.44	-25.41	S81, G118, Y121, E199, S200, Y334,
	TFD	7.20	-3.70	1.74	-165.67	-83.16	-241.91	-18.35	S286, R288
BChE	HLR	5.09	-2.38	4.83	-92.17	-24.4	-151.4	-24.7	D70, W82, E197, S198, P285, L286, Y332, W430,
	LRG	6.52	-2.11	4.91	-102.12	-28.1	-161.6	-19.6	W82, N83, T120, G116, G117, S287, W231, P285
	TFD	4.12	-2.81	5.12	-166.7	-44.4	-212.1	-14.0	D70, S72, G116, S198, A199, P285
TYR	HLR	4.41	-1.49	1.23	-98.21	33.14	-121.10	-34.45	N260, R268
	LRG	5.98	-4.73	7.10	-170.3	62.053	-319.8	-6.22	H85, N260, E322
	TFD	7.80	-2.62	5.56	-148.0	32.933	-191.8	-4.97	H85, H244, H259, H263, R268

<sup>a</sup> Cscore is a consensus scoring which uses multiple types of scoring functions to rank the affinity of ligands, <sup>b</sup>Crash-score revealing the inappropriate penetration into the binding site, <sup>c</sup>Polar region of the ligand, <sup>d</sup>D-score showing hydrogen bonding, complex (ligand–protein), and internal (ligand–ligand) energies, <sup>e</sup>PMF-score indicating the Helmholtz free energies of interactions for protein–ligand atom pairs (Potential of Mean Force, PMF), <sup>f</sup>G-score for charge and van der Waals interactions between the protein and the ligand, <sup>g</sup>Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

forms the strongest complex with the AChE enzyme among the studied ligands. Other compounds, such as LRG and TFD have also shown a comparable binding affinity towards the AChE. In the case of BChE-ligand bonded systems, compounds HLR and TFD demonstrated almost similar docking scores (cScore: 5.01 and 5.12, respectively), while the compound HLR demonstrated the highest binding affinity towards BChE (cScore: 6.52). Among TYR-ligand complexes, TFD showed substantial binding potential towards TYR (cScore: 7.80, whereas compounds HLR and LRG demonstrated weak to moderate binding affinity towards the TYR enzyme. To get detailed insight into the docking results, the docking scores and the list of residues involved in H-bond interactions are summarized in Table 5. Furthermore, the simplified 2D ligand interaction diagrams for all docking poses are depicted in Fig. 4 to get better insight into the interaction pattern of ligand-receptor complexes.

The best docking conformations were saved for each ligand-receptor complex and graphically inspected to determine the ligand-protein interactions responsible for the variation in binding affinity. Moreover, only the top-ranking docking complexes for each ligand-protein system are presented in Fig. 5 (A-I) to reveal the important molecular interactions responsible for the superior binding affinities of top-scored ligands for their corresponding molecular target. Graphical analysis of all docking complexes (Figs. 3 and 4) demonstrates that all ligands occupy the same binding cleft to adopt identical binding orientations in their respective molecular target. However, the differences in binding affinities

may originate from variations in the interaction pattern of the ligand-bound system. As presented in Fig. 5A-C, all three ligands (HLR, LRG, and TFD) penetrate deeply into the two main binding subsites (CAS and PAS) of AChE. In the active site of AChE, both ligands HLR, LRG, and TFD acquire such a binding conformation to establish several interactions with surrounding residues Y70, D72, Y121, W279, S286, I287, R289, F290, F330, F331, G335, Y334, and H440 (Fig. 5A and 2B). In the HLR-bonded system, HLR covers a major area of AChE's active site to establish at least six H-bond interactions with D72, Y121, S286, Y334, and H440. In addition to the conserved H-bond contacts with Y121 and Y334, compound LRG also establishes four H-bond interactions with S81, G118, E199, and S200. Compound TFD was found to establish only three H-bond contacts with residues S286 and I287. Despite fewer H-bonds, considering the binding affinity of TFD towards AChE might be attributed to van der Waals's contact of TFD with nearby residues D72, W84, L282, F331, Y334. In addition, the benzyl moiety faces the aromatic indole moiety of W279 to establish pi-pi contact. Hence, these nonbonding interactions may play a crucial role in improved binding affinities of HLR, LRG, and TFD towards AChE. In BChE-ligand bonded systems, compound LRG demonstrated the highest binding affinities among studied ligands, which a network of nine H-bonds may explain between LRG and residues W82, N83, T120, G116, G117, S287, W231, P285 of BChE. Compound LFD displays the least binding affinity towards BChE by establishing fewer H-bond contacts. Interestingly, LFD demon-

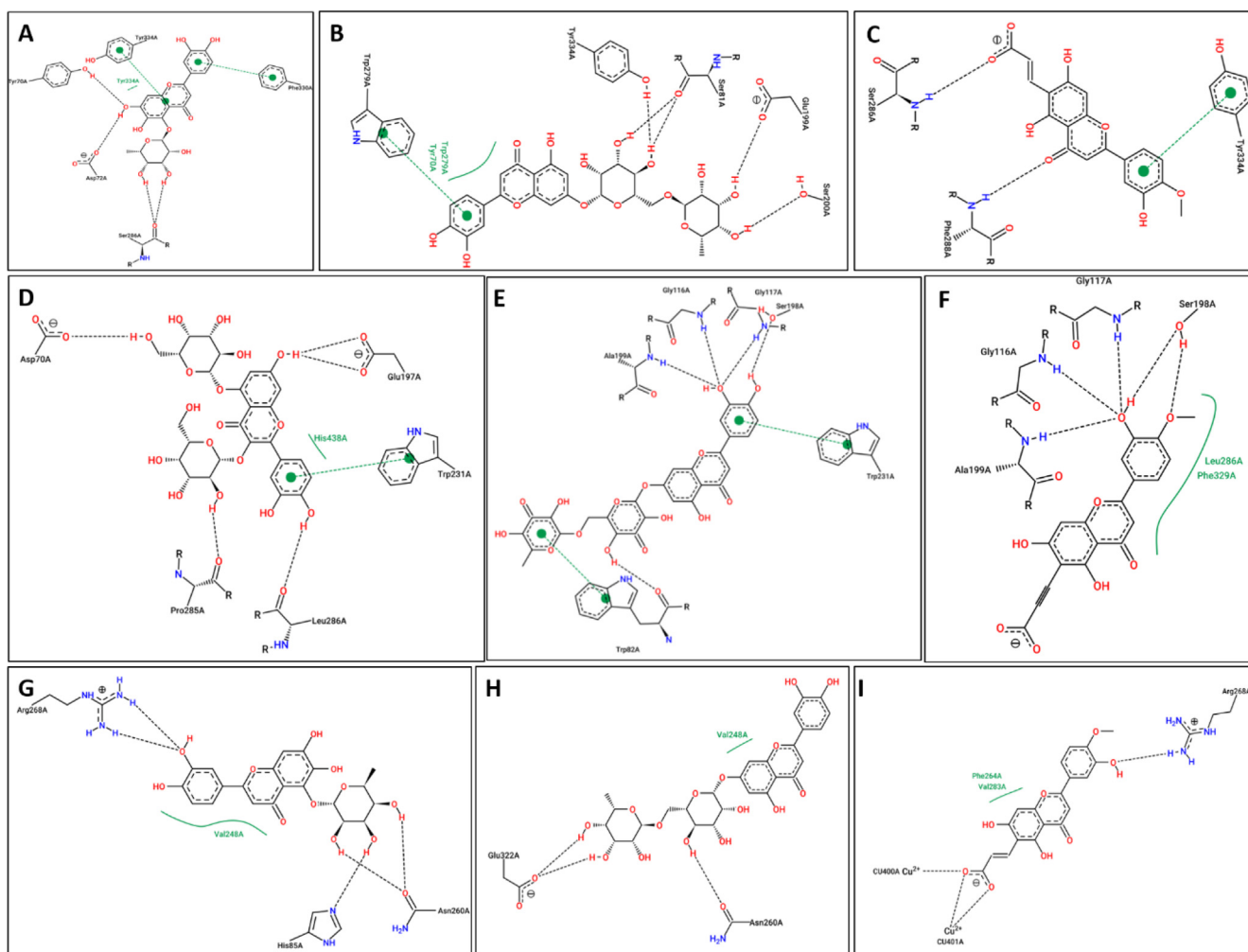
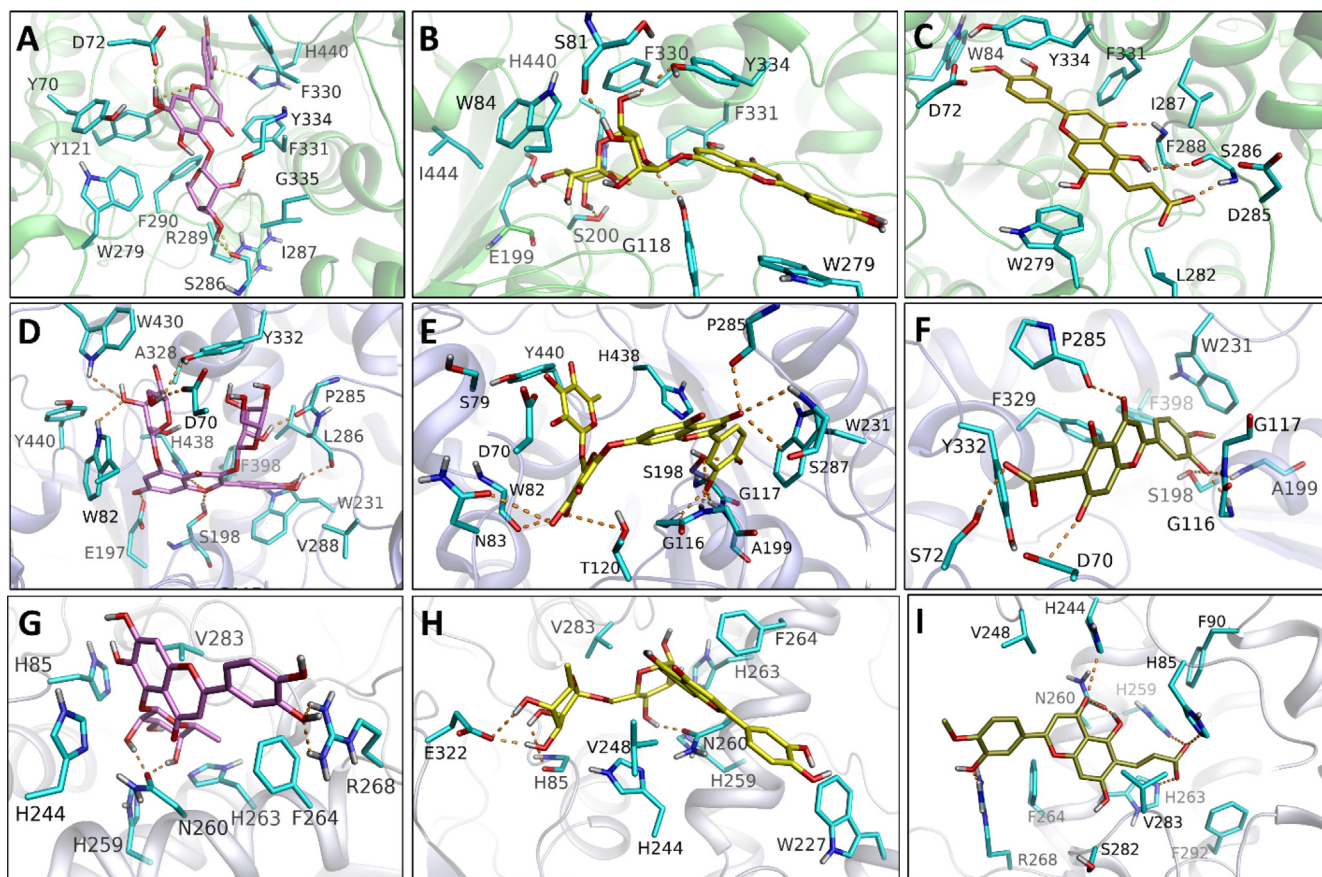


Fig. 4. 2D-interaction diagram of studied ligands in complex with AChE, BChE and TYR enzymes. (A) AChE-HLR, (B) AChE-LRG, (C) AChE-TFD, (D) BChE-HLR, (E) BChE-LRG, (F) BChE-TFD, (G) TYR-HLR, (H) TYR-LRG, (I) TYR-TFD.





**Fig. 5.** Docking generated complexes of proteins bonded to their tested compounds: (A) AChE-HLR, (B) AChE-LRG, (C) AChE-TFD, (D) BChE-HLR, (E) BChE-LRG, (F) BChE-TFD, (G) TYR-HLR, (H) TYR-LRG, (I) TYR-TFD.

**Table 6**  
Effect of oral administration of methanol extract of *P. nodiflora* on haematological parameters of chicks.

Parameters	Group (ZP)				
	Control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
<b>Erythrocyte counts</b>					
Day 7	2.5 ± 0.12	2.63 ± 0.08	2.43 ± 0.08	2.48 ± 0.08	2.74 ± 0.07
Day 14	2.60 ± 0.13	2.46 ± 0.07	2.70 ± 0.02	2.43 ± 0.04	2.48 ± 0.05
Day 21	2.40 ± 0.07	2.54 ± 0.07	2.74 ± 0.07	2.73 ± 0.07	2.56 ± 0.05
<b>Leucocyte count (103/ul)</b>					
Day 7	197.38 ± 3.62	193.9 ± 2.45	190.8 ± 5.05	195.3 ± 4.31	222.2 ± 5.30
Day 14	202.8 ± 6.11	196.1 ± 3.73	193.5 ± 4.25	213.0 ± 10.9	233.5 ± 3.75
Day 21	213.3 ± 16.3	221.3 ± 3.82	198.1 ± 4.90	224.7 ± 2.81	229.4 ± 6.31
<b>Lymphocyte count (10<sup>3</sup> / μ L)</b>					
Day 7	168.98 ± 2.85	168.90 ± 2.60	214.10 ± 3.64	174.90 ± 11.89	167.50 ± 3.48
Day 14	167.6 ± 3.28	170.4 ± 1.67	211.0 ± 2.23	215.0 ± 0.89	216.2 ± 0.71
Day 21	213.3 ± 16.3	209.8 ± 3.79	210.8 ± 2.67	162.1 ± 5.18	159.7 ± 2.58
<b>Haemoglobin (gm/dl)</b>					
Day 7	10.14 ± 0.37	10.50 ± 0.28	11.36 ± 0.49	11.40 ± 0.18	10.14 ± 0.47
Day 14	10.28 ± 0.63	10.34 ± 0.37	9.46 ± 0.26	11.28 ± 0.28	10.8 ± 0.29
Day 21	10.34 ± 0.37	10.54 ± 0.34	9.58 ± 0.22	11.43 ± 0.43	11.10 ± 0.27
<b>MCV (FL)</b>					
Day 7	125.10 ± 0.96	123.90 ± 1.33	137.24 ± 1.07	129.20 ± 2.90	124.80 ± 1.89
Day 14	124.10 ± 1.18	116.80 ± 0.77	134.20 ± 1.08	136.70 ± 1.78	136.90 ± 1.78
Day 21	136.30 ± 1.91	137.3 ± 2.73	132.7 ± 1.57	135.0 ± 1.49	137.0 ± 1.15
<b>MCHC</b>					
Day 7	31.60 ± 0.29	31.20 ± 0.14	38.20 ± 2.85	40.80 ± 0.57	42.20 ± 1.06
Day 14	29.86 ± 1.90	31.50 ± 0.40	37.40 ± 2.72	38.30 ± 1.90	41.10 ± 0.73
Day 21	30.10 ± 0.28	31.70 ± 0.18	40.40 ± 0.62	41.20 ± 0.53	41.60 ± 0.57
<b>HCT (%)</b>					
Day 7	31.48 ± 0.23	31.66 ± 0.65	30.98 ± 1.10	33.58 ± 2.41	36.74 ± 1.23
Day 14	31.90 ± 1.49	30.74 ± 0.87	29.92 ± 0.33	29.64 ± 1.53	37.60 ± 1.12
Day 21	32.10 ± 0.91	32.12 ± 0.68	30.0 ± 0.49	31.90 ± 0.77	32.80 ± 2.03

strated the highest binding affinity in TYR-ligand complexes by acquiring six H-bonds with residues H85, H244, H259, H263, and R268 of the TYR enzyme. Moreover, the aromatic moiety in LFD is sandwiched between V283 and H263 to contribute to sigma-pi and pi-pi contacts, respectively. Compounds of HLR and LRG with a moderate affinity towards AChE develop three to four H-bonds with the nearby residues in the active site of the TYR enzyme (Fig. 5G and H). Overall, the docking analysis is consistent with the results and effectively explains the influence of interaction changes on the binding affinities of chosen inhibitors.

### 3.5. Toxicological studies

The PN-M extract was subjected to toxicological studies to assess the plant's toxic effects, including different hematological, biochemical, and physical parameters. The effect of oral administration of methanol extract of *P. nodiflora* on the haematological parameters of chicks is shown in Table 6, while the effect of oral administration of methanol extract of *P. nodiflora* on the biochemical parameters of chicks is shown in Table 7; likewise, the effect of oral administration of methanol extract of *P. nodiflora* on body organs of chicks is shown in Table 8. Broiler chicks were selected and distributed in four experimental groups. They were orally fed with the extract for 21 days. The chicks were physically evaluated, and the number of chicks remained the same with no abnormality.

**Table 7**  
Effect of oral administration of methanol extract of *P. nodiflora* on biochemical parameters of chicks.

Biochemical parameters	Control	Plant extract			
		150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
Alkaline Phosphatase	2650.0 ± 111.10	2637.2 ± 144.50	3066.5 ± 153.60	2345.0 ± 141.90	2012.5 ± 141.90
Aspartate Aminotransferase	6.0 ± 0.47	8.50 ± 1.44	10.50 ± 0.64	8.75 ± 0.85	7.0 ± 0.85
Alanine Aminotransferase	149.4 ± 13.44	174.50 ± 7.90	174.25 ± 7.43	183.75 ± 6.20	160.0 ± 7.43
Urea level	14.0 ± 1.08	21.0 ± 2.08	17.0 ± 1.88	18.0 ± 0.85	14.0 ± 0.85
Creatinine	0.25 ± 0.01	0.18 ± 0.03	0.34 ± 0.03	0.39 ± 0.02	0.40 ± 0.04

**Table 8**  
Effect of oral administration of methanol extract of *P. nodiflora* on body organs of chicks.

Parameters/Day	Group (PN)				
	Control	150 mg/kg	200 mg/kg	250 mg/kg	300 mg/kg
<b>Liver weight</b>					
Absolute	53.11 ± 1.70	52.62 ± 1.83	44.73 ± 0.85	37.09 ± 1.32	35.30 ± 1.37
Relative	2.10 ± 0.08	2.33 ± 0.13	1.88 ± 0.02	1.67 ± 0.04	1.68 ± 0.05
<b>Kidney weight</b>					
Absolute	4.03 ± 0.01	12.4 ± 0.94	3.73 ± 0.43	4.16 ± 0.26	3.5 ± 0.20
Relative	0.48 ± 0.03	0.55 ± 0.05	0.15 ± 0.01	0.18 ± 0.009	0.16 ± 0.003
<b>Lung's weight</b>					
Absolute	4.03 ± 0.01	11.36 ± 0.82	4.43 ± 0.08	3.73 ± 0.26	3.73 ± 0.31
Relative	0.41 ± 0.03	0.17 ± 0.008	0.16 ± 0.01	0.18 ± 0.002	0.50 ± 0.02
<b>Thymus weight</b>					
Absolute	4.59 ± 0.54	5.36 ± 0.31	3.66 ± 0.17	4.4 ± 0.36	4.1 ± 0.11
Relative	0.18 ± 0.02	0.19 ± 0.005	0.19 ± 0.01	0.15 ± 0.006	0.23 ± 0.007
<b>Intestine weight</b>					
Absolute	143.47 ± 10.7	141.7 ± 18.50	146.6 ± 5.89	149.0 ± 13.01	132.2 ± 13.11
Relative	5.62 ± 0.28	6.85 ± 1.51	6.64 ± 0.29	6.30 ± 0.58	5.89 ± 0.82
<b>Heart weight</b>					
Absolute	10.44 ± 0.76	10.60 ± 0.85	8.53 ± 0.54	10.96 ± 0.74	8.56 ± 0.17
Relative	0.41 ± 0.03	0.40 ± 0.01	0.49 ± 0.03	0.36 ± 0.02	0.47 ± 0.05
<b>Spleen weight</b>					
Absolute	3.27 ± 0.03	1.97 ± 0.16	2.35 ± 0.02	1.64 ± 0.14	1.36 ± 0.15
Relative	0.13 ± 0.002	0.08 ± 0.005	0.09 ± 0.005	0.07 ± 0.007	0.06 ± 0.004
<b>Trachea weight</b>					
Absolute	3.43 ± 0.31	3.65 ± 0.43	2.51 ± 0.08	2.59 ± 0.17	2.46 ± 0.26
Relative	0.13 ± 0.010	0.16 ± 0.020	0.10 ± 0.004	0.11 ± 0.008	0.11 ± 0.006
<b>Bursa weight</b>					
Absolute	1.17 ± 0.01	1.19 ± 0.005	1.77 ± 0.18	1.07 ± 0.08	1.04 ± 0.07
Relative	0.04 ± 0.002	0.05 ± 0.001	0.07 ± 0.007	0.04 ± 0.004	0.05 ± 0.005

After comparing the weights of different groups, no significant variation was found. On day 21, absolute and relative weights of other body organs, including kidney, heart, spleen, trachea, liver, intestine, thymus, and lungs, were also done with no significant variation. Hematological and biochemical parameters were evaluated after collecting blood samples on days 7, 14, and 21. On account of statistical analysis, *P. nodiflora* was non-toxic, as there was no significant difference in experimental values to that of the control group. Overall, we have used the methanolic extract (at different concentrations of 150 mg/kg, 200 mg/kg, 250 mg/kg, and 300 mg/kg) of *P. nodiflora* plant in order to check the in-vivo toxicity. The results have indicated that the tested extract was found safer even at the higher doses of 300 mg/kg.

### 4. Conclusion

Based on the results of current study, methanolic and dichloromethane extracts of *P. nodiflora* exhibit varying inhibitory effects on enzymes and antioxidant properties. More precisely, the methanol extract showed better antioxidant activity than the dichloromethane extract. In addition, weak inhibiting propensities were exhibited by the studied extracts against the screened enzymes. Significant quantities of TPC and TFC were yielded from the extracts. UHPLC-MS and HPLC-PDA analysis conform to the presence of several important phenolics, flavonoids, terpenes, and gly-

cosides. The molecular docking highlighted the interaction between the identified phytochemicals and tested enzymes. In acute toxicity studies, oral doses of methanolic extract did not result in death or adverse effects on general behavior. Almost no significant changes were observed in any of the hematological and biochemical parameters of the animals. This medicinal plant was found to be effective as an enzyme inhibitor and antioxidant, making it a possible option for further advanced research into its pharmacological capabilities.

### 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.

### Acknowledgment

This research was funded by Scientific Research Deanship at University of Hail- Saudi Arabia through project number RG-23076.

### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2023.105233>.

### References

- Acquaviva, A., S. C. Di Simone, Nilofar, A. Bouyahya, G. Zengin, L. Recinella, S. Leone, L. Brunetti, A. I. Uba and O. Guler (2023). "Screening for Chemical Characterization and Pharmacological Properties of Different Extracts from *Nepeta italica*." *Plants* **12**(15): 2785.
- Ahmed, F., Selim, M., Das, A., Choudhuri, M., 2004. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Die Pharm.-Int. J. Pharma. Sci.* **59** (4), 329–330.
- Al-Snafi, A., Faris, A., 2013. Anti-inflammatory and antibacterial activities of *Lippia nodiflora* and its effect on blood clotting time. *J. Thi-Qar Sci.* **4** (1).
- Al-Snai, A., 2019. Pharmacological and therapeutic effects of *Lippia nodiflora* (*Phyla nodiflora*). *IOSR J. Pharm.* **9** (8), 15–25.
- AO, T. and B. SL (2012). "EFFECTS OF *LIPPIA NODIFLORA* EXTRACTS ON MOTOR COORDINATION, EXPLORATORY BEHAVIOR PATTERN, LOCOMOTOR ACTIVITY, ANXIETY AND CONVULSIONS ON ALBINO MICE." *Asian Journal of Pharmaceutical and Clinical Research* **4**(3): 133-138.
- Arumanayagam, S., Arunmani, M., 2015. Hepatoprotective and antibacterial activity of *Lippia nodiflora* Linn. against lipopolysaccharides on HepG2 cells. *Pharmacogn. Mag.* **11** (41), 24.
- Ashokkumar, D., Thamilselvan, V., Mazumder, U.K., Gupta, M., 2008. Antioxidant and free radical scavenging effects of *Lippia nodiflora*. *Pharm. Biol.* **46** (10–11), 762–771.
- Balakrishnan, G., Janakaraman, L., Balakrishnan, A., Lakshmi, B.S., 2010. Molecular basis of the anti-inflammatory property exhibited by cyclo-pentano phenanthrenol isolated from *Lippia nodiflora*. *Immunol. Invest.* **39** (7), 713–739.
- Balamurugan, R., Duraipandiyani, V., Ignacimuthu, S., 2011. Antidiabetic activity of sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. *Eur. J. Pharmacol.* **667** (1), 410–418.
- Balamurugan, R., Ignacimuthu, S., 2011. Antidiabetic and hypolipidemic effect of methanol extract of *Lippia nodiflora* L. in streptozotocin induced diabetic rats. *Asian Pac. J. Trop. Biomed.* **1** (1), S30–S36.
- Balick, M.J., Cox, P.A., 1996. *Plants, people, and culture: the science of ethnobotany*. Scientific American Library.
- Balunas, M.J., Kinghorn, A.D., 2005. Drug discovery from medicinal plants. *Life Sci.* **78** (5), 431–441.
- Barnabas, C., G. Gunasingh and S. Nagarajan (1980). FLAVONOIDS FROM THE FLOWERS OF *PHYLA-NODIFLORA LINN*, COUNCIL SCIENTIFIC INDUSTRIAL RESEARCH PUBL & INFO DIRECTORATE, NEW DELHI 110012, INDIA. **19**: 822–822.
- Bibi Sadeer, N., Sinan, K.I., Cziáky, Z., Jekó, J., Zengin, G., Jeewon, R., Abdallah, H.H., AlDhaheri, Y., Eid, A.H., Mahomoodally, M.F., 2022. Towards the pharmacological validation and phytochemical profiling of the decoction and maceration of *Bruguiera gymnorhiza* (L.) Lam.—A traditionally used medicinal halophyte. *Molecules* **27** (6), 2000.
- Chang, X., Ye, Y., Pan, J., Lin, Z., Qiu, J., Peng, C., Guo, X., Lu, Y., 2022. Comparative analysis of phytochemical profiles and antioxidant activities between sweet and sour wampee (*Clausena lansium*) fruits. *Foods* **11** (9), 1230.
- Chen, P., Ran, H., Li, J., Zong, J., Luo, Q., Zhao, T., Liao, Z., Tang, Y., Fu, Y., 2022. Antioxidant activity of phenolic extraction from different sweetpotato (*Ipomoea batatas* (L.) Lam.) blades and comparative transcriptome analysis reveals differentially expressed genes of phenolic metabolism in two genotypes. *Genes* **13** (6), 1078.
- Cheong, B., Teoh, P., 2014. Antiproliferative and apoptotic effects of *Phyla nodiflora* extracts on human breast cancer cell line. *Asian Pac. J. Trop. Disease* **4** (3), 238.
- Cheung, J., Rudolph, M.J., Burshteyn, F., Cassidy, M.S., Gary, E.N., Love, J., Franklin, M. C., Height, J.J., 2012. Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J. Med. Chem.* **55** (22), 10282–10286.
- Chohan, T.A., Chen, J.-J., Qian, H.-Y., Pan, Y.-L., Chen, J.-Z., 2016. Molecular modeling studies to characterize N-phenylpyrimidin-2-amine selectivity for CDK2 and CDK4 through 3D-QSAR and molecular dynamics simulations. *Mol. Biosyst.* **12** (4), 1250–1268.
- Chohan, T.A., Qian, H.-Y., Pan, Y.-L., Chen, J.-Z., 2016. Molecular simulation studies on the binding selectivity of 2-anilino-4-(thiazol-5-yl)-pyrimidines in complexes with CDK2 and CDK7. *Mol. Biosyst.* **12** (1), 145–161.
- Dodoala, S., Diviti, R., Koganti, B., Prasad, K., 2010. Effect of ethanolic extract of *Phyla nodiflora* (Linn.) Greene against calculi producing diet induced urolithiasis. *Indian J. Nat. Prod. Resour.* **1** (3), 314–321.
- Durairaj, A.K., Vaiyapuri, T.S., Mazumder, U., Gupta, M., 2007. Antimicrobial and lipid peroxide scavenging activity of *Lippia nodiflora* (Verbenaceae). *Pharmacologyonline* **3**, 177–189.
- Durairaj, A., Vaiyapuri, T.S., Kanti, M.U., Malaya, G., 2008. Protective activity and antioxidant potential of *Lippia nodiflora* extract in paracetamol induced hepatotoxicity in rats. *Iran. J. Pharmacol. Ther.* **7** (1), 83–89.
- Elakovich, S.D., Stevens, K.L., 1985. Volatile constituents of *Lippia nodiflora*. *J. Nat. Prod.* **48** (3), 504–506.
- Gadhvi, R., Mishra, G.J., Reddy, M., Nivserkar, M., 2012. Antihypertensive efficacy of *Lippia nodiflora* whole plant on uninephrectomized doca salt hypertensive rats. *IOSR J. Pharm.* **2** (6), 24–28.
- Ghershi, D., Sanchez, R., 2011. Beyond structural genomics: computational approaches for the identification of ligand binding sites in protein structures. *J. Struct. Funct. Genomics* **12** (2), 109–117.
- Gopal, R., Balkrishna, K., Vasanth, S., Bhima Rao, B., 1996. Activity of *Lippia nodiflora* essential oil on bacteria. *Semin Research in Ayurveda and Siddha*, New delhi.
- Hassan, H.M.A., 2015. A short history of the use of plants as medicines from ancient times. *CHIMIA Int. J. Chem.* **69** (10), 622–623.
- Ismaya, W.T., Rozeboom, H.J., Weijn, A., Mes, J.J., Fusetti, F., Wichers, H.J., Dijkstra, B. W., 2011. Crystal structure of *Agaricus bisporus* mushroom tyrosinase: identity of the tetramer subunits and interaction with tropolone. *Biochemistry* **50** (24), 5477–5486.
- Jabeen, M., Jillani, U., Chaudhary, B.A., Uzair, M., 2016. Phytochemical and pharmacological studies of *Phyla Nodiflora* (Verbenaceae): A review. *Pak. J. Pharm. Res.* **2**, 49–54.
- Jain, A.N., 1996. Scoring noncovalent protein-ligand interactions: a continuous differentiable function tuned to compute binding affinities. *J. Comput. Aided Mol. Des.* **10** (5), 427–440.
- Jain, A.N., 2003. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* **46** (4), 499–511.
- Jain, A.N., 2007. Surflex-Dock 2.1: robust performance from ligand energetic modeling, ring flexibility, and knowledge-based search. *J. Comput. Aided Mol. Des.* **21** (5), 281–306.
- Khalil, A., Lahloub, M., Salama, O., 1995. Phenolic compounds from *Lippia nodiflora*. *J. Pharm. Sci.* **11** (2), 256–265.
- Ko, H.-H., Chiang, Y.-C., Tsai, M.-H., Liang, C.-J., Hsu, L.-F., Li, S.-Y., Wang, M.-C., Yen, F.-L., Lee, C.-W., 2014. Eupafolin, a skin whitening flavonoid isolated from *Phyla nodiflora*, downregulated melanogenesis: Role of MAPK and Akt pathways. *J. Ethnopharmacol.* **151** (1), 386–393.
- Kurmukov, A.G., 2013. *Phytochemistry of Medicinal Plants*. Medicinal Plants of Central Asia, Uzbekistan and Kyrgyzstan, Springer, pp. 13–14.
- Lan, J.-S., Liu, L., Zeng, R.-F., Qin, Y.-H., Hou, J.-W., Xie, S.-S., Yue, S., Yang, J., Ho, R.J., Ding, Y., 2021. Tumor-specific carrier-free nanodrugs with GSH depletion and enhanced ROS generation for endogenous synergistic anti-tumor by a chemotherapy-photodynamic therapy. *Chem. Eng. J.* **407**, 127212.
- Li, C., B. Shi, X. Li, W. e. Zhang and X. Pan (2022). "Changes of phenolics contents, antioxidant activities, and enzyme activities in pellicles of *Juglans sigillata* Dode during fruits development." *International Journal of Food Properties* **25**(1): 2133-2145.
- Locatelli, M., Zengin, G., Uysal, A., Carradori, S., De Luca, E., Bellagamba, G., Aktumsek, A., Lazarova, I., 2017. Multicomponent pattern and biological activities of seven Asphodeline taxa: potential sources of natural-functional ingredients for bioactive formulations. *J. Enzyme Inhib. Med. Chem.* **32** (1), 60–67.
- Malathi, R., Cholarajan, A., Karpagam, K., Jaya, K., Muthukumar, P., 2011. Antimicrobial studies on selected medicinal plants (*Coleus amboinicus*, *Phyla nodiflora* and *Vitex negundo*). *Asian J. Pharma. Technol.* **1** (2), 53–55.
- Meden, A., Knez, D., Jukić, M., Brazzolotto, X., Gršič, M., Pišlar, A., Zahirović, A., Kos, J., Nachon, F., Svetec, J., 2019. Tryptophan-derived butyrylcholinesterase inhibitors as promising leads against Alzheimer's disease. *Chem. Commun.* **55** (26), 3765–3768.
- Onufriev, A., Bashford, D., Case, D.A., 2004. Exploring protein native states and large-scale conformational changes with a modified generalized born model. *Proteins Struct. Funct. Bioinf.* **55** (2), 383–394.
- Patel Janki, B., Shah Kinjal, H., Patel Rashmika, C., 2005. Evaluation of antibacterial activity of methanolic extract of seeds of *PHYLA NODIFLORA LINN*. *Microbiology* **43** (2), 295–300.

- Pau, J.C., 2022. "Toxicity and Teratogenicity Effects of Aqueous Leaf Extract of *Phyla nodiflora* in Zebrafish (*Danio rerio*) Embryos". *Egypt. Acad. J. Biol. Sci. B Zool.* 14 (2), 439–447.
- Petrovska, B.B., 2012. Historical review of medicinal plants usage. *Pharmacogn. Rev.* 6 (11), 1.
- Phillipson, J.D., 2001. Phytochemistry and medicinal plants. *Phytochemistry* 56 (3), 237–243.
- Pirzada, A., Iqbal, P., Shaik, W., Kazi, T., Ghani, K., 2005. Studies on the elemental Composition and antifungal activity of medicinal plant *Lippia nodiflora* L. against skin fungi. *J. Pak. Assoc. Dermatol.* 15, 113–118.
- Pizzino, G., N. Irrera, M. Cucinotta, G. Pallio, F. Mannino, V. Arcoraci, F. Squadrito, D. Altavilla and A. Bitto (2017). "Oxidative stress: harms and benefits for human health." *Oxidative medicine and cellular longevity* **2017**.
- Powell, M. J. (1978). A fast algorithm for nonlinearly constrained optimization calculations. *Numerical analysis*, Springer: 144–157.
- Rahman, H., Javaid, S., Ashraf, W., Rasool, M., Anjum, S., Saleem, H., Siddique, F., Chtita, S., Sivandzade, F., Alqahtani, F., 2021. Neuropharmacological investigation, ultra-high performance liquid chromatography analysis, and in silico studies of *Phyla nodiflora*. *J. Physiol. Pharmacol.* 72 (4).
- Ravikanth, V., Ramesh, P., Diwan, P., Venkateswarlu, Y., 2000. Halleridone and Hallerone from *Phyla nodiflora* as taxonomic markers. *Biochem. Syst. Ecol.* 28 (9), 905–906.
- Regupathi, T., Chitra, K., 2015. "Antidandruff activity of *Eclipta alba* (L.) Hassk. and *Lippia nodiflora* linn". *Int. J. Pharma. Phytopharmacol. Res.*
- Saleem, H., Zengin, G., Locatelli, M., Ahmad, I., Khaliq, S., Mahomoodally, M.F., Hussain, R., Rengasamy, K.R., Mollica, A., Abidin, S.A.Z., 2019. Pharmacological, phytochemical and in-vivo toxicological perspectives of a xero-halophyte medicinal plant: *Zaleya pentandra* (L.) Jeffrey. *Food Chem. Toxicol.* 131, 110535.
- Sharma, R., Singh, R., 2013. A review on *Phyla nodiflora* Linn.: a wild wetland medicinal herb. *Int. J. Pharmaceut. Sci. Rev. Res.* 20 (1), 57–63.
- Shukla, S., Patel, R., Kukkar, R., 2009. Study of phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of *Phyla nodiflora* Linn. *Int. J. Pharm. Pharm. Sci.* 1 (1), 85–91.
- Siddiqui, B.S., Ahmed, F., Ali, S.K., Perwaiz, S., Begum, S., 2009. Steroidal constituents from the aerial parts of *Lippia nodiflora* Linn. *Nat. Prod. Res.* 23 (5), 436–441.
- Suroowan, S., Llorent-Martínez, E., Fakurazi, S., Zengin, G., Khalid, A., Ahmed, I.E., Makeen, H.A., Alhazmi, H.A., Albratty, M., Mohan, S., 2023. Unveiling the phytochemical and pharmacological potential of *Mimusops maxima* (Poir.) Vaughan—an endemic plant with potential therapeutic effects. *Process Biochem.* 132, 157–165.
- Thirupathy, K.P., Tulshkar, A., Vijaya, C., 2011. Neuropharmacological activity of *Lippia nodiflora* Linn. *Pharmacogn. Res.* 3 (3), 194.
- Vanajothi, R., Sudha, A., Manikandan, R., Rameshthangam, P., Srinivasan, P., 2012. *Luffa acutangula* and *Lippia nodiflora* leaf extract induces growth inhibitory effect through induction of apoptosis on human lung cancer cell line. *Biomed. Prev. Nutr.* 2 (4), 287–293.
- Wairata, J., Fadlan, A., Purnomo, A.S., Taher, M., Ersam, T., 2022. Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiparasitic activities of *Garcinia forbesii* King: A correlation study. *Arab. J. Chem.* 15, (2) 103541.
- Yen, F.-L., M.-C. Wang, C.-J. Liang, H.-H. Ko and C.-W. Lee (2012). "Melanogenesis inhibitor (s) from *Phyla nodiflora* extract." *Evidence-Based Complementary and Alternative Medicine* **2012**.
- Zekri, N., Zerkani, H., Elazzouzi, H., Zair, T., El Belghiti, M.A., 2021. Extracts of *M. pulegium* (L.) and *M. spicata* (L.): Effect of extraction conditions on phenolics and flavonoids contents and their antioxidant power. *Egypt. J. Chem.* 64 (3), 1447–1459.
- Zengin, G., Ak, G., Ceylan, R., Uysal, S., Llorent-Martínez, E., Di Simone, S.C., Rapino, M., Acquaviva, A., Libero, M.L., Chiavaroli, A., 2022. Novel perceptions on chemical profile and biopharmaceutical properties of *Mentha spicata* extracts: Adding missing pieces to the scientific puzzle. *Plants* 11 (2), 233.
- Zengin, G., M. F. Mahomoodally, E. Yildiztugay, S. Jugreet, S. U. Khan, S. Dall'Acqua, A. Mollica, A. Bouyahya and D. Montesano (2022). "Chemical Composition, Biological Activities and In Silico Analysis of Essential Oils of Three Endemic Prangos Species from Turkey." *Molecules* **27**(5): 1676.
- Zengin, G., Á. Fernández-Ochoa, M. d. I. L. Cádiz-Gurrea, F. J. Leyva-Jiménez, A. Segura-Carretero, F. Elbasan, E. Yildiztugay, S. Malik, A. Khalid and A. N. Abdalla (2023). "Phytochemical Profile and Biological Activities of Different Extracts of Three Parts of *Paliurus spina-christi*: A Linkage between Structure and Ability." *Antioxidants* **12**(2): 255.