Antioxidant and antibacterial Potential of Phytochemicals extracted from *Ehretia laevis*–an *in vitro* and *in silico* analysis

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Abstract

The present study aims to identify antibacterial and antioxidant phytochemical drug from Ehretia laevis. This study reports detailed phytochemical and biological investigations of newly identified compounds from E.laevis. Soxhlet extraction, qualitative and quantitative phytochemical, antibacterial assay and antioxidant property of crude extract were used as preliminary screening. The secondary screening docking was performed followed by GCMS. Data suggest that the phytochemical analysis reveals presence of phenols, tannins, saponins, glycosides, flavonoids and alkaloids in ethyl acetate extraction. Protein, flavonoids saponins, steroids, terpenoids and Alkaloids in chloroform were identified. High amounts of phenol, flavonoid and alkaloid were recorded in ethylacetate extract than chloroform extract. MIC was best at EA extract recorded between 12.5 to 25 µg. GCMS reveals presence of 20-30 compounds with some novel phytochemicals like amyrin, 6-bromo-5-oxo-3-methylhexan, 1,3-heptadiene, 7-azido-3-(1-ethoxyethoxy)-, (e)-(.+-.)-, 1H-furo[3,4-c]pyrrole-4-carboxylic acid, 6-(2-furanyl)hexahydro-1,3-dioxo-4-phenyl-, methyl ester etc.

In silico docking of amyrin and 1H-furo[3,4-c]pyrrole-4-carboxylic acid reveals that both compounds are capable to form strong hydrogen bonds comparatively equal to standard drug and thus inhibit tRNA synthetases and nitric oxide synthase protein. The newly isolated triterpenoid amyrin and carboxlic acid 1H furan derivative may serve as potential leads for the development of novel anti-iNOS agents and antibacterial compound and have drug likeliness properties.

Keywords: Antibacterial, Antioxidant, SORS, NO synthase, Tyrosyl-tRNA synthetases

Introduction

This herbaceous plant contains a variety of chemicals that have antiviral and antibacterial qualities. They also possess antioxidant qualities which are advantageous for enhancing defenses towards infections caused by viruses. *Ehretia laevis* Roxb. has been used traditionally as a wound treatment herb. Its efficacy has also been investigated on wounds other than long-term varicose ulcers. Plants include a variety of chemical substances that can help to promote wound healing. Though it is also known as Ajan Vruksha, Khandu Chakka is the plant's eponymous[16]. Numerous studies have demonstrated naturally occurring substances in higher plants. Numerous substances derived from plants have been suggested to have antioxidant properties.

Given their shown ability to scavenge reactive oxygen species (ROS), flavonoids and other phenolic compounds derived from plants are considered potential therapeutic therapies for conditions brought on by ROS[9].Its usual applications are reasonably justified by pharmacological research conducted on different sections of plants, crude extracts/fractions and isolated *E. laevis* molecules. Recent research has focused on the investigation of compounds with antibacterial, analgesic, anti-inflammatory, anti-arthritic, antioxidant and wound-healing qualities. Its antimicrobial properties promoted its use as medicine for skin conditions, dental problems, fissure therapy, diarrhea, dysentery, cuts and wound healing on *E. Laevis*[15].

Because of the plant's numerous bioactive metabolites, which have been isolated from its crude extracts, include pentacyclic triterpenoids, phenolics, flavonoids, tannins, fatty acids, vitamins, minerals, amino acids and carbohydrates. It has also been documented in traditional medical systems like Ayurveda and Siddha[1]. A few classes of bioactive phytochemicals have been investigated by the researchers as a result of their quantitative investigations. Nonetheless, phenolic substances and pentacyclic triterpenoids attracted the interest of multiple studies[5].

In order for the newly discovered bioactive metabolites from *E. laevis* to become viable therapeutic options for the treatment of a variety of illnesses, more thorough phytochemical investigations must be conducted to identify and investigate those[10]. Its bark and leaves were extracted and isolated along with its major metabolites, using petroleum ether, chloroform and methanolic extracts.

These include flavonoids, alkaloids, tannins, phenolic components and pentacyclic triterpenoids[18]. The process of protein translation is a biological mechanism that has been

extensively verified as a target for anti-infective drugs in a variety of bacteria.

Targeting protein translation, the majority of antibiotics engage in interactions with microbial ribosomes by directly binding to ribosomal component proteins or rRNA[3]. Synthase does not catalyze. One of the key pathological chains is NO. The arginine binding site of nitric oxide synthase was the target of nitric oxide synthase inhibitors[2]. The goal of the current study was to screen phytochemicals and to conduct docking experiments on the NOS enzyme and tRNA synthetases.

Material and Methods

Collection and preparation of Plant extract: The plant samples were collected from Perundurai, Erode district, Tamil nadu, India. The air dried fine powder was then subjected to extraction with Soxhlet apparatus using ethyl acetate and chloroform. The extracts obtained with final volume after maceration and separation were concentrated under vacuum evaporator and redissolved at 1mg/mL for further *in vitro* studies.

Preliminary Phytochemical Screening: Preliminary phytochemical analysis was performed to screen secondary metabolites present in the extract of *Ehretia laevis* by standard methods. The screening was performed for proteins, carbohydrate, flavonoids, saponins, tannins, terpenoids, steroids, alkaloids and glycosides. The precipitate formation or the color intensity was considered as analytical output for these tests. The secondary phytochemical analysis was performed using a Shimadzu QP 5050A mass spectrometer. . Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Ionization mode was electron Impact ionization and the scanning range was from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec. interval

Total alkaloid Estimation: 10g of plant extract was weighed and diluted in 10 mL of 2N HCL. 5 ml of this solution was added to a separatory funnel and rinsed three times with 10 ml of chloroform. This solution's pH was brought to neutral by adding 0.1 N NaOH. This solution was then mixed with 5 ml of BCG solution and 5 ml of phosphate buffer. The mixture was vigorously agitated to extract the complex, which was then collected in a 10 ml volumetric flask and diluted with 5 ml chloroform. Atropine standard solution was transferred to different separatory funnels and was extracted with 5 ml of chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer.

Total flavonoid estimation: Separately, 1.5 mL of 2% aluminium chloride and 0.5 mL of diluted standard quercetin solutions were combined. The mixture was then left at room temperature for 60 minutes. Using a Varian UV-Vis spectrophotometer, the absorbance of the reaction mixtures was measured against a blank at 420 nm. Using quercetin as the standardization substance, the calibration plot was used to determine the concentration of total flavonoid content in the test samples. The stock quercetin solution was made by dissolving 1 mg of quercetin in 1 mL of methanol and the standard quercetin solutions were made by serially diluting the stock solution with methanol (10–200 g/mL).

Total Phenol estimation: Folin-Ciocalteu reagent was diluted 1:10 to evaluate the total phenolic content of the plant extracts. Gallic acid calibration curve was plotted using the standard as a guide. 2000 mL of Folin-Ciocalteu reagent (1 N) were combined with 0.5 mL of the extract solution (1000 g/mL). The reaction mixture was allowed to stand at room temperature for 30 min with occasional shaking for colour development after 3 min at a standstill. 2 mL of 2% sodium carbonate was added. A UV-vis spectrophotometer was then used to measure absorbance at 750 nm. The results were given as mg of gallic acid equivalent (mg GAE/g) per g of extract. Gallic acid at a concentration of 0–100 g was used to create a calibration curve.

Determination of Antibacterial activity of *Ehretia laevis* leaf extract: The antibacterial efficacy of the *E. laevis* leaf extract was assessed against several bacterial strains including *Bacillus subtilis*, *Klebsiella pneumonia Pseudomonas aeruginosa* and *Staphylococcus aureus*. This assessment was carried out utilizing the well-diffusion method using Muller-Hinton agar plates. Initially, these plates were inoculated with respective selected bacterial strains using sterile swabs and wells were made carefully with sterile cork-borer without further contaminations (by proper sterilization) in the subsequent plates. Gel puncture was employed to create wells in each plate into which antibiotic discs and varying concentrations (20, 40 and 60 mg/ml) of EAEL were introduced. Following this, the plates were kept for incubation at 35°C for 24 h and the observed inhibitory zones were measured (in mm).

Minimum inhibitory concentration: The MIC of these extracts was determined by broth dilution technique in the range of 100, 50, 25, 12.5, 6.25 µg/ml. Each dilution was seeded with bacterial suspension (1×10^6cfu/ml) and incubated for 24 h at 37°. After incubation, the growth of the bacterial isolates in the test tubes was observed as turbidity viability test with resazurin oxidation.

Antioxidant activity

DPPH radical scavenging activity: DPPH was dissolved in 0.1 mM methanol and then 1 mL of the solution was mixed with 1 mL of the EAEL and CEEL respectively in methanol solution at various concentrations. Following a 30 min of incubation, the absorbance of the samples was recorded in a spectrophotometer at a wavelength of 517 nm. Blank was considered as without adding extract.

The various concentrations of quercetin were considered as the standard references. The absorbance was found to be lower than that of the reaction which indicates a higher level

of DPPH radical scavenging activity. The ability to scavenge the DPPH radical was calculated using the following formula:

DPPH radical scavenging activity (%) = [(Control – Sample) / Control] x 100

Superoxide radical scavenging (SORS) activity: The assay mixture comprised of a sample mixed with 0.1 mL of nitro blue tetrazolium (1.5 mM NBT solution), 0.2 mL of EDTA (0.1 M EDTA), 0.05 mL of riboflavin (0.12 mM) and 2.5 mL of phosphate buffer (0.067 M phosphate buffer). The control group did not contain the sample but included DMSO. The SORS activity was exposed to light for 30 minutes and then measured spectrophotometrically at 560 nm. Ascorbic acid served as the standard reference for evaluating SORS activity. The percentage of inhibition in SORS activity was determined using the following formula:

Superoxide radical scavenging activity (%) = [(Control – Sample) / Control] x 100

Hydroxyl radical scavenging (HRS) activity: Different concentrations of extracts were placed in separate test tubes and evaporated until dry. Next, each tube received 1 mL of an iron-EDTA solution consisting of 0.13% ferrous ammonium sulfate and 0.26% EDTA along with 0.5 mL of EDTA (0.018%) and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid and the test tubes were incubated in a water bath at a temperature ranging from 80°C to 90°C for 15 minutes. To stop the reaction, 1 mL of ice-cold TCA (17.5% w/v) solution was introduced. Afterward, 3 mL of Nash reagent was added to all the tubes.

Nash reagent was prepared by combining 75 mL of ammonium acetate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone and then adjusting the total volume to 1 L with distilled water. The tubes were left at room temperature for 15 minutes to facilitate color development. Quercetin served as the standard reference for assessing HRS activity. Following the reaction, a yellow color developed and was subsequently measured using spectrophotometry at 412 nm:

OH radical scavenging activity (%) = [(Control – Sample) / Control] x 100

Hydrogen peroxide scavenging activity: A hydrogen peroxide solution was prepared at a concentration of 40 mM in a phosphate buffer with a pH of 7.4. Subsequently, an extract with varying concentrations (1 mL) was introduced along with 0.6 mL of the H_2O_2. The total volume was adjusted to 3 mL using phosphate buffer. A blank solution was prepared by using a phosphate buffer without the addition of H_2O_2.

Then incubate the samples at room temperature for 10 min and spectrophotometrically read at 230 nm. Ascorbic acid with various concentrations was used as the standard reference. The hydrogen peroxide scavenging activity of percentage was calculated as follows:

H_2O_2 scavenging activity (%) = [(Control – Sample) / Control] x 100

Preparation of Receptor Proteins and docking: The Protein Data Bank (https://www.rcsb.org/) provided three-dimensional (3D) structures of the tyrosyl-tRNA synthetase (PDB 1JII) and NO synthase PDB 6NGJ in the.pdb format for docking. The receptor proteins' binding pockets were calculated using the Molecular Computing Environment program. The water atoms were removed, hydrogen atoms were added, any previously bound ligands were removed (if any), the protonation procedure was carried out and energy minimization was done in order to further adapt the receptor proteins for molecular docking.

The molecular docking of ligands to the active amino acids of the binding pocket of the receptor proteins was done using the Auto dock software. The relationships between the receptor proteins and important active substances were visualized using the UCSF chimera1.1 program. Drug like ADME properties of ligand was done in SWIS ADME forum.

Statistical Analysis: All the data were considered for the statistical analysis and their mean and standard error of mean were presented accordingly for the antibacterial, antioxidant and anticancer activity. The histogram and charts were made using Prism software.

Results and Discussion

The preliminary qualitative phytochemical analysis of *Ehretia laevis* extracted with ethylacetate and chloroform reveals presence of various phytochemicals. Both extract showed 60% positive results. Out of 10, alkaloid, phenols, tannins, flavonoids, saponins and glycosides were identified from ethyl acetate extract whereas alkaloids, flavonoids, saponins, protein, steroids and terpenoids were identified from chloroform extract. Glycosides, tannin and phenols were present only in ethyl acetate extract. Likewise protein, steroids and terpenoids were only found in chloroform extract (Table 1). The data correlates with the findings[13]. The quantitative phenolic estimation of plant *Ehretia laevis* among ethyl acetate was 286 mg/g and 154 mg/g of chloroform extract.

Total flavonoid content was found to be 340mg in ethyl acetate extraction and 210 mg/g in chloroform extract. Concentration of alkaloid in EA extract was 232 mg/g where as in chloroform extraction, it was calculated as 184mg/g. The quantitative assay of extraction was given in figure 1. GC-MS analysis was used to identify the phytochemical components found in the ethyl acetate and chloroform extract of the leaves of the plant *Ehretia laevis* (Figures 2 and 3). Tables 2 and 3 represent the list of active chemical

components together with their retention time (RT), molecular formula and molecular weight (MW). In ethyl acetate extract, thirty phytochemical components corresponded to 26 compounds. The maximum was 55.97 % of tetrapentacontane (RT30.948 min) followed by 4, 4, 6a, 6b, 8a, 11, 12, 14b-octamethyl-1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a,14b-octadecahydro-2h-picen-3-one(13.41%; RT 33.268min) was identified. The spectrum also reveals presence of novel compounds alpha and betaAmyrin, Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl, 6-bromo-5-oxo-3-methylhexan-1,3-diol, 3-heptadiene7-azido-3-(1-ethoxyethoxy). Chloroform extract spectrum reveals 20 peaks corresponding to 19 compounds.1,4-epoxynaphthalene-1(2h)-methanol, 4,5,7-tris(1,1-dimethyle thyl)-3,4-dihydro is the major compound (46.96 %) eluted at 39.10min followed by 15% of 1-bromo-8-methylhexacosane at 35.8min.1-bromo-8-methylhexa cosane, n-heptylhexanamide, 1h-furo[3,4-c]pyrrole-4-carboxylic acid, 14,19-dioxoundecacyclo. 1,4-diphosphorin are novel compounds detected from the peaks. The presence of cyclohexasiloxane, benzoic acid, 4-methyl-ester, carboxylic acid derivatives was also reported earlier[14].

Antibacterial activity: Antibacterial activities of the ethyl acetate extracts of *Ehretia laevis* (EAEL) at different concentrations (20, 40 and 60 µg/mL) inhibited the growth of all bacterial strains. The zone of inhibition was given in table 4 showing noteworthy antibacterial action against *P. aeruginosa* (24 mm) followed by 22 mm against two Gram positive *Bacillus* sp and *S.aureus* at 60µg. Moderate antibacterial effect was recorded against *E.coli* and *K.penumoniae.* The activity at 20µg was less significant (<15mm) mm and moderate inhibition was recorded at 40µg (>15 to <20mm). Chloroform extract antibacterial activity among test pathogens is given in table 5. At concentration 20 and 40µg, a moderate antibacterial effect was noted (>15 to <20mm) except *K.penumoniae*.

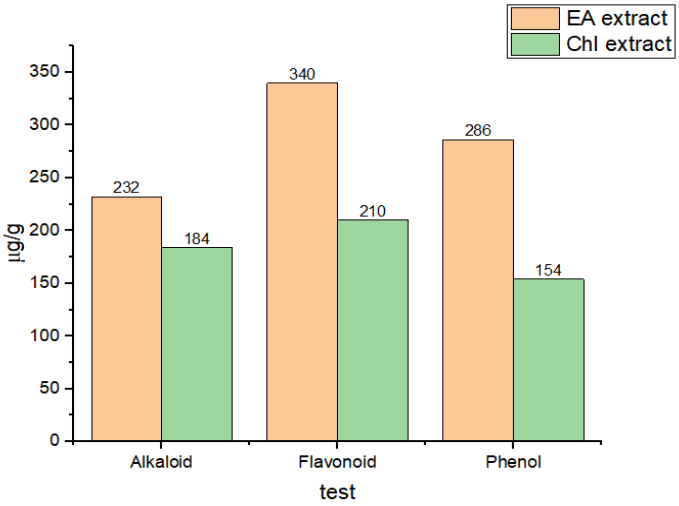

Figure 1: Quantitative phytochemical estimation

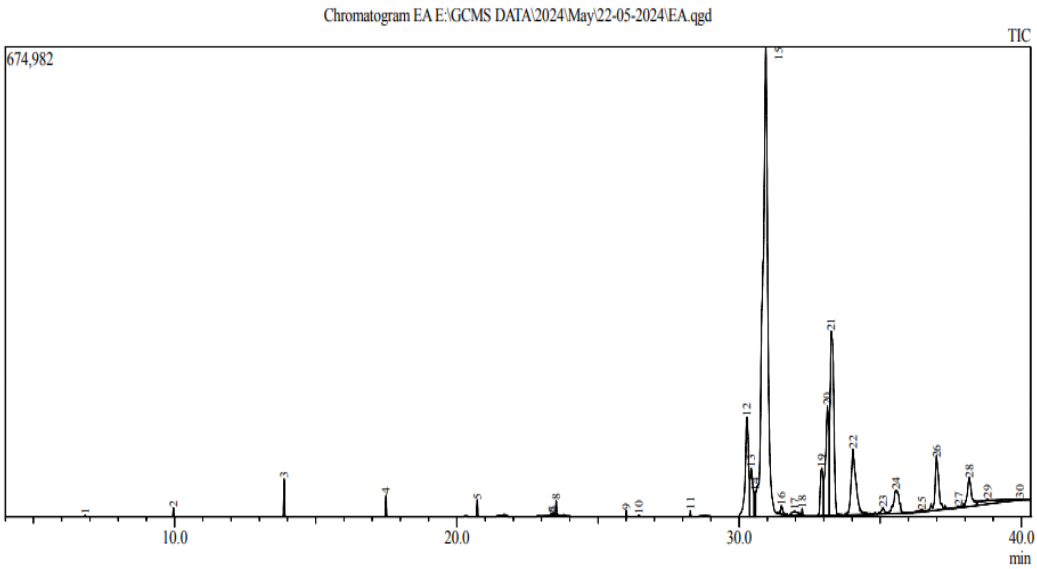

Figure 2: GCMS of crude extracted with ethyl acetate

Table 1
Phytochemical Screening Quantitative Analysis of Crude extracts of *Ehretia laevis*

S.N.	Phytochemicals	Ethyl Acetate	Chloroform
1	Protein	-	+
2	Carbohydrate	-	-
3	Phenols	+	-
4	Tannis	+	-
5	Flavonoids	+	+
6	Saponins	+	+
7	Glycosides	+	-
8	Steroids	-	+
9	Terpenoids	-	+
10	Alkaloids	+	+

Table 2
NIST matched compound extracted with ethyl acetate

Peak	Retention time	Area %	Name
1	6.833	0.02	2-keto-butyric-acid
2	9.961	0.13	benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester
3	13.882	0.58	Cyclohexasiloxane, dodecamethyl-
4	17.49	0.32	Cycloheptasiloxane, tetradecamethyl-
5	20.724	0.25	benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester
6	23.375	0.06	2(3h)-naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-, [4r-(4.alpha.,4a.alpha.,6.beta.)]-
7	23.41	0.08	6-(1,3-Dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-ene
8	23.519	0.21	Cyclohexasiloxane, dodecamethyl-
9	26.004	0.08	silicate anion tetramer
10	26.449	0.04	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-
11	28.273	0.07	benzoic acid, 2,6-bis(trimethylsiloxy)-, trimethylsilyl ester
12	30.273	6.63	Tetrapentacontane
13	30.429	2.69	Tetrapentacontane
14	30.565	0.63	3-(t-Octylamino)propionitrile
15	30.948	46.65	Tetrapentacontane
16	31.496	0.37	beta.-Amyrin
17	31.973	0.56	thiophene, 2-(4-methoxy-3,5-dimethylphenyl)-
18	32.236	0.12	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-icosamethylcyclodecasiloxane #
19	32.929	2.59	4, 4,6a,6b,8a,11,12,14b-octamethyl 1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2h-picen-3-one
20	33.132	7.01	4,4,6a,6b,8a,11,12,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2h-picen-3-one
21	33.268	13.41	4,4,6a,6b,8a,11,12,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2h-picen-3-one
22	34.035	6.15	methyl commate a
23	35.105	0.44	1,7-dioxaspiro[5.5]undecane-4,5-diol, 2-ethyl-3-methyl-10-(phenylmethoxy)-8-[(phenylmethoxy)methyl]-, 5-acetate, [2r-[2.alpha.,
24	35.566	2.62	alpha.-Amyrin
25	36.48	0.07	butane, 2,2-dimethyl-
26	37.006	3.89	Dotriacontane, 1-iodo-
27	37.78	0.05	6-bromo-5-oxo-3-methylhexan-1,3-diol
28	38.161	2.48	Octacosane, 1-iodo-
29	38.805	1.29	tri-o-trimethylsilyl, n-pentafluoropropionyl derivative of terbutaline
30	39.958	0.52	1,3-heptadiene, 7-azido-3-(1-ethoxyethoxy)-, (e)-(.+-.)-

Table 3
NIST matched compound extracted with chloroform

Peak	Retention time	Area %	Name
1	9.959	2.83	benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester
2	13.88	9.36	cyclohexasiloxane, dodecamethyl-
3	17.487	3.72	3-butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
4	20.723	1.98	benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester
5	23.517	1.1	phosphonous dibromide, [2,2,2-trifluoro-1-(trifluoromethyl)-1-[(trimethylsilyl)oxy]ethyl]-
6	26	0.52	1,3-diphenyl-1-((trimethylsilyl)oxy)-1(z)-heptene
7	35.7	1.84	n-heptylhexanamide
8	35.852	15.3	1-bromo-8-methylhexacosane
9	36.385	2.68	cyclobutanecarboxylic acid, 2-adamantyl ester
10	36.477	7.04	1.beta.-carboxymethyl-6.beta.-hydroxy-2-(2'.alpha.-hydroxy-2'.beta.-methyl-5'.alpha.-isopropylcyclopentyl)-1.alpha.,4-dimethylc
11	36.53	0.5	1-oxaspiro[3.5]non-5-ene, 6-(3,3-dimethyl-1-butynyl)-2,2,3,3-tetramethoxy-
12	37.46	0.46	1-methyl-2-carboxaldehyde-3(1-carboxaldehyde-ethyl)cyclopentanec
13	38.215	0.38	4-methyl-4-nitrosopentanenitrile
14	38.293	0.57	methyl 3-methyl-5-oxy-2-phenoxyhexanedithioate
15	38.41	0.63	1h-furo[3,4-c]pyrrole-4-carboxylic acid, 6-(2-furanyl)hexahydro-1,3-dioxo-4-phenyl-, methyl ester, (3a.alpha.,4.beta.,6.beta.,6
16	38.455	1.37	14,19-dioxoundecacyclo[9.9.0.0(1,5).0(2,12).0(2,18).0.(3,7).0(6,10).0(8,12).0(11,15).0(13,17).0(16,20)]i cosane-4-syn,9-syn-dica
17	38.575	0.92	1,4-diphosphorin, 1,4-dihydro-1-phenyl-4-(phenylmethyl)-, cis-
18	38.625	0.3	7-7-methoxyphenyl)-1-aza-3,8-dioxa-4-tricyclo[5.3.0.0(2,6)]decen-10-one
19	39.104	46.96	1,4-epoxynaphthalene-1(2h)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro-
20	39.245	1.55	2h-oxocin, silane deriv.

Table 4
Antibacterial activity of ethyl acetate extract of *Ehretia laevis*

S.N.	Test pathogen	20µg/ml	40µg/ml	60µg/ml	NC
1.	*S.aureus*	16.5±0.7	20.25±0.31	22.25±0.26	2.25±0.07
2.	*B.cereus*	11.25±0.27	16.25±0.52	22.25±0.38	1.25±0.07
3.	*P.aeruginosa*	14.25±0.37	19.25±0.35	24.25±0.12	2.5±0.35
4.	*E.coli*	12.25±0.42	17.25±0.37	21.25±0.17	2.5±0.24
5.	*K.penumoniae*	13.25±0.18	16.25±0.35	19.25±0.31	2.5±0.24

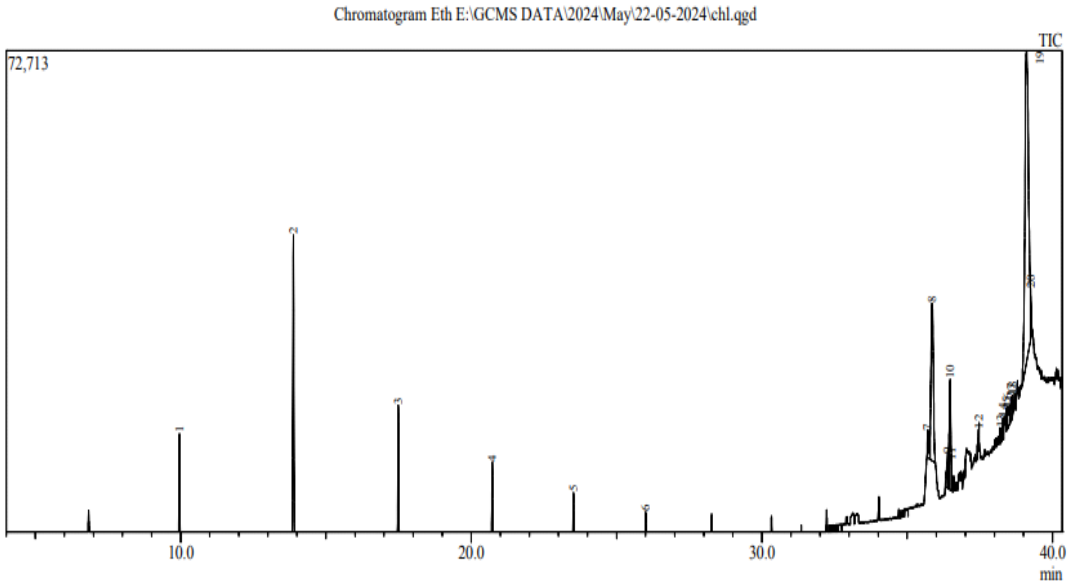

Figure 3: GCMS of crude extracted with chloroform

Table 5
Antibacterial activity of chloroform extract of *Ehretia laevis*

S.N.	Test pathogen	20µg/ml	40µg/ml	60µg/ml	NC
1.	*S.aureus*	18.25±0.7	21.5±0.31	23.5±0.5	5.25±0.29
2.	*B.cereus*	16.5±0.62	19.5±0.14	20.5±0.42	5.25±0.7
3.	*P.aeruginosa*	15.25±0.3	17.25±0.17	21.5±0.48	6.5±0.35
4.	*E.coli*	16.25±0.16	18.25±0.35	20.5±0.16	6.5±0.6
5.	*K.penumoniae*	12.25±0.28	15.5±0.3	19.25±0.23	6.5±0.5

The results disclose that all the extracts have significant antibacterial action against Gram-negative and Gram-positive bacteria, establishing a broad spectrum of activity. At 60 µg/ml, maximum zone of inhibition was recorded as 23.5±0.5mm on *S.aureus* and minimum as 19.25±0.23 mm against *K.penumoniae.* The experiment confirms that both the extracts have significant antibacterial action against Gram-negative and Gram-positive bacteria, establishing a broad spectrum of activities by concentration dependent manner. Figure 4 represents the minimum inhibitory concentration (MIC) of extract against test pathogens. It was found that the EA extract has 12.5 to 25 µg of MIC and chloroform extract has 25-50µg/mL.

The MIC of EA extract was 12.5, 12.5,12.5,25 and 25 respectively on *S.aureus, E.coli, P.aeruginosa, B.cereus and K.penumoniae* whereas for chloroform extracted compound, it was recorded as 50,25,25,50,50µg/mL. The MIC values shows that ethyl acetate extracted compound was found to be superior to chloroform extract. Antimicrobial activity of extracts of *E. laevis* leaves has been evaluated and reported earlier[12]. Similar study confirmed the antimicrobial action of methanolic extract of *E. laevis* against microflora using agar well diffusion method at 400 -800 µg/mL[4]. Activity of *E. laevis* against *Pseudomonas aeruginosa and Staphylococcus aureus* was reported[6]. Antimicrobial activity and minimum inhibitory concentration of *E. laevis* leaves extracts against different microorganisms were reported[11].

Antioxidant activity of extract: Two successive extracts of the *Ehretia laevis* were subjected to DPPH, superoxide, hydroxyl and hydrogen peroxide scavenging activity at 10-100µg/mL and the data is compared in figure 5. It was noted that the antioxidant activity was increased by increasing the concentration and reached higher activity at 100µg. The DPPH scavenging potential of samples compared with quercetin standard denotes that the percentages of free radical scavenging were 59.16±0.9, 66.09±1.5 and 96.6±0.8 among EAEL, CEL and standard (Fig. 5a). Similarly, the SORS activity among EAEL, CEL and ascorbic acid varied among concentration between 10 to 100 and reached maximum activity at 100 µg recorded as 66.6±1.0, 69.1±1.4 and 92.0±.6 % (Fig. 5b.).

Study on hydroxyl radical scavenging activity at 100 µg concentration was 65.93333±0.4, 59.03±0.3 and 96.55667±0.8 percentage and 17.4±0.6, 22.48±1.08 and 22.45±0.92 percentage at 10 µg respectively on EAEL, CEL and ascorbic acid (Fig. 5c). The possible DPPH radical scavenging antioxidant activity of the methanol extract *E.laevis* was already reported[8] along with the nitric oxide scavenging activity of *Ehretia laevis* [14].

Hydrogen peroxide scavenging activity among samples and standard represented on figure 5d denotes increasing the radical inhibition toward concentration found to be maximum at high concentration. At 10 µg, the activity was recorded as 17.61667±0.7, 24.53333±0.9 and 27.63667±0.9 % whereas at 100µg, 66.49333±0.8, 58.22333±0.6 and 91.17667±0.7% are for EAEL, CEL and standard. Comparing to plant samples, standard sowed more than 50% inhibition at 60µg level where as extract required 70-80µg. The IC50 value of scavenging activity towards samples and standard reveals that EACE extract was found to be better than other sample and standard. Polyphenolic chemicals in the extraction could be responsible for the hydroxyl radicals' ability to scavenge since they can donate electrons to H_2O_2, neutralizing it to water[19].

The data (Fig. 6) represent that the EAEL has IC50 of 64.6, 49.6, 300.2 and 171.2µg/mL and 293.0,156.9, 369.6 and 338.0 µg/mL for chloroform extract. The IC50 values of standard were 85, 156.9,369.6, 338.0 µg/mL respectively for DPPH, SORS, hydroxyl scavenging H_2O_2 scavenging. There is scientific evidence to support the use of *Ehretia laevis* in the treatment of a variety of diseases because the degree of inhibition of the NO free radicals was found to increase with increasing levels of the extracts[17].

Molecular docking antibacterial: Due to their necessity as vital enzymes for all living things, tyrosyl-tRNA synthetase (TyrRSs) makes excellent therapeutic target docked with alpha amyrin and mupirocin. The formation of hydrogen bond, ligand and receptor atoms and affinity of binding docking score were predicted. Mupirocin showed formation of 7 hydrogen bond in cavity C1 with ASP195, Gly193, Cys37, Gly38, His50, Thr75 and the docking score was -7.6 kcal/mol.

In addition, Asp, Al, Pro took part in hydrophobic interaction. The O1 atom of ligand and OD1/OD2, N0, SG, O0, NE2, OG1 atom of above amino acids interacted and formed hydrogen bond (table 6). The binding interaction between mupirocin and receptor was given in figure 7a.

The binding energies calculated by molecular docking between amyrin and TyrRSs were -9, -8.4 and -6.5 kcal/mol respectively at cavity of C1, C2 and C4 pockets. Leu128,

GLy38, Asn109 and Lys105 amino acids interacted with amyrin and formed hydrogen bonds (Table 7). In addition, the ligand also showed pi pi interaction, weak hydrogen bond and hydrophobic interaction with amino acids present in cavity. Figure 8 a–c illustrated the docked conformations of α-amyrin in the binding site of C1, C2, C4 cavities of tRNA synthetases enzyme with 6 hydrogen bonds, 15 hydrophobic interactions, 1 pi pi interaction and 3 weak hydrogen bond formations. Table 8 denotes the binding affinity data of 1H-Furo[3,4-c]pyrrole-4-carboxylic acid (1HFC) with TyrRSs.

Figure 9 represents two pocket C1 and CE residues interaction with ligand and formation of hydrogen bond. C1 nitrogen atom of ASP 40 and LYS84 interacted with oxygen atom of ligand and the binding affinity was -6.9kcal/mol. C3 cavity oxygen atom of Leu128 interacted with nitrogen atom of ligand and the docking score was -5.6kcal/mol. pi pi interaction with His, hydrophobic contact among phe, Ala His, Ile and Leu and weak hydrogen bonding in Ile and Gly were other interactions found in 1HFC. tRNA synthetases have been identified as potential targets for compounds being researched as antibacterial and antiparasitic medicines[8]. No scientific data among the selected ligands was found on the database over tyrosyl-tRNA synthetases inhibition.

Molecular docking antioxidant: Ascorbic acid inhibitor selected as standard for docking and the interaction is represented in figure 10. The binding affinity and amino acid, atoms of ligand were given in table 9. The docking score for large cavity 1 is -6.0 kcal/mol and 4 hydrogen bonded residues were ARG414, Asp675, Ser413 and Asp697 along with a weak H bond Thr. The docking score of small cavity C4 was- 4.9 kcal/mol and 8 hydrogen bonded 7 amino acid residues showed hydrogen bonding: Lys319, Arg669, bLys319, His407, Tyr403, Gln364, Ser367 along with one hydrophobic contact with Ser. The integration of molecular docking simulations has unveiled the potential of ascorbic acid as highly promising therapeutic agents for addressing oxidative stress[7]:

Table 6
Binding affinity of mupirocin with Tyrosyl-tRNA synthetase PDB 1JII

Cur Pocket ID	Vina score	Cavity volume (Å^3)	Ligand	Amino acid	Acceptor	Other interaction
C1	-7.6	1294	O1	ASP195 Gly193 Cys37 Gly38 His50 Thr75	OD1/OD2 N0 SG O0 NE2 OG1	HydrophobicASP C1(2), Ala C1,Pro C1

Table 7
Binding affinity of alpha Amyrin with Tyrosyl-tRNA synthetase PDB 1JII

Cur Pocket ID	Vina score	Cavity volume (Å^3)	Ligand	Amino acid	Acceptor	Other interaction
C1	-9	222	O1	Leu128	O	Pi pi: His-C11 HPB: Thr-C12, Tyr-C23, Leu-C26,Leu-C8, Phe-C16,Leu-C9
C2	-8.4	1294	O1	Leu 128 , GLy38, Glu251	O/0	WHB Ile-C11 Gly-O1 HPB:Ala –C27, Leu-C30, Val-C26, Asp-C21 Phe-C12
C4	-6.5	191	O1	Asn109 Lys105,	O	Hpb:Lys -C27, Ala -C21 Asp- C30, Ala -C16 WHB:Asn-C19

Table 8
Binding affinity of 1H-Furo[3,4-c]pyrrole-4-carboxylic acid, 6-(2-furanyl)hexahydro-1,3-DIOXO-4-phenyl-, methyl ester, (3A.alpha.,4.beta.,6.beta.,6 with Tyrosyl-tRNA synthetasePDB 1JII

Cur Pocket ID	Vina score	Cavity volume (Å^3)	Ligand	Amino acid	ACCEPTOR	Other interaction
C1	-6.9	1294	O1 O2	ASP 40 LYS84	N0 NZ	Pi pi: His-C11 HPB: phe, Ala HIS-C12
C3	-5.6	222	N1	Leu128	O	WHB Ile-C11 Gly-O1 HPB:Ile-C3, Leu-C7, Leu-C13 Phe-C12

Table 9
Binding affinity of ascorbic acid with NO synthase PDB 6NGJ

Cur Pocket ID	Vina score	Cavity volume (Å³)	Ligand	Amino acid	ACCEPTOR	Other interaction
C1	-6.0	17897	O1	ARG414 Asp675 Ser413 Asp697	NH2 OD2 OG OD2	Weak H bond Thr O1
C4	-4.9	348	O1	Lys319 Arg669 Lys319 His407 Tyr403 Gln364 Ser367	Nz0 NH2/NE NZ NE OH O0 OG	Weak H ondSer O1

Table 10
Binding affinity of alpha amyrin with PDB 6NGJ NO synthase

Cur Pocket ID	Vina score	Cavity volume (Å³)	Ligand	Amino acid	ACCEPTOR	Other interaction
C2	-8.7	560	O1	Trp678	O/0	HPB: TRP C13,C20,Glu C27, Ala C25
C1	-8.9	17897	O1	Trp678	O/0	GluC9, Arg C21,C28, Trp C13,C20,Ala C25

Table 11
Binding affinity of 1H-furo[3,4-C]pyrrole-4-carboxylic acid and NO synthase

Cur Pocket ID	Vina score	Cavity volume (Å³)	Ligand	Amino acid	ACCEPTOR	Other interaction
C1	-8.0	4947	O2/O1/N1 O6	Ser413 Asn697	N0/OG ND	Weak H bond: Ala O2,
C3	-5.7	359	O2 O3/O4 O6	Lys319 Tyr403 Arg410	NZ OH NH2	HPB Arg C7

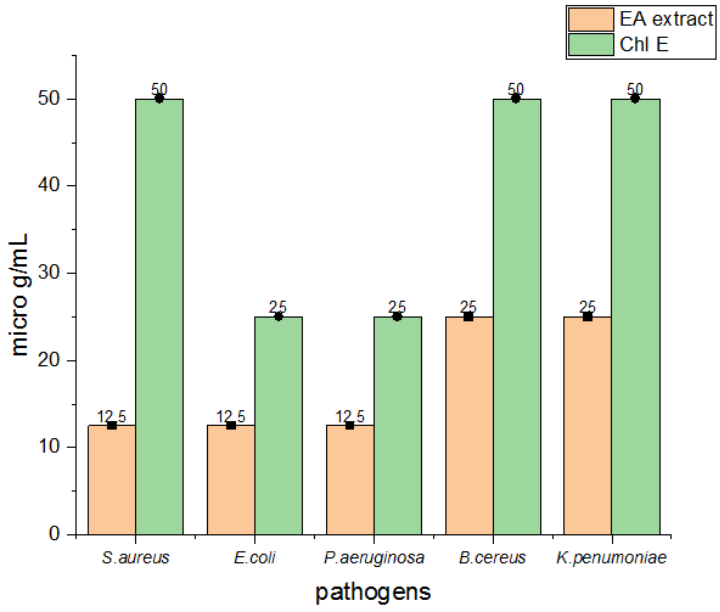

Fig. 4: Minimum inhibitory concentration of extract against test pathogens

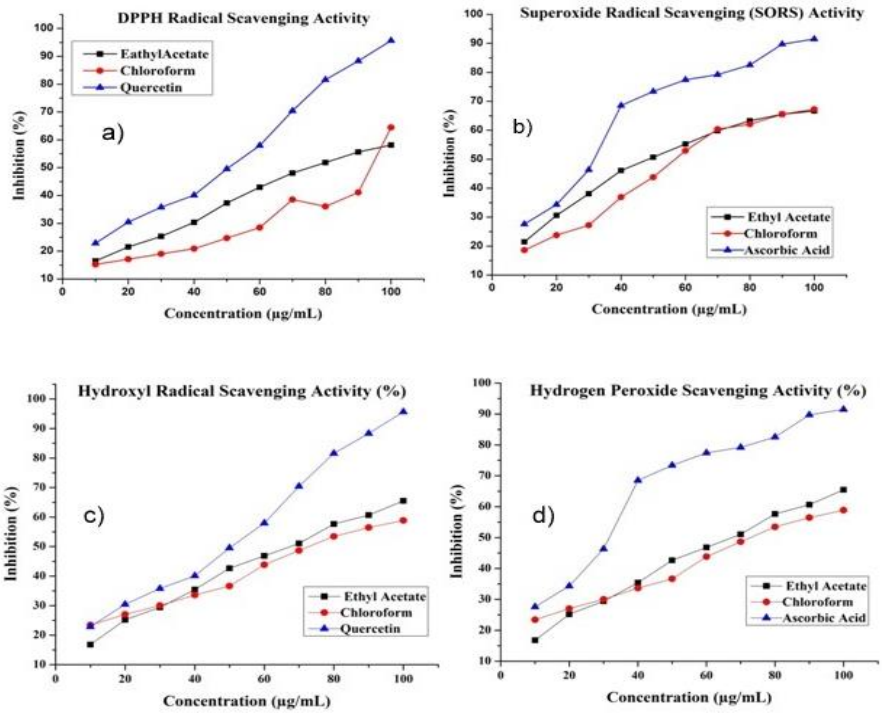

Figure 5: Free radical scavenging activity of crude extract of *E laevis*

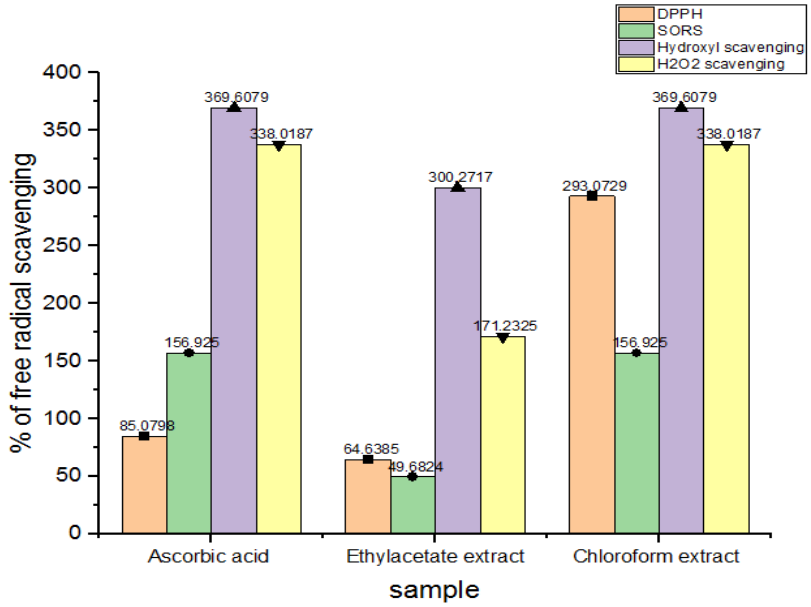

Figure 6: Half maximal inhibitory concentration (IC50) of extract

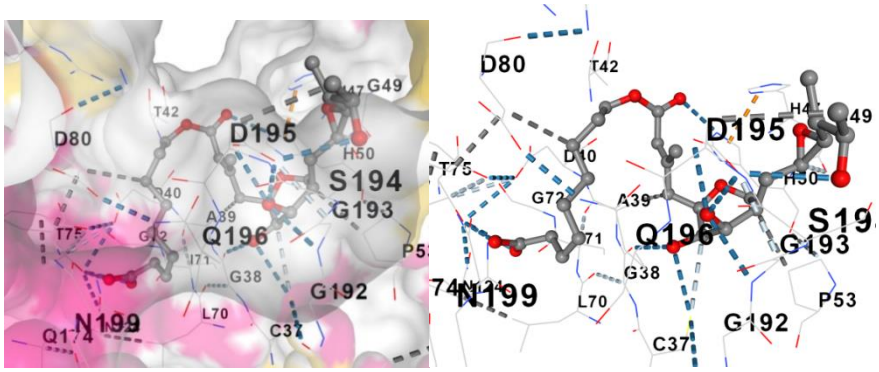

Figure 7: Interaction of C1 cavity TyrRSs and mupirocin

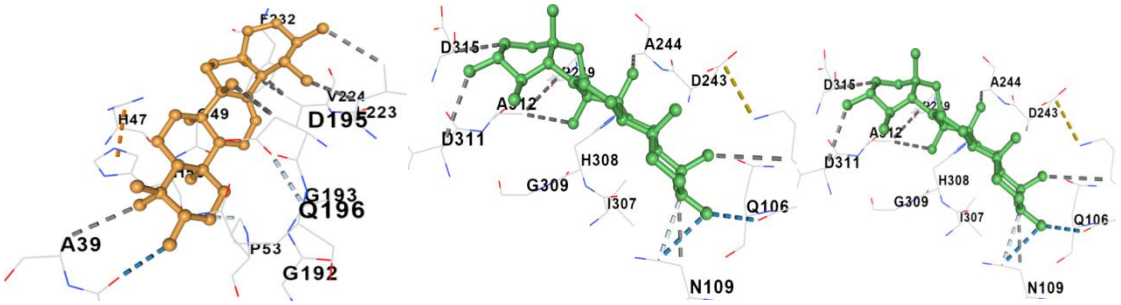

Figure 8: Interaction of TyrRSs(C1/C2/C4) and alpha amyrin

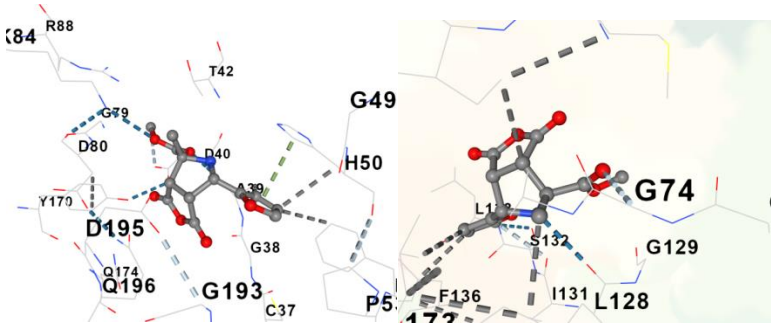

Figure 9: Interaction of TyrRSs(C1/C3) with1h-furo[3,4-c]pyrrole-4-carboxylic acid

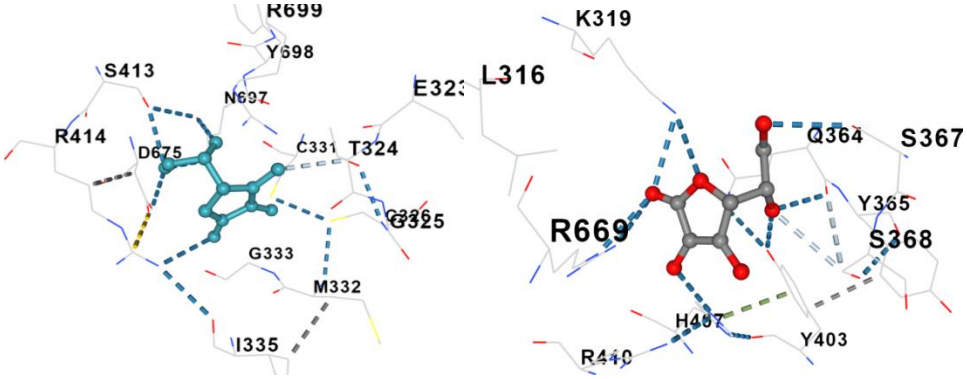

Figure 10: Interaction of C1/C4 cavity NO synthase and L Ascorbic acid

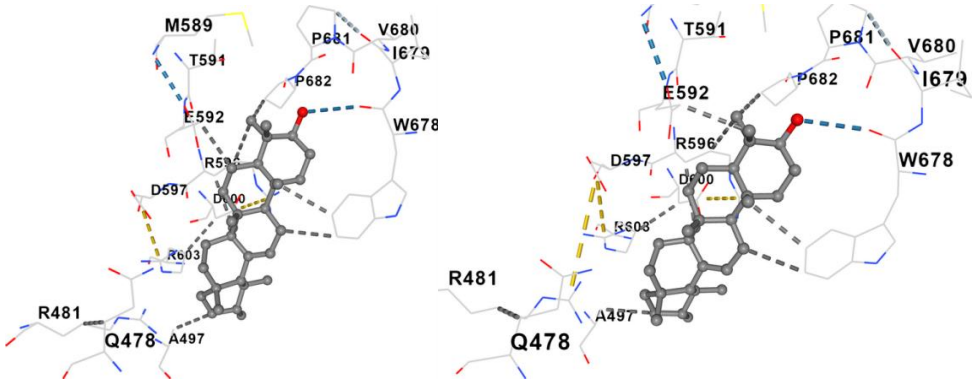

Figure 11: Interaction of C1/C4 cavity NO synthase and amyrin

A strong binding affinity of alpha amyrin with NO synthase was noted mainly by interaction with Trp aminoacid (table 9). Docking score of cavity C2 was -8.7 kcal/mol by forming hydrogen bond between oxygen atom of Trp and ligand amyrin along with hydrophobic contact respectively with Trp, Glu, Ala. At cavity C1, the docking score was -8.9 kcal/mol by forming hydrogen bond between oxygen atom of Trp and ligand along with hydrophobic contact on Glu, Arg,Trp,Ala (Fig. 11). Molecular docking studies revealed the highest binding affinity (− 8.4 Kcal/mol) of α-amyrin for determination of antioxidant and anticancer activity of amyrin[20]. The compounds of 1H-Furo[3,4-c]pyrrole-4-carboxylic acid were subjected to docking using NOS and the data as given in table 11. Two cavities showed strong interaction and formation of stable hydrogen bonds (Fig. 12a).

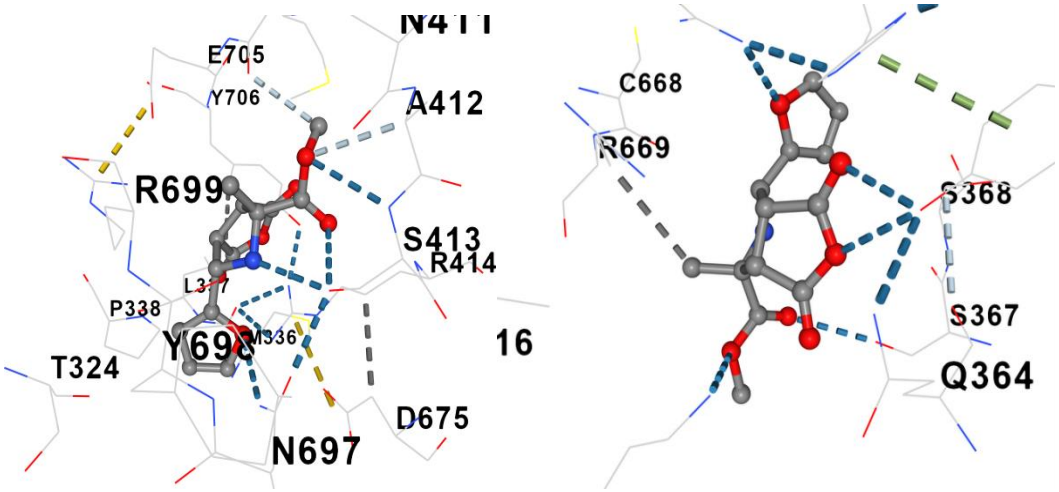

Figure 12: Interaction of C1/C3 cavity NO synthase with1H-furo [3, 4-c] pyrrole-4-carboxylic acid

Large cavity C1 showed interaction of ligand atom O2/O1N11 with Ser413 and O6 atom with Asn697. Total 4 hydrogen bonds, one weak hydrogen bond with Ala were identified. The docking score was calculated as -8.0kcal/mol. Cavity 3 size 359A showed hydrogen bonding among Lys319-O2, Tyr403 with O3/O4 and Arg410 –O6 interaction (Fig. 12b) with hydrophobic contact on Arg. The docking score was predicted as -5.7 kcal/mol. Previous literature survey confirms that there was no report on 1H-Furo[3,4-c]pyrrole-4-carboxylic acid derivatives. The drug-likeness test in this study provides information regarding the potential of the drug that could be used orally following the Lipinsky rule. Drug likeness of Mupirocin shows water soluble class, low GI absorption, BBB permeant. P-gp substrate therefore reduces drug absorption and has 1 violation on Lipinski: (MW>500,). Similarly alpha amyrin molecular weight is 426.72 g/mol. P-gp substrate may increase drug absorption and: was found violation on MLOGP>4.15. 1H-Furo[3,4-c]pyrrole-4-carboxylic acid, 6-(2-furanyl)hexahydro-4-methyl-1,3-dioxo-, methyl ester, have Molecular weight 279.25 g/mol, Num. H-bond acceptors 7, Num. H-bond donors1, High, GI absorption, neither act as P-gp substrate or CYP1A2 inhibitor and 0 violation on Lipinski rule.L-ascorbic acid is the L-enantiomer of ascorbic acid.

ADME reveals molecular weight 176.12 g/mol H-bond acceptors 6, Num. H-bond donors 4, soluble, High GI absorption, do not act as P-gp substrate and inhibit CYP1A2 inhibitor and thus follows Lipinsk rule with zero violation.

Conclusion

According to the current investigation, the ethyl acetate and chloroform extract of *Ehretia laevis* have a high polyphenolic content, novel phytochemical amyrin and 1H-Furo [3,4-c]pyrrole-4-carboxylic acid found to be potent protein synthesis and NO synthase inhibitor. The extract has significant antibacterial and anti-oxidant properties when compared to the standard. The findings confirm the plant *Ehretia laevis's* potential as a natural antioxidant and antibacterial source.

Acknowledgement

The authors are thankful to the management of Nandha Arts and Science College for extending the facilities and necessary support.

Reference

1. Dhenge S. and Khandare K., Efficacy of local application of Khanduchakka (*Ehretia laevis* roxb) Ghrita in Dushtavrana—A case report, *Int. J. Ayurveda Integr. Med*., **7**, 3726–3731 **(2016)**

2. García-Mediavilla V. et al, The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells, *Eur. J. Pharmacol*., **557**, 221-229 **(2007)**

3. Ibba M. and Söll D., Aminoacyl-tRNA Synthesis, *Annual Review of Biochemistry*, **69**, 617-650 **(2000)**

4. Jadhav M.V., Deshpande R.R., Dadpe M., Mahajan P., Kakade P., Kamble G. and Deshpande N.R., Screening of antimicrobial activity of active compounds of Embelia basal, chlorhexidine and amoxicillin against salivary microflora of mixed dentition age group, *Res. J. Pharm. Biol. Chem*., **3**, 1334–1339 **(2012)**

5. Joshi U.P. and Wagh R.D., GC-MS analysis of phytochemical compounds present in the bark extracts of *Ehretia laevis* Roxb., *Int. J. Res. Dev. Pharm. Life Sci*., **7**, 3150–3154 **(2018)**

6. Jyothirmai N., Nagaraju B., Deepika M.Y., Kumar J.N. and Rani G.S., Evaluation of anti-inflammatory and antibacterial activities of different solvent extracts of *Ehretia laevis* Roxb., *Int. J. Pharm. Sci. Res*., **8**, 715–720 **(2016)**

7. Hina Raza, Ali Abrar, Asmara Ashraf, Suryyia Manzoor, Rahat Shamim, Farhan Siddique, Ahmad Mohammad Salamatullah, Mohammed Bourhia and Gezahign Fentahun Wondmie, Design, Synthesis, Evaluation and Molecular Docking Study of Ascorbic Acid Dual-Coated Omeprazole Pellets and the Antioxidative Effect of Ascorbic Acid on Omeprazole-Induced Renal Injury in an Animal Model, *ACS Omega*, **9(1)**, 1143-1155 **(2024)**

8. James S. Pham, Karen L. Dawson, Katherine E. Jackson, Erin E. Lim, Charisse Flerida A. Pasaje, Kelsey E.C. Turner and Stuart A. Ralph, Aminoacyl-tRNA synthetases as drug targets in eukaryotic

parasites, *International Journal for Parasitology: Drugs and Drug Resistance*, **4(1)**, 1-13 **(2014)**

9. Kang Dae Gill, Yun keun Chi and Lee Sub Ho, Screening and comparison of antioxidant activity of solvent extracts of herbal medicines used in Korea, *Journal of Ethnopharmacology*, **87**, 2-3 **(2003)**

10. Li L., Yong P., Xia Y., Li-Jia X., Ta-Na W., Yong L., Ren-Bing S. and Pei-Gen X., Chemical constituents and biological activities of plants from the genus Ehretia Linn., *Chin. Herb. Med*., **2**, 106–111 **(2010)**

11. Murarkar K., Bande D. and Chandak A., Antimicrobial activity and minimum inhibitory concentration of *Ehretia laevis* leaves extracts against different microorganisms, *World J. Pharm. Res*., **7**, 691–697 **(2018)**

12. Mutha M., Deshpande R.R., Patil V., Shep S., Deshpande N.R. and Torne R., Comparative evaluation of antibacterial properties of different extracts of MimusopsElengi (Bakul) and *Ehretia laevis* (Ajaan) against salivary microflora, *Asian J. Pharm. Clin. Res*., **8**, 217–219 **(2015)**

13. Rao H., Rao I., Saeed L., Aati H.Y., Aati S. and Zeeshan M., Phytochemical analysis and bioactivity assessment of five medicinal plants from Pakistan: Exploring polyphenol contents, antioxidant potential and antibacterial activities, *Saudi Journal of Biological Sciences*, **30(10)**, 103783 **(2023)**

14. Sharma P., Shri R., Ntie-Kang F. and Kumar S., Phytochemical and Ethnopharmacological Perspectives of Ehretia laevis, *Molecules*, **26(12)**, 3489 **(2021)**

15. Thakre Rushikesh et al, AjanVruksha/ KhanduChakka (*Ehretia laevis* Roxb). Plant Leaves as A Effective Healer in Chronic Varicose Vein Ulcer. – A Case Report, *Indian Journal of Forensic Medicine & Toxicology*, **15(2)**, 703-707 **(2021)**

16. Torane Rasika C., Adsul Vaishali B., Shendkar Chandrakant D. and Deshpande Nirmala R., Evaluation of In Vitro Nitric Oxide Scavenging Activity of Medicinal Plant- Ehretia laevis, *Asian J. Research Chem*., **4(12)**, 1864-1866 **(2011)**

17. Velappan S. and Thangaraj P., Phytochemical constituents and antiarthritic activity of *Ehretia laevis* Roxb., *J. Food Biochem*., **38**, 433–443 **(2014)**

18. Vibhuti Raj Kamal, Kumar Yatin and Pramanik Avijit, In silico analysis of putative hemolysin proteins in the genome of Vibrio alginolyticus ATCC 17749 and their structure prediction, *Res. J. Biotech.*, **19(1)**, 55-58 **(2024)**

19. Yildirim A., Mavi A., Oktay M., Kara A.A., Algur O.F. and Bilaloglu V., Comparision of antioxidant and antimicrobial activities of Tilia (TiliaargenteaDesf Ex DC), Sage (SaviatrilobaL.) and Black tea (Camellia sinensis) extracts, *J Agric Food Chem*., **48(10)**, 5030-5034 **(2000)**

20. Warake R.A. et al, Evaluation of in vitro antioxidant, anticancer activities and molecular docking studies of Cappariszeylanica Linn. Leaves, *Futur J Pharm Sci*, **7**, **76 (2021)**.

(Received 27th January 2025, accepted 01st March 2025)