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RESEARCH ARTICLE

Characterisation and *in silico* evaluation of secondary metabolites reported in *Nyctanthes arbor-tristis* L. with anti-arthritic properties

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Abstract

The current study made an attempt to explore bioactive secondary metabolites as an alternative or complementary source for the treatment of rheumatoid arthritis. It aimed at the characterisation of secondary metabolites present in methanolic leaf extract of *Nyctanthes arbor-tristis* (MLEN) having anti-arthritic properties using thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). Further, *in silico* evaluation of bioactive principles was performed against rheumatoid arthritis factors visfatin, resistin, chemerin, leptin, adiponectin and lipocalin 2 using AutoDock Vina. Two colour bands with Rf values of 0.44 and 0.88 were resolved with the mobile phase of n-butanol: acetic acid: water (50:10:40) in TLC. HPLC metabolite profiling and ^{1}H and ^{13}C NMR spectroscopic analyses reported a total of eleven phenolic compounds (gallic acid, syringic acid, ferulic acid, luteolin, benzoic acid, kaempferol, ellagic acid, chrysin, phenylacetic acid, chlorogenic acid and p-coumaric acid) with luteolin being most abundant (73 µg/mL). Through *in silico* evaluation, the identified compounds have shown favourable binding affinities with the targets. Chlorogenic acid has shown the highest binding affinity of -10.2 kcal/mol with visfatin (PDB ID 5LX5), -8.2 kcal/mol with chemerin (PDB ID 7YKD) and -7.8 kcal/mol with resistin (PDB ID 1RFX) confirming its strong affinity to inhibit inflammation due to arthritis. These findings infer that MLEN has promising anti-arthritic activity. Further research in the field of *in vitro* and *in vivo* anti-arthritic activity, followed by toxicity parameters, may authenticate the therapeutic potential of the extract in the treatment of arthritis.

Keywords: HPLC; *in silico* analysis; MLEN; NMR; phenols; secondary metabolites

Introduction

Arthritis is a widespread noncommunicable disease that affects joints with symptoms such as joint pain and stiffness (1). More than 100 types of arthritis exist in various forms, of which osteoarthritis (degenerative joint disease) and rheumatoid arthritis are commonly prevalent. Osteoarthritis shows effect mainly on knees, fingers and hips. Rheumatoid arthritis is characterized by chronic inflammation due to synovial hyperplasia, which further progresses to massive irreversible bone destruction (2). Symptoms include redness, warmth, swelling and decreased range of motion of the affected joints (3). A recent epidemiological study reveals that about 1 % of people all over the world are now affected by rheumatoid arthritis and it is more prevalent among women rather than men, though this fact has not been scientifically proven. Strong evidence suggests the involvement of cytokines is important in disease progression since cytokines play a fundamental role in various inflammatory processes, articular destruction and rheumatoid arthritis- associated comorbidities (4–6). Leptin, adiponectin, visfatin, resistin, chemerin and lipocalin 2 are some of the proinflammatory cytokines that play a key role in arthritis (7, 8).

Although allopathic medicines provide some relief from pain, their use for longer periods results in several side effects such as respiratory infections, edema and urinary tract infections (9). This led the researchers to find an alternative medicine. Herbal medicines have come up as safe alternatives for the treatment of arthritis. Herbs such as *Zingiber officinale, Curcuma longa, Salvia miltiorrhiza* and *Tripterygium wilfordii* show medicinal potential as ancillaries in RA therapy (10). Natural phytochemicals extracted from medicinal plants can reduce the arthritic effect by collaborating and changing the expression of pro-inflammatory signaling on the inflammation pathway (11).

Nyctanthes arbor-tristis L. commonly known as harsingar in Hindi, is a member of the family Oleaceae, native to South Asia and Southeast Asia. The plant is mostly found in the Himalayas in India and has been discovered in areas of Nepal, to the east of Assam, Tripura, Bengal, Jammu and Kashmir, as well as in the central region of Godavari in the south (12). Several phytochemical constituents, including carbohydrates, steroids, flavonoids, iridoid glycosides, terpenes and alkaloids are available in different parts of *N. arbor-tristis* (13). It has been widely used in Ayurveda, Siddha and Unani in India for centuries to treat a number of diseases. Previous

research showed that the plant parts exhibit antioxidant, analgesic, anti-inflammatory, anti-nociceptive, hepatoprotective, antibacterial, antifungal, anti-cancer and anticholinesterase properties, which may be attributed to the various phytochemical constituents, including alkaloids, phenols, tannins and glycosides (14–16). The anti-arthritic activity of the methanolic extract of *N. arbor-tristis* has been reported in Freund's Complete Adjuvant (FCA)-induced arthritis model in rats (17). The stem bark powder is used to cure malaria, as an expectorant and to relieve rheumatic joint pain. The flowers are used as an expectorant, hair tonic, stomachic, carminative, astringent to the intestines and to cure piles and other skin problems. The seeds are used as anthelmintics, to treat alopecia and to treat bilious fevers (14). The leaves of the plant are used to treat a variety of illnesses, including chronic fever, sciatica, rheumatism and internal worm infections. Numerous pharmacological benefits of the leaf extract have been demonstrated, including antibacterial, analgesic, anti-inflammatory, anti-diabetic, anti-arthritic, antioxidant, hepatoprotective and antispasmodic properties (18, 19). However, there is less evidence of phytochemical compounds being evaluated against adipokines using *in silico* docking. The present study focused on screening and structural characterisation of the secondary metabolites of *N. arbor-tristis* and the *in silico* evaluation of their biological activity against arthritic adipokines.

Materials and Methods

Collection of the plant material and authentication

Fresh leaves of *N. arbor-tristis* were collected in February 2021 from Eluru town in the Eluru district of Andhra Pradesh, India. The plant specimen is deposited in the Department of Botany, Acharya Nagarjuna University, Guntur and the voucher specimen number is ANUBH02101. The taxonomic identity of the plant was authenticated by Prof. S. M. Khasim, Department of Botany and Microbiology, Acharya Nagarjuna University.

Extraction of the plant material

Fresh leaves (810 g) of the plant were cleaned, allowed to dry at room temperature and were pulverized into a coarse powder (258 g) using a mechanical grinder. The leaf extract was prepared with 3100 mL of methanol and 258 g of leaf powder. The Soxhlet apparatus was used to prepare the methanolic extract (MLEN) as methanol was a high polar solvent with a low molecular size and excellent cell wall penetration ability and is more efficient for extracting more phytochemicals. It was then concentrated to 1 mg/mL (W/V) using a rotary evaporator at reduced pressure. It was then stored at 4 °C until it was needed (20).

Thin layer chromatography (TLC)

Thin layer chromatography studies were performed on MLEN to identify compounds in the extract. Silica gel 60 F_{254} TLC aluminium sheets from Merck, Germany, were used. Three solvent systems were employed. The first solvent combination consisted of petroleum ether and ethyl acetate (7:3), followed by ethyl acetate, methanol and water (50:10:40) and the third combination with n-butanol, acetic acid and water (50:10:40) respectively. The TLC plates were observed under UV light immediately after elution. Rf values were calculated (21).

High performance liquid chromatography (HPLC)

Chemicals, reagents and standards

HPLC grade methanol and acetonitrile were purchased from Thermo Fisher Scientific, India Pvt. Ltd., Mumbai. The purity of all standards (more than 95 %) was procured from Chemdyes Corporation, Rajkot, India.

Preparation of standards and sample

Following precise weighing, 100 mg of gallic acid, syringic acid, ferulic acid, benzoic acid, luteolin, kaempferol, ellagic acid, chrysin, phenylacetic acid, chlorogenic acid, p-coumaric acid and dried methanolic extract were each transferred into 100 mL volumetric flasks. The volume was then increased to 100 mL using HPLC-grade methanol. For HPLC injection, the stock solution was appropriately diluted to get different concentrations of standards (0.1–100 ug/mL) to draw the standard calibration curve (22).

Method development

A Zodiac C18 column (100 × 4.6 mm, 5 µm GL Sciences, Tokyo, Japan) was utilized for chromatographic separation. Two different mobile phases were used (acetonitrile and methanol; acetonitrile and water) in a 50:50 ratio and the programming was done with four ways: a) acetonitrile and methanol (50:50) at 210 nm and 60 min run time; b) acetonitrile and water at 210 nm wavelength and 60 min run time; c) acetonitrile and water at 280 nm wave length and 60 min run time; d) acetonitrile and water at 253 nm and 20 min run time with a flow rate of 0.5 mL/min. A sample volume of 20 µL was injected into the mobile phase at pH 5.5 with elution in isocratic conditions. The run time was 60 min. The components were detected at 210 nm. Chromatograms were also obtained at 280 nm with a 60 min run time and 253 nm with a 20 min run time respectively. The system suitability test was assessed by three replicates of gallic acid, chlorogenic acid, chrysin, kaempferol, luteolin, p-coumaric acid and syringic acid. The limit of detection (LOD) and limit of quantification (LOQ) were determined as per ICH guidelines. The concentration of the compounds in the samples was calculated through the standard calibration curve equation and was expressed as µg/mL of extract weight.

Nuclear magnetic resonance (NMR)

Five mg of dried sample was dissolved in 0.6 mL of deuterated solvent and transferred into a 5 mm NMR tube to record ^{1}H NMR spectra and for ^{13}C NMR, 20 mg was dissolved in 0.6–0.8 mL deuterated solvent. ^{1}H NMR and ^{13}C NMR spectroscopy was conducted with instrument ID: SA/AD/014. Deuterated methanol (CD_3OD) was used as a solvent for MLEN. Pre-saturation was not done during the experiment. For ^{1}H NMR spectroscopy, the frequency of the spectrometer was maintained at 400 MHz. A total of 128 scans were taken with an acquisition time of 4 sec and a relaxation delay of 1 sec was incorporated after each scan. The spectral width was 7183.9 Hz. For ^{13}C NMR spectroscopy, the frequency of 100.547 MHz was maintained. An acquisition time of 1.311 sec was used for a total of 3000 scans. The relaxation delay was 3 sec. The spectral width was 25000 Hz. In 3000 scans, a total of 65536 points were collected. The spectrum unveiled the presence of 17 carbon peaks. The metabolites identified were well documented (23).

In silico analysis

Preparation of protein

PDB files of three-dimensional structures of leptin, visfatin, adiponectin, resistin, chemerin and lipocalin 2 were acquired from the Protein Data Bank (PDB) (https://www.rcsb.org).

Preparation of ligands

The phenolic compounds in PDB format were downloaded from PubChem.

Interactions between bioactive compounds (ligands) and proteins

The phenolic compounds identified in HPLC and NMR of MLEN (chlorogenic acid, ferulic acid, syringic acid, ellagic acid, benzoic acid, luteolin, phenylacetic acid, p-coumaric acid and gallic acid) were docked with adipokines visfatin (PDB ID 5LX5), leptin (PDB ID 1AX8), adiponectin (PDB ID 6U66), chemerin (PDB ID 7YKD), resistin (PDB ID 1RFX) and lipocalin 2 (PDB ID 3BX7), which are commonly found in rheumatoid arthritis. Using AutoDock Vina, the phenolics were docked as ligands into the active site of protein receptors. The PDB ID was downloaded from PDB and the phenolic compound SMILES ID was downloaded from PubChem (24). The best-docked conformation between the ligand and the target receptor protein with the most favourable (least free) binding energy was chosen using the docking method.

Results

Thin layer chromatography (TLC)

In our results, two prominent colour bands were resolved in TLC with Rf values of 0.44 and 0.88 using n-butanol: acetic acid: water (50:10:40) as the mobile phase while a single colour band with Rf value of 0.2 was observed in ethyl acetate: methanol: water (50:10:40), indicating that the solubility of phytochemicals is solvent specific.

High performance liquid chromatography (HPLC)

As shown in Fig. 1, the HPLC chromatogram showed eleven prominent phenolic compounds with varied retention times at different wavelengths and mobile phases. Based on the NIST database library, eleven compounds gallic acid, syringic acid, chlorogenic acid, luteolin, benzoic acid, kaempferol, ellagic acid, chrysin, phenylacetic acid, ferulic acid and p-coumaric acid, were identified in different methods applied as described in the methodology. The characteristics of the phenolic compounds of MLEN identified in four different solvent systems showed variations in their concentrations, peak area and retention time.

Method validation

The developed method was validated as per ICH guidelines, which include the parameters such as linearity and range, LOD and LOQ.

Linearity and range

The area under curve data versus the concentration of gallic acid, syringic acid, chlorogenic acid, chrysin, kaempferol, luteolin and p-coumaric acid were treated by linear correlation coefficient. The calibration curve equations of gallic acid, syringic acid, chlorogenic acid, chrysin, kaempferol, luteolin and p-coumaric acid were obtained as y = 107396x - 11319(R^2 = 0.9839), y = 99532x + 1947.6 (R^2 = 0.9872), y = 15898x + 2412 (R^2 = 0.9773), y = 98799x + 46543 (R^2 = 0.9925), y = 106176x + 1482.4(R^2 = 0.9924), y = 1757.6x - 9251 (R^2 = 0.9816), y = 509.5x - 515 (R^2 = 0.9956) respectively. This method was found to be linear in the range of 0.1–100 µg/mL for all the compounds. R^2 values of 0.99, 0.98 and 0.97 in the tested range of 0.1–100 µg/mL concentrations indicate good to acceptable linearity of response.

Quantification of phenols in MLEN

Quantification of the phenols gallic acid, syringic acid, chlorogenic acid, chrysin, kaempferol, luteolin and p-coumaric acid was done by performing HPLC analysis of the sample solution. The amount of gallic acid, syringic acid, chlorogenic acid, chrysin, kaempferol, luteolin and p-coumaric acid present in MLEN was calculated using linear regression analysis. Luteolin was found to be most abundant, with a concentration of 73 µg/mL, gallic acid with 0.82 µg/mL syringic acid with 0.16 µg/mL, chlorogenic acid with 0.12 µg/mL and p-coumaric acid peak with 3.78 µg/mL (Table 1).

Table 1. Phenols reported in HPLC of MLEN showing retention time, area (%) and concentration (µg/mL)

S. No	Name	RT (min)	Area (%)	Concentration (µg/mL)
1.	Gallic acid [b]	13	13.05	0.821
2.	Syringic acid [a]	20	9.40	0.16
3.	Ferulic acid [c]	25	0.05	negligible
4.	Luteolin [a]	34	62.20	73
5.	Benzoic acid [a]	31	0.03	negligible
6.	Kaempferol [a]	46	0.87	0.001
7.	Ellagic acid [b]	22	0.03	negligible
8.	Chrysin [b]	55	0.54	0.44
9.	Phenyl acetic acid[c]	32	0.02	negligible
10.	Chlorogenic acid [d]	08	6.21	0.1229
11.	P-coumaric acid [d]	13	2.01	3.78

RT: Retention time, (a, b, c, d) denotes mobile phase mentioned in methodology.

Limit of detection (LOD)

LOD was calculated using the following formula:

$$LOD = 3.3\,\sigma/S$$

Where σ = standard deviation of the intercepts of the calibration curve and S is the slope of the calibration curve. The LOD of gallic acid, syringic acid, chlorogenic acid, chrysin, kaempferol, luteolin and p-coumaric acid was found to be 0.0026, 0.0034, 0.0092, 0.0015, 0.0045, 0.026 and 0.2332 µg/mL respectively.

Limit of quantification (LOQ)

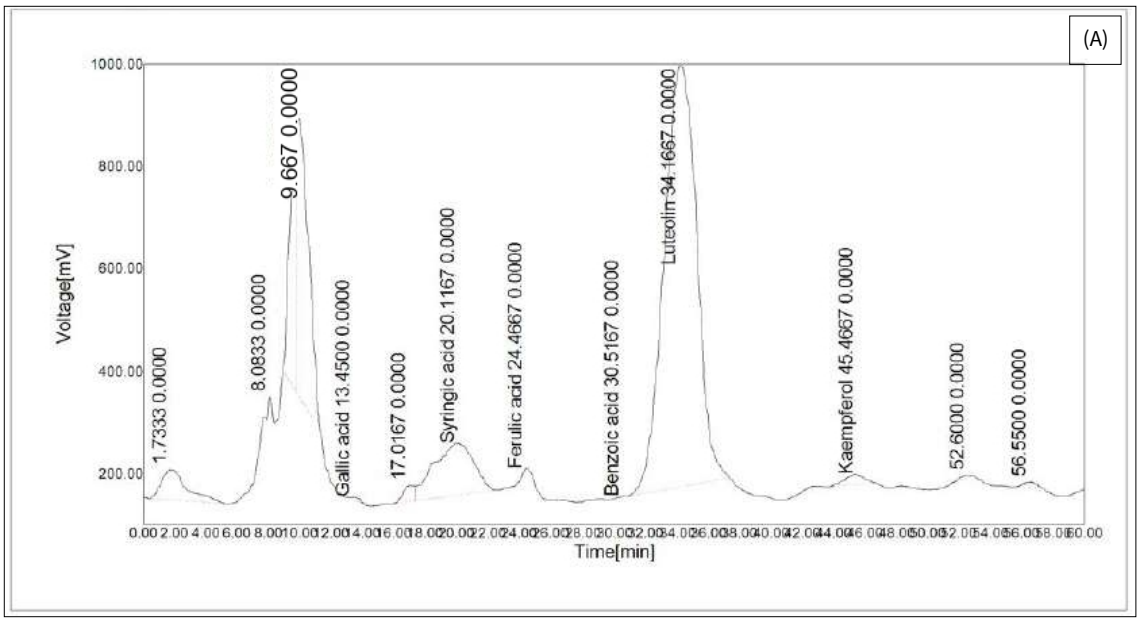
LOQ was calculated by the formula:

$$LOQ = 10\,\sigma/S$$

LOQ of gallic acid, syringic acid, chlorogenic acid, chrysin, kaempferol, luteolin and p-coumaric acid was found to be 0.0078, 0.0102, 0.027, 0.0047, 0.0137, 0.0796 and 0.7067 µg/mL respectively.

Nuclear magnetic resonance (NMR)

^{1}H NMR analysis of MLEN showed peaks at 4.888 ppm with intensity (109.5), 3.348 ppm (intensity 50.4), 3.313 ppm (intensity 8.3), 3.310 ppm (intensity 10.9) and 3.306 ppm (intensity 8.4). The ^{13}C NMR of MLEN showed chemical shifts at 206.253 ppm, 130.647 ppm, 129.584 ppm, 77.905 ppm, 72.927 ppm, 49.000 ppm and 48.788 ppm respectively (Fig. 2).


(A)
Voltage[mV]
Time[min]
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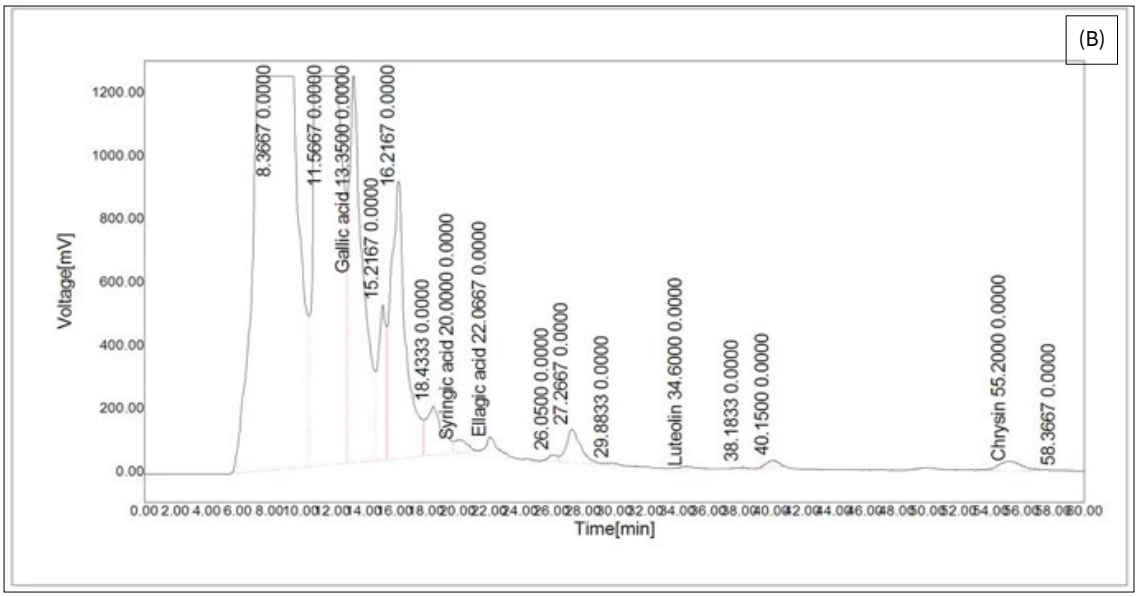
8.0833 0.0000
9.667 0.0000
Gallic acid 13.4500 0.0000
17.0167 0.0000
Syringic acid 20.1167 0.0000
Ferulic acid 24.4667 0.0000
Benzoic acid 30.5167 0.0000
Luteolin 34.1667 0.0000
Kaempferol 45.4667 0.0000
52.6000 0.0000
56.5500 0.0000


(B)
Voltage[mV]
Time[min]
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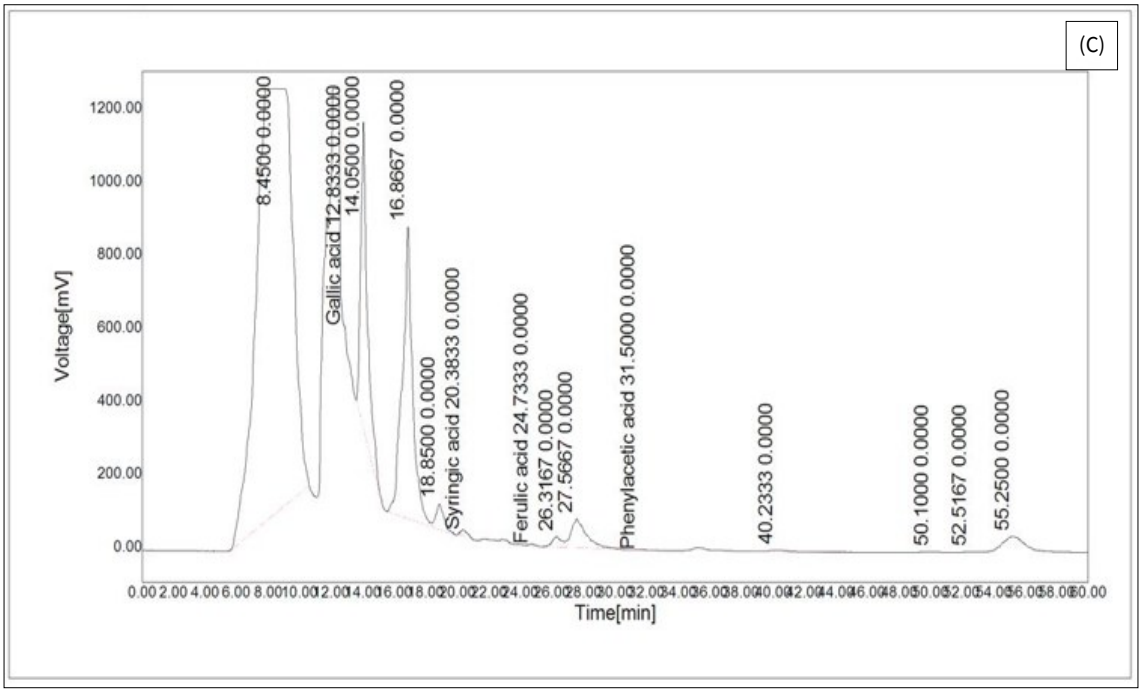
11.5667 0.0000
Gallic acid 13.3500 0.0000
15.2167 0.0000
16.2167 0.0000
18.4333 0.0000
Syringic acid 20.0000 0.0000
Ellagic acid 22.0667 0.0000
26.0500 0.0000
27.2667 0.0000
29.8833 0.0000
Luteolin 34.6000 0.0000
38.1833 0.0000
40.1500 0.0000
Chrysin 55.2000 0.0000
58.3667 0.0000


(C)
Voltage[mV]
Time[min]
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Gallic acid 12.8333 0.0000
14.0500 0.0000
16.8667 0.0000
18.8500 0.0000
Syringic acid 20.3833 0.0000
Ferulic acid 24.7333 0.0000
26.3167 0.0000
27.5667 0.0000
Phenylacetic acid 31.5000 0.0000
40.2333 0.0000
50.1000 0.0000
52.5167 0.0000
55.2500 0.0000

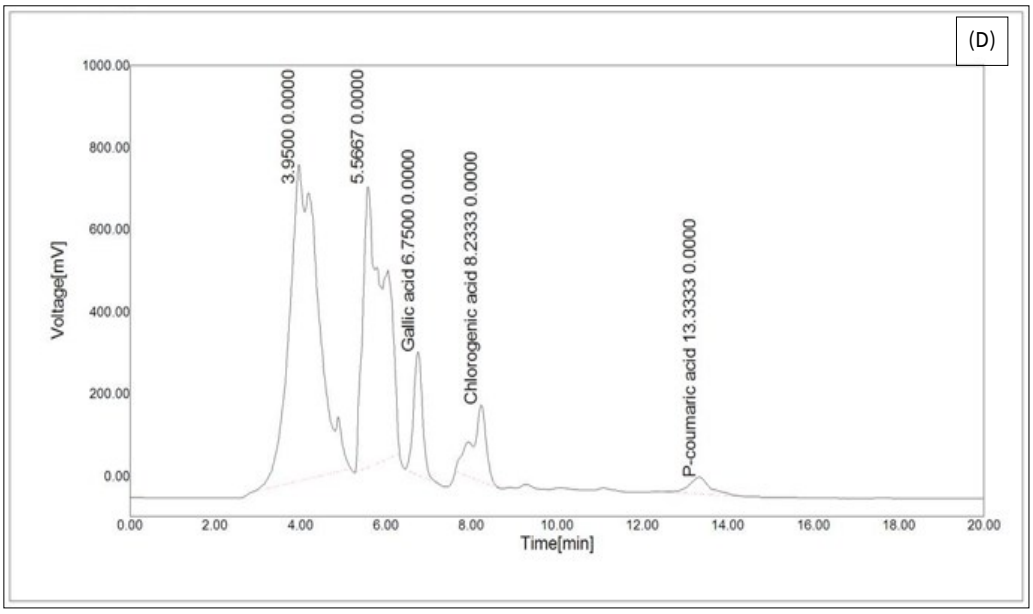

Fig. 1. HPLC chromatograms of phenols in methanolic leaf extract of *N. arbor-tristis* L.(MLEN) showing the presence of gallic acid, syringic acid, chlorogenic acid, luteolin, benzoic acid, kaempferol, ellagic acid, chrysin, phenylacetic acid, ferulic acid and p-coumaric acid. (A) acetonitrile and methanol (50:50) as mobile phase at 210 nm and 60 min run time; (B) acetonitrile and water (50:50) as mobile phase at 210 nm and 60 min run time; (C) acetonitrile and water (50:50) as mobile phase at 280 nm and 60 min run time; (D) acetonitrile and water (50:50) as mobile phase at 253 nm and 20 min run time.

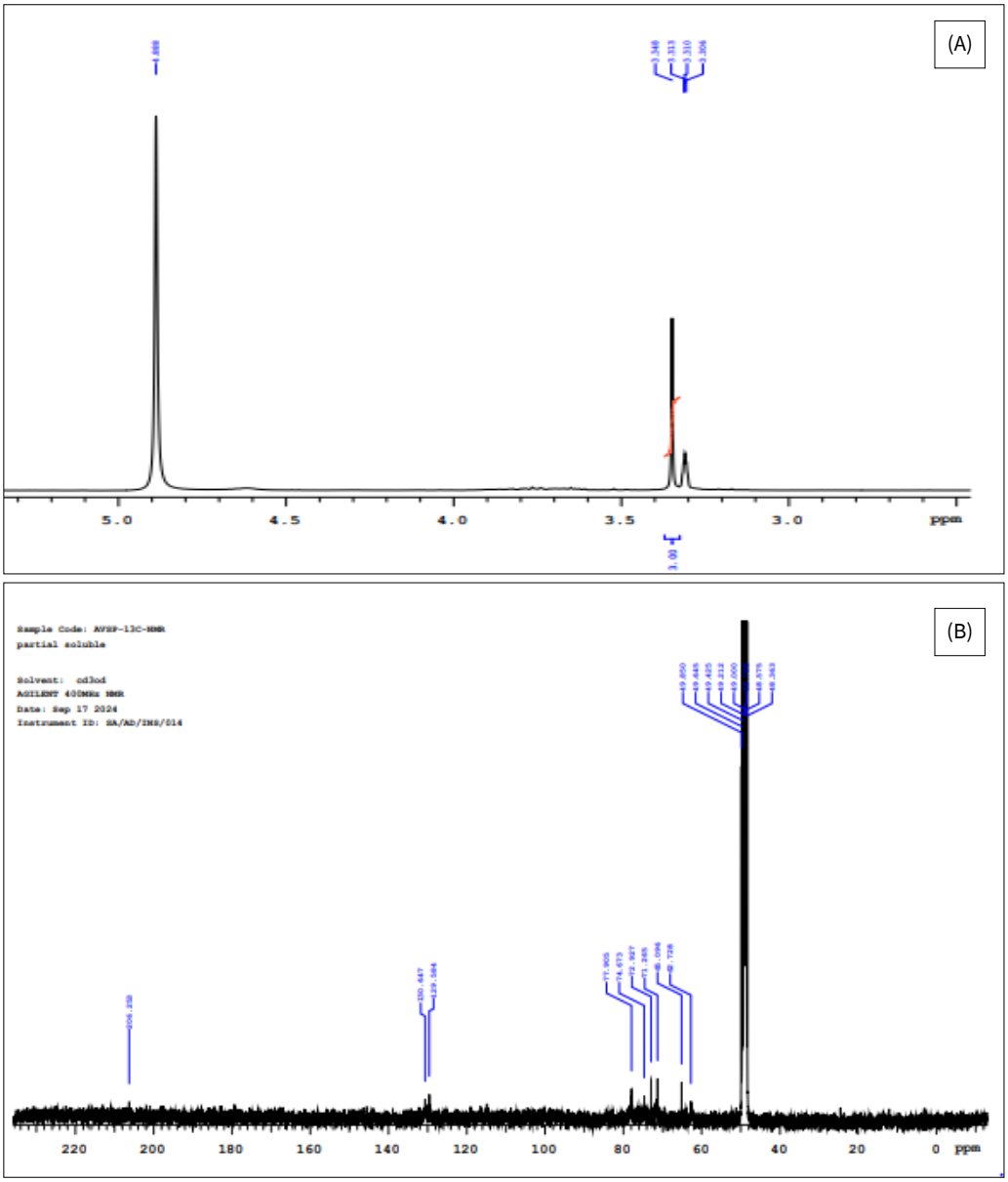

Fig. 2. (A) 400 MHz ^{1}H NMR spectrum of MLEN dissolved in CD_3OD; (B) 400 MHz ^{13}C NMR spectrum of MLEN dissolved in CD_3OD.

In silico analysis

Molecular docking was performed to predict the binding affinity and interaction patterns of bioactive molecules of MLEN (ligands) and rheumatoid arthritis factors such as visfatin, resistin, chemerin, leptin, adiponectin and lipocalin 2 (Fig. 3) using AutoDock Vina.

In our results, the binding energy of the ligand to the protein varied among the individual rheumatoid arthritis factors. About 48 runs were performed in molecular docking to obtain the best docking pose based on the docking score list of the ligand. Ligands with the least binding energies were the best to retard the target proteins. Most of the phenols docked reported best binding affinities with visfatin, followed by resistin and chemerin (Fig. 4). Fig. 5 visualizes the binding energies and docking results between ligands and target proteins. Table 2 shows the individual bioactive molecules of MLEN (ligand) and their interactions in terms of binding energy, number of hydrogen bonds and number of binding sites. Chlorogenic acid has shown the highest binding affinity of -10.2 with visfatin (PDB ID 5LX5) followed by luteolin the most abundant phenol in MLEN with binding affinity of -9.2 with visfatin (PDB ID 5LX5).

Discussion

Our HPLC analysis identified eleven prominent phenolic compounds, gallic acid, syringic acid, chlorogenic acid, luteolin, benzoic acid, kaempferol, ellagic acid, chrysin, phenylacetic acid, ferulic acid and p-coumaric acid, with luteolin being the most abundant (Fig. 1).

In NMR studies (Fig. 2) ^{1}H NMR showed a peak at 4.888 ppm which indicates that a methylene group adjacent to an electronegative atom may support the presence of ferulic acid (methoxy group -OCH_3). ^{1}H NMR has shown peaks between 3.306 and 3.348 ppm (-CH_2-OH, -CH_2-O-R, -CH2-N) and 4.888 ppm(-CH-O-), which may indicate the presence of chlorogenic acid, luteolin and p-coumaric acid (Libre Texts Chemistry). The peaks obtained at different retention times in ^{13}C NMR represent the presence of C=O, C in aromatic rings, alkyne, allylic, benzylic and ketone groups respectively (Libre Texts Chemistry), confirming the presence of chlorogenic acid, luteolin, p-coumaric acid, gallic acid, syringic acid and ferulic acid (25 –28).

Phenolic compounds are well known for their antioxidant and anti-inflammatory activities (15, 16). Flavonoids show anti-bacterial, antiviral, anti-inflammatory and anti-allergic properties (29). Tannins, terpenoids and phenols are found to possess anti-oxidant, antimicrobial, anti-inflammatory and anticancer properties (30).

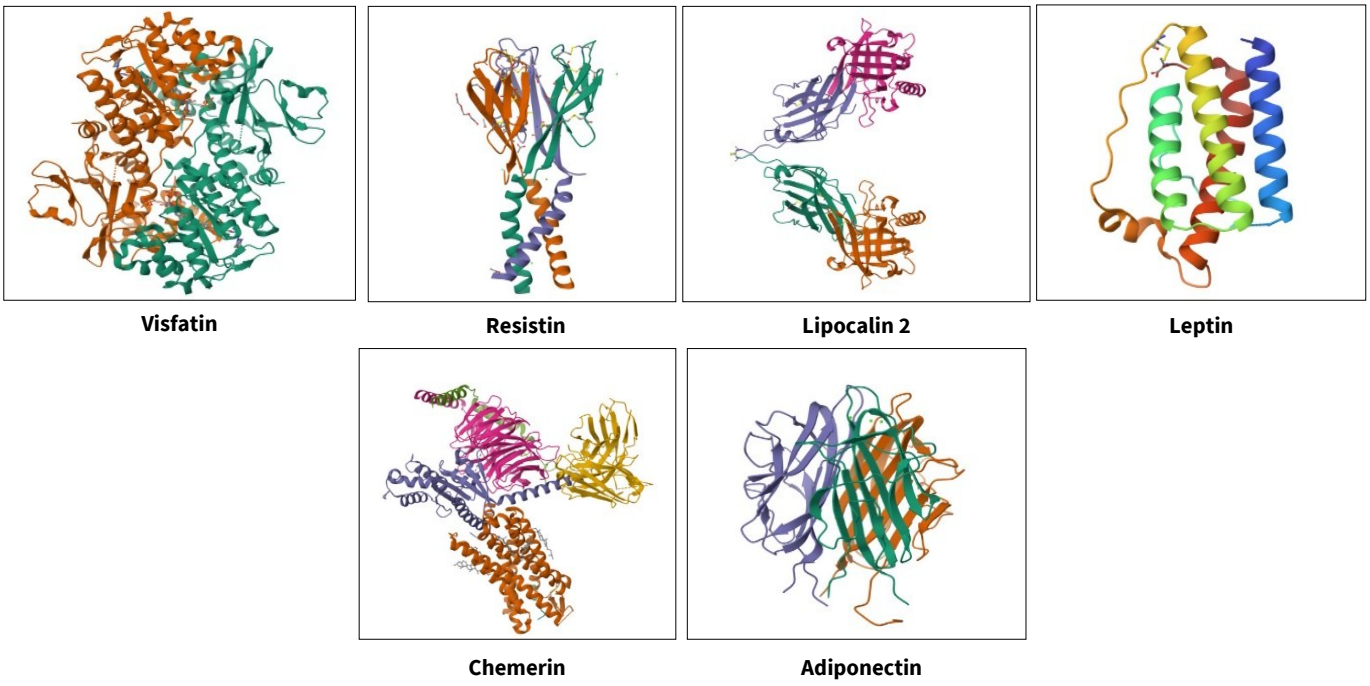

Fig. 3. Structure of adipokines acting as rheumatoid arthritis factors obtained from PDB.

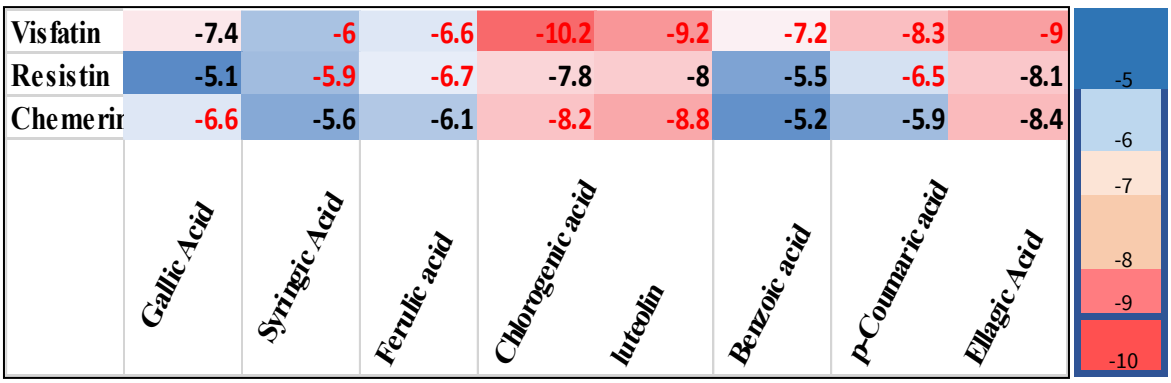

Fig. 4. Heat map of interaction between identified phenols and adipokines reporting significant binding energies.

Table 2. Molecular docking of identified phenols with visfatin reporting binding affinities, hydrogen interactions and hydrophobic interactions

Sl. No.	Bioactive compound	Ligand/SMILE ID	Receptor/ target protein (PDB ID)	Disease	Binding affinity	Residue involved in hydrogen interactions (Ligand atom receptor)	Residue involved in hydrophobic interactions (Ligand atom receptor)
1.	Gallic acid (3,4,5-trihydroxybenzoic acid)	C1=C (C= C(C(=C1O) O) O) C(=O) O	5LX5	Rheumatoid arthritis	-7.4	O3-F193(A) O O1-D219(A) OD2 O5-D313(A) OD2 O5-G353(A) O O3-D16(B) OD1 O1-D16(B) OD1 O3-R196(A) NE O3-R196(A) NH2	C6-F193(A) CB
2.	Syringic acid (4-Hydroxy-3,5-dimethoxybenzoic acid)	COC1=CC (=CC(=C1O) OC) C(=O) O	5LX5	Rheumatoid arthritis	-6	O5-V356(A) O O4-V356(A) O O4-S382(A) OG O4-G383(A) N O4-S382(A) OG O4-G385(A) N O1-R392(B) NH2 O4-T37(C) OG1 O5-S57(C) OG O5-S57(C) OG O4-T37(C) OG1 O2-I67(A) N	No interactions
3.	Ferulic acid	COC1=C (C=CC (=C1)/C=C/C (=O) O) O	5LX5	Rheumatoid arthritis	-6.6	O1-H191(A) ND1	C8-F193(A) CZ C8-A244(A) CB C6-I309(A) CD C4-I351(A) CD
4.	Chlorogenic acid	C1[C@H] ([C @H] ([C@@H] (C[C@@]1 (C(=O) O) O) OC (=O)/C=C/C2=CC (=C(C=C2) O) O) O) O	5LX5	Rheumatoid arthritis	-10.2	O9-D219(A) OD2 O3-Y18(B) OH O2-K400(B) NZ O4-K400(B) NZ	C15-D219(A) CB
5.	Luteolin	C1=CC (=C (C=C1C2=CC (=O) C3=C (C=C (C=C3O2) O) O) O) O	5LX5	Rheumatoid arthritis	-9.2	O2-H191(B) NE2 O3-H191(B) NE2 O2-D219(B) OD1 O2-S241(B) OG O4-S275(B) OG O5-V350(B) O O3-H191(B) NE2 O2-S241(B) OG O2-V242(B) N O2-H191(B) NE2 O4-S275(B) OG	C11-F193(B) CE2 C1-V242(B) CG2 C4-I309(B) CD C8-I351(B) CD C9-A379(B) CB
6.	Ellagic acid	C1=C2C3= C(C(=C1O) O) OC (=O) C4=CC (=C(C(=C43) OC2=O) O)	5LX5	Rheumatoid arthritis	-9	O6-G353(A) O O4-G353(A) O O4-G381(A) O O3-T391(B) OG1 O5-T391(B) OG1 O5-D393(B) OD1 O3-D393(B) OD2 O5-D393(B) OD2 O7-R392(B) NE O7-R392(B) NH2 O3-T391(B) OG1 O3-D393(B) N O5-T391(B) OG1 O4-V356(A) N	No interactions
7.	Benzoic acid	C1=CC=C(C=C1) C(=O) O	5LX5	Rheumatoid arthritis	-7.2	O2-D219(A) OD2	No interactions
8.	p-Coumaric acid	C1=CC(=CC=C1/C=C/ C(=O)O)O	5LX5	Rheumatoid arthritis	-8.3	O1-F193(A) O O2-S275(A) OG O3-S275(A) OG O1-D16(B) OD1 O2-S275(A) OG O3-S275(A) OG O1-R196(A) NE O1-R196(A) NH2	C5-F193(A) CB C8-F193(A) CZ C8-Y18(B) CD1 C7-Y18(B) CE1 C2-Y18(B) CE2

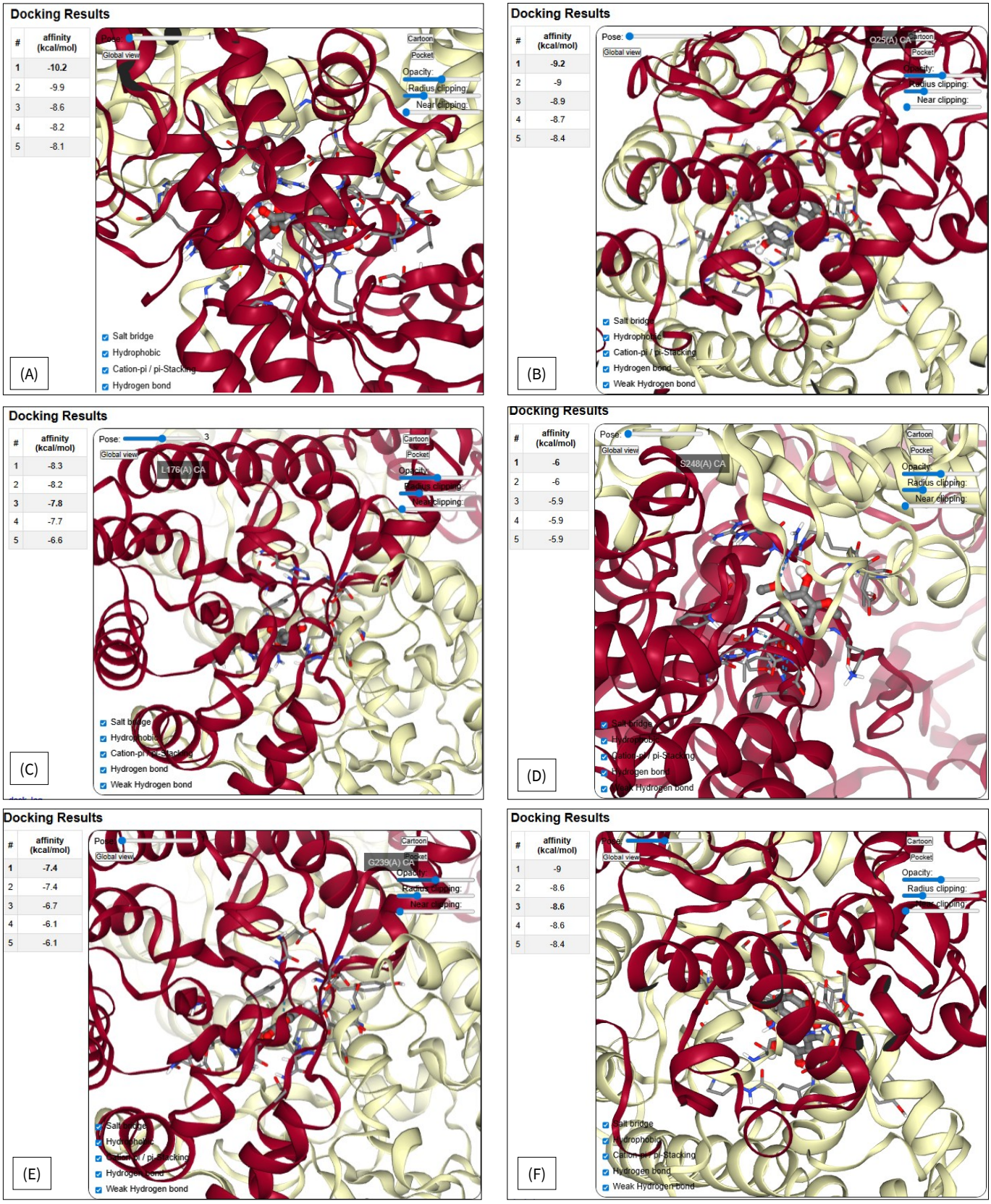

Fig. 5. Ligands visualising the docking results with visfatin (5LX5) an adipokine acting as rheumatoid arthritis factor.

Adipokines play an important role in the pathogenesis of RA. Adiponectin, visfatin and chemerin increase the secretion as well as activity of interleukin-6 (IL-6), IL-8 and stimulates the production of matrix metalloproteinases (MMPs). Leptin increases tumour necrosis factor- α (TNF-α), IL-6 (31). TNF-α, IL-1β and IL-6 were found in high concentrations in synovial fluids of RA patients (2). TNF-α, stimulates the production of IL-1β, IL-6, activates NF-Kb signalling and induces MMPs (32).

The molecular docking studies of gallic acid, syringic acid, chlorogenic acid, luteolin, benzoic acid, kaempferol, ellagic acid, chrysin, phenylacetic acid, ferulic acid and p-coumaric acid conducted against these adipokines revealed that all the compounds have shown potential anti- arthritic activity against leptin, adiponectin, resistin, visfatin, chemerin and lipocalin 2. Phenols suppress the production of adipokines and inhibit the production of TNF-α, IL-1β, IL-6 (33, 34). Flavonoids inhibit the inflammatory enzymes cyclooxygenase (COX) and lipoxygenase

(LOX) and inhibit the NF-κB, MAPK activation (35, 36). Phenolic acids reduce inflammation by inhibiting inflammatory enzymes COX-2 and inducible nitric oxide synthase (iNOS) (37). Phenolic acids, flavonoids and tannins scavenge the reactive oxygen species (ROS) thus supporting antioxidant-mediated anti-inflammatory activity (38).

Chlorogenic acid has shown the highest binding affinity of -10.2 with visfatin (PDB ID 5LX5). Chlorogenic acid suppressed pro-inflammatory cytokines including TNF-α, IFN-γ and IL-1β (39). It lowers the visfatin in visceral fat through an adiponectin receptor-mediated signalling pathway (40). CGA shows anti-inflammation, antioxidant activities and a hepato-protective effect by suppressing Toll-like receptor 4 signalling (41). Luteolin which has been reported in high concentration in our analysis has shown the highest binding affinity of -9.6 with visfatin (PDB ID 5LX5). Luteolin is reported to show antioxidant and anti-inflammatory properties. The strong antioxidant activity of luteolin is confirmed by reducing the ROS level and blocking the mitochondrial apoptosis pathway induced by ROS (42). Luteolin inhibits LPS induced activation of NF kappa B and AP-1 activity, suppresses the production of pro-inflammatory cytokines and enzymes TNF, IL-6, COX-2 and iNOS (43). Ferulic acid down-regulated adipokines leptin, chemerin and showed significant anti-adipogenic activity (33). Syringic acid scavenges DPPH free radicals effectively and also exhibits anti-inflammatory effects by regulating the NF-κB, iNOS and COX-2 signalling pathways (44, 45). Kaempferol shows potential anti-osteoarthritis (OA) effects, exhibiting the down-regulation of miR-146a and repressing the expression of decorin (46, 47). P-coumaric acid affects the adipokines related to insulin resistance and also plays a key role in stimulating electrical factors of biological membranes (48–50). Hydrogel made with gallic acid and agarose exhibited antibacterial, anti-inflammatory and wound healing activity (51).

The present study focussed on identifying the phenols of MLEN and predicting their anti-arthritic activity computationally. The results gave a positive report on their inhibitory potential against the target adipokines. This provides strong support for further experimental studies with *in vivo* models or *in vitro* cell line studies on anti-arthritic activity of MLEN.

Conclusion

HPLC and NMR analyses of MLEN reported the presence of different phenols like gallic acid, syringic acid, ferulic acid, chlorogenic acid, luteolin, kaempferol, chrysin, p-coumaric acid, benzoic acid, phenyl acetic acid and ellagic acid. The molecular docking studies reported potential anti-arthritic activity of these phytoconstituents particularly chlorogenic acid with a binding affinity of -10.2 kcal/mol, followed by luteolin with -9.2 kcal/mol and ellagic acid with -9 kcal/mol against visfatin (PDB ID 5LX5), a proinflammatory cytokine of arthritis. All the other phenols have also shown the highest binding affinity with visfatin (PDB ID 5LX5) supporting the anti-arthritic activity of bioactive compounds in MLEN. Further *in vivo* and *in vitro* studies are required to confirm the anti-arthritic activity of MLEN.

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Authors' contributions

VSPT contributed to conceptualization, methodology, software, data curation, visualization and original draft preparation. AVA contributed to supervision and resources. Both AVA and VSPT contributed to validation, formal analysis, investigation and review and editing of the manuscript. Both the authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

In silico evaluation of bioactive compounds of methanolic leaf extract of *Nyctanthes arbor-tristis* L. on adipokines was carried out using SEAM DOCK web server.

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