Research Article

In silico analysis of *Trichosanthes lobata* extracts: A promising source of antioxidants for therapeutic applications

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(Received: April 2023 Revised: May 2023 Accepted: June 2023)

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ABSTRACT

Introduction and Aim: *Trichosanthes lobata* is one of the species which belongs to Chinese traditional medicine for the therapeutic purpose of antioxidant properties. Free radicals' production by the body has numerous beneficial roles including in immune systems, cellular signaling pathways, mitogenic response, and synthesis of cellular structures. This study aimed to evaluate the therapeutic efficacy of the *T. lobata* secondary metabolites toward COX proteins and validate their antioxidant potential.

Materials and Methods: We extracted the plant using Soxhlet and subjected it to various assays like DPPH and TEAC, followed by *in silico* analysis. The molecular docking and dynamic simulation have been analyzed with respect to the protein of interest against selected phytochemicals from *T. lobata.*

Results: We observed the significant outcome from DPPH and TEAC assays like reducing the capability in contrast to *T. lobata* followed by docking and dynamic stability.

Conclusion: The current findings have unveiled that the investigated flora, namely *T. lobata*, is a bastion of secondary phytochemicals. The plant's exceptional antioxidant capacity is attributable to the occurrence of one or more of these secondary metabolites, which exert their respective or synergistic effects.

Keywords: *Trichosanthes lobata;* antioxidants; DPPH; molecular docking; free radicals.

INTRODUCTION

Despite its essentiality for the sustenance of aerobic organisms, molecular oxygen can have deleterious effects when administered in excessive quantities. Oxidation, the process of electron loss from an atom, ion, or molecule resulting in the creation of species possessing numerous outer shell electrons, referred to reactive oxygen species (ROS), is a consequence thereof. These reactive species are synthesized as a byproduct of metabolic processes (1), such as energy production from food, or exposure to harmful conditions like tobacco smoke, prolonged exposure to ultraviolet (UV) radiation, and so on. Once generated, they can interact with various biomolecules and participate in a host of biochemical pathways. When a free radical produced endogenously aims to acquire an electron from a nearby stable atom/molecule, it triggers the transformation of the latter into another free radical (2). This initiates a series of reactions that ultimately culminate in an escalation in the number of free radicals present. Consequently, free radicals bring about oxidative stress, a phenomenon that can instigate cellular damage which is counteracted by stabilizing the electron-deficient species through electron transfer. Antioxidants are entities that facilitate the stabilization of oxidatively burdened targets, thus forestalling or delaying the damage triggered by free radicals. These antioxidants may be either of natural or synthetic origin (3). A comprehensive classification of antioxidants is presented in fig. 1. In terms of oxidative stress, the interplay between production and clearance of oxidation in the organism. The overall controlled production of free radicals or ROS plays a multifarious role in immune systems, cellular signaling pathways, mitogenic response, and synthesis of cellular structures. Conversely, their superfluous production culminates in oxidative stress with deleterious effects on the structural integrity of cellular components such as lipids, proteins, and DNA, resulting in chronic or degenerative pathologies such as cancer (4), cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, cataracts, age-related macular degeneration, and so forth. Biological systems have antioxidant defense mechanisms to control damage caused by enzymatic and non-enzymatic natures to inactivate ROS (5). Normal production of ROS is controlled by the antioxidants enzymes whereas, when an excess concentration of ROS is produced, the endogenous antioxidant defense system is compromised resulting in the need for exogenous antioxidants to be supplied through food, nutritional supplements, and pharmaceuticals. Hence nutritional antioxidant supplements are one of the sources of reducing oxidative stress. The subtropical vine *Trichosanthes lobata* is a common culinary ingredient in Indian culinary and a major component of polyherbal Siddha formulation "*Punarvasavam*".

Traditionally the leaves and fruits of the plants are used as an active therapeutic to address sinuses (6), fever, dermal allergies, inflammation, ulcerative colitis, and hepatic conditions. In the current study, the plant extracts were subjected to *in vitro* antioxidants to evaluate their therapeutic efficacy. The work is further extended with an *in-silico* evaluation of the therapeutic efficacy of the *T. lobata* secondary metabolites toward COX proteins to validate their antioxidant potential (7).

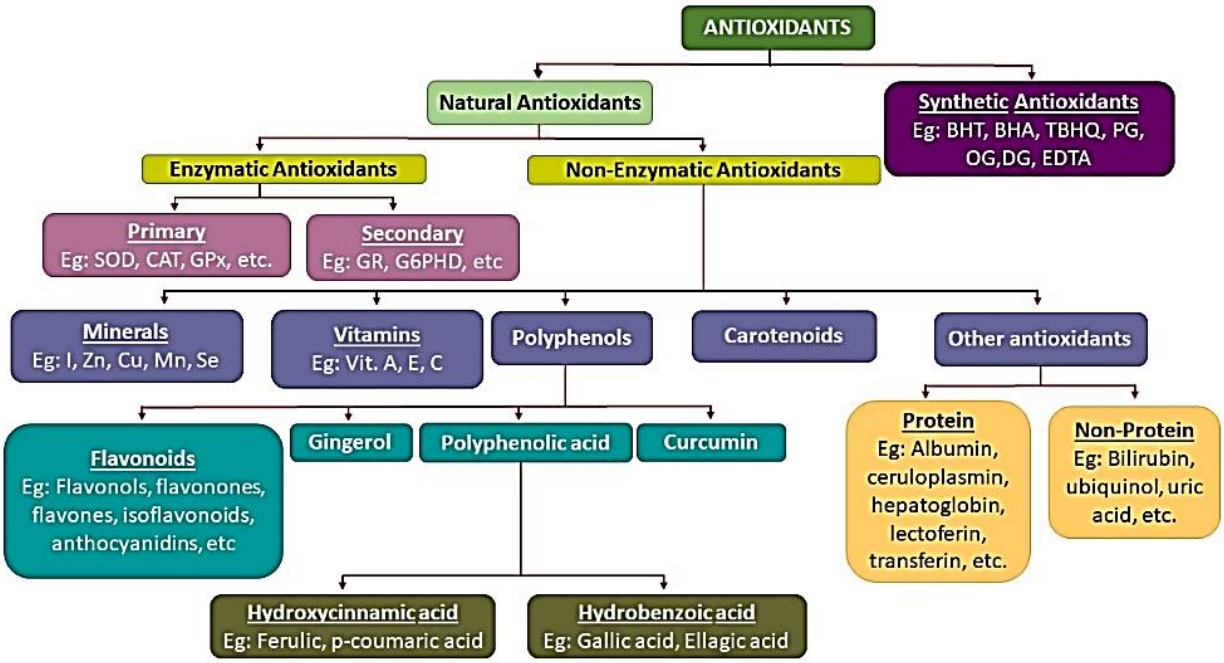


Fig. 1: Classification of antioxidants

MATERIALS AND METHODS

Plant collection

A specimen of *Trichosanthes lobata* Roxb., was collected from a wooded area near Vattavada Panchayat, Devikulam in the Idukki District of Kerala, India (coordinates: 10o10'38.7" N, 77o15'33" E). The authenticity of the test sample was confirmed by the BSI (Botanical Survey of India), Southern Circle in Coimbatore, Tamil Nadu, India with the reference number BSI/SRC/5/23/2016/Tech./213. A voucher specimen (BU BOT/H/007880 dated 10/03/2021) has been submitted at the Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India.

Preparation of crude plant extracts

Using a sophisticated Soxhlet apparatus, finely ground plant specimens weighing 50 grams were subjected to a gradual extraction process that involved the use of various solvents indulging chloroform, petroleum ether, ethyl acetate and methanol. The extracted samples were then lyophilized using a VirTis Benchtop K 4K TXL-75 apparatus to remove any residual aqueous content and incorporate reduced pressure using a state-of-the-art Supervac R-185 rotary vacuum evaporator (India). The dried samples were subsequently cryopreserved at a temperature of -20°C for future use in both phytochemical screening and the assessment of antioxidant and *in vitro* anti-diabetic enterprise.

DPPH Assay

This method of Blois (1958) and Lokapur *et al.*, (2022) was used to determine the antiradical effectiveness through the DPPH methodology. This approach uses a persistent methanol-soluble free radical DPPH to evaluate the efficacy of antioxidants or other radical species. The reduction of DPPH results in the loss of its absorption band at 515 nm. To assess the photometric measurement, different extract volumes were prepared in different test tubes with varying concentrations (ranging from 0-250 µg for PE and CHL extracts of leaf and stem, 0-100 µg for EA extracts of leaf and stem, 0-100 µg for methanol extracts of the stem, and 0-20 µg for methanol extract of the leaf). The uptake of methanol was prepared at 100 µL, and after adding 5.0 ml of 0.1 mM DPPH, the mixture was thoroughly shaken. The incubation for tubes was kept at 27ºC for 20 minutes. A control was prepared to employ the same procedure as the sample extract, but methanol solvent was used to adjust the standard values. The colorimetric absorbance readings in the samples were determined at 517 nm through the application of linear regression analysis, the antioxidant activity of the extracts was extrapolated and expressed as half-life Inhibitory Concentration, which is the quantity of extract, measured in micrograms, required to absorb half-life of the DPPH radicals. It is noteworthy that the lower the IC_{50} value, the greater the antioxidant capacity (8, 9).

Superoxide radical scavenging activity

The modified Beauchamp and Fridovich method was used to produce superoxide radicals. This was done to evaluate the sample's ability to scavenge superoxide radicals produced in the riboflavin-light-NBT system, which inhibits the formation of formazan. The reaction mixture, consisting of 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT, and varying extract concentrations (ranging from 0-20 µg for methanol leaf extract and 0-25 µg for other extracts), was prepared with a total volume of 3 ml. After 90 seconds of illumination, the reaction was initiated. The absorbance was measured at 590 nm immediately after illumination. The reaction mixture was completely enclosed in a box with an aluminum foil liner, while a blank tube with a similar reaction mixture stored in the dark was used. The experimental procedure was replicated thrice to ensure accuracy. The graph generated from plotting the percentage inhibition against the sample concentration was utilized to obtain the IC_{50} value, which represents the concentration of the sample needed to achieve a 50% inhibition of the reaction under the specified test conditions (10).

Trolox equivalent antioxidant capacity (TEAC) assay

The modified ABTS˙+ method by Manian *et al*., (11) was used to measure antioxidant activity. ABTS radical cation was created by the reaction of 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate for 16 hours at room temperature in the dark. Prior to the analysis, the solution was diluted in ethanol (approximately 1:89 v/v) and allowed to equilibrate at 30°C until the absorbance reached 0.700±0.02 at 734 nm. A 10µl aliquot of each dilution was added to the assay, and the stock solution of the sample extracts was reduced to produce a 20–80% suppression of the blank absorbance. After precisely 30 minutes of initial mixing, 10 ml of sample extracts were mixed with 1 ml of diluted ABTS˙+ solution, and the absorbance was measured at 30°C. Appropriate solvent blanks were used in the experiment. The concentration of Trolox after each standard dilution was carried out in triplicate and presented as the percentage suppression of the blank absorbance at 734 nm, indicating TAA (Total Antioxidant Activity). TAA is expressed as the number of Trolox having equivalent antioxidant activity and is given as mol/g sample extracts on a dry weight basis. The entire process was performed in triplicate to ensure accuracy and precision (10).

Nitric oxide radical scavenging assay

The assessment of the nitric oxide scavenging activity of *T. lobata* leaf and stem extracts was carried out using a method described by Maji et al., (2018) and Sreejayan and Rao (2018). To scavenge the nitric oxide radical, different concentrations of solvent extracts (ranging from 100-500 µg for PE and CHL extracts, 20-100 µg for EA extracts and methanol extract of the stem, and 2-20 µg for methanol extract of a leaf) were added to 3 ml of 10 mM sodium nitroprusside in 0.2 µl of phosphate buffered saline (pH 7.4). The mixture was incubated for 2.5 hours at room temperature, followed by the addition of 0.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% H_3PO_4) to form a chromophore (purple azo dye). The absorbance of the chromophore was measured at 546 nm. The analysis was conducted thrice, and a graphical plot of the inhibition percentage against the sample concentration was utilized to calculate the concentration of the sample that can cause 50% inhibition (also known as IC_{50}) under the prevailing test conditions (12, 13).

Hydroxyl radical scavenging assay

The method used to assess hydroxyl radical scavenging activity in the leaf and stem extract was a modified version of the method developed by Klein *et al*., To avoid plagiarism, appropriate adjustments were made. In this study, 20 µg of each solvent extract was mixed with a solution of iron-EDTA, EDTA, and DMSO. The reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid and incubated in a water bath at 80 to 90 ˚C for 15 minutes. After incubation, 1 ml of ice-cold 7.5% w/v TCA was added. Then, 3 ml of Nash reagent, containing ammonium acetate, glacial acetic acid, and 2 ml of acetylacetone, was added to the mixture, and allowed to equilibrate at room temperature for 15 minutes. The resulting pink color intensity was measured using a spectrophotometer at 532 nm against a reagent blank (14).

Lipid peroxidation

The method used for the determination of lipid peroxidation was based on the procedure initially developed by Ohkawa *et al*., (15) with some modifications. Briefly, different concentrations of extracts ranging from 0 to 50 µg/ml (0 to 20 µg/ml for methanol leaf extract) were mixed with 0.1 ml of 25% rat liver homogenate containing 30 mM KCl and incubated. After incubation, the mixture was treated with SDS, TBA, and acetic acid and then heated in a boiling water bath for an hour at 100°C. Subsequently, 5 ml of a 15:1 pyridine:butanol solution was added to the cooled mixture, mixed well, and centrifuged at 3000 rpm for ten minutes. The clear supernatant was then examined for its absorbance at 532 nm against pyridine:butanol. From the graph of the inhibition percentage against sample concentration, the sample concentration that provides 50% inhibition (IC_{50}) under the assay conditions was determined (14).

Phosphomolybdenum assay

The evaluation of the antioxidant activity of the extracts was carried out using the phosphomolybdenum method as described by Prieto *et al*., and Lokapur *et al.*, (2020) with some

modifications to avoid plagiarism. Briefly, 0.5 ml of the leaf and stem extracts, both at a concentration of 20 µg/ml, were mixed separately with 3 ml of the reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was incubated at 95°C for 90 minutes and the absorbance was measured at 695 nm. The results were expressed in terms of ascorbic acid equivalents (16, 17).

Metal chelating assay

The current study aimed to evaluate the capability of the extracts in inhibiting the formation of iron-(II)-ferrozine complex, which was assessed using the iron II chelating assay. This assay was conducted based on the protocol established by Dinis *et al*., with some minor modifications. To start the experiment, 100 µl of the extracts were added to 50 µl of 2 mM FeCl2 solution. Afterward, 200 µl of 5 mM ferrozine was added to the test tubes, and the reaction was allowed to proceed for 10 min at room temperature. The reaction mixture was then measured spectrophotometrically at 562 nm against a blank containing methanol. To compare the results, a negative control consisting of deionized water was used. The extracts' potential to chelate ferrous ions was expressed as mg EDTA equivalents/g extract, which represents the extracts' ability to form stable complexes with iron ions (18).

Reducing power

In terms of reducing power concentration, different concentrations of leaf and stem extracts were prepared and mixed separately with 2.5 ml of 0.2 M phosphate buffer (pH 6.6). To each mixture, 2.5 ml of 1% potassium ferrocyanide solution was added, and the mixture was incubated at 50°C for 20 minutes. Following this, 2.5 ml of 10% trichloroacetic acid (TCA) was added, and the contents were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected, and to each mixture, 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride were added. The absorbance of the reaction mixture was measured at 700 nm.

***In silico* studies**

The three-dimensional structures of the ligands used in this study were obtained from the PubChem database. The protein sequences of the target proteins (COX2, aldose reductase, lipoxygenase, and xanthine oxidase) were obtained from NCBI databases since their three-dimensional structures were not available in the Protein Data Bank. To predict the protein structures, the Homology modeling technique was utilized with SWISS-MODEL. The molecular docking technique was employed using PyRx (AutoDockVina) to identify the active binding sites of the target proteins that are specific regions for interaction. The ligands were docked with the protein receptors, and the best inhibitors were categorized based on their binding affinities and the amino acids involved in antioxidant activities. Additionally, all the compounds were subjected to ADMET analysis to evaluate their pharmacological properties.

RESULTS

Antioxidant activity

We measured the nitric oxide scavenging activity of different extracts of *T. lobata* leaves and stem. The results showed that all the extracts exhibited scavenging activity in a dose-dependent manner. The methanol leaf extract exhibited the highest activity, with an IC50 value of 18.86 ± 0.2 µg/ml extract, followed by the ethyl acetate extract of the leaf, which showed an IC_{50} value of 91.88 ± 0.3 µg/ml extract. In contrast, the chloroform extract of the stem displayed the least scavenging activity, with an IC_{50} value of 1098.9 ± 0.2 µg/ml extract. We found the scavenging activity of the *T. lobata* extracts to be appreciable. We also assessed the antioxidant capacity of all the extracts of *T. lobata* leaves and stem for their IC_{50}, using the lipid peroxidation assay.

We observed that the methanol leaf extract (10.99 ± 0.1 µg/ml extract) exhibited higher antioxidant potential than the other extracts, followed by the ethyl acetate leaf extract (38.49 ± 0.2 µg/ml extract). The petroleum ether extract of the stem showed the lowest activity (137.17 ± 0.3 µg/ml extract). To measure the Trolox equivalent antioxidant capacity (TEAC) of the *T. lobata* extracts, we used the improved $ABTS^{\bullet+}$ decolorization assay. The results showed that the extracts were able to quench ABTS•+ with values ranging between 47.2 ± 5.8 to 2642 ± 10.1 µmol trolox/g extract. The methanol leaf extract (2642 ± 10.1 µmol trolox/g extract) demonstrated the highest potency in scavenging the radicals, followed by the ethyl acetate leaf extract. The petroleum ether extract of the stem showed the least quenching activity, as listed (Table 1).

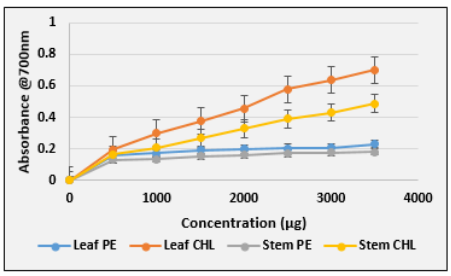
Our analysis of *T. lobata* leaf and stem extracts revealed that the methanol leaf extract had the highest hydroxyl scavenging ability, with an inhibition rate of 79.97 ± 0.2 AAE/g extracts. This suggests that *T. lobata* has a scavenging ability against highly reactive oxygen species. In addition, we conducted a phosphomolybdenum assay to measure the antioxidant potential of the extracts. The methanol leaf extract (47.46 ± 0.2 AAE/g extracts) demonstrated higher activity than other extracts, followed by the methanol stem extracts (46.69 ± 0.1 AAE/g extracts). The petroleum ether extract of the stem and leaf showed the least activity. To further evaluate the antioxidant capacity of *T. lobata* leaves and stems, we conducted a metal chelating assay. The methanol extract of the leaves (59.22 ± 0.1 mg EDTA/g extract) exhibited the highest activity, followed by the methanol stem (46.69 ± 0.1 mg EDTA/g extract), ethyl acetate leaves (40.05 ± 0.1 mg EDTA/g extract), and ethyl acetate stem (34.45 ± 0.1 mg EDTA/g extract). Fig. 2 displays the

dose-response curves for reducing the powers of different *T. lobata* extracts. It was observed that the reducing power increased with increasing concentration of the extracts. The methanol leaf extract showed the highest reducing power, with absorbance at 700 nm ranging from 0.28 ± 0.01µg/ml to 1.21 ± 0.01µg/ml, while the petroleum ether extract of the stem exhibited the lowest reducing power, with absorbance ranging from 0.13 ± 0.11 µg/ml to 0.18 ± 0.04 µg/ml.

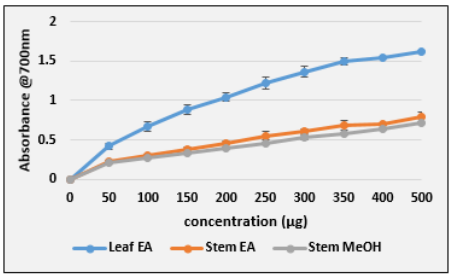
Table 1: *In vitro* antioxidant activity of leaf and stem parts of *T. lobata*[*]

Parameters	Leaf				Stem			
	PE	CHL	EA	MeOH	PE	CHL	EA	MeOH
DPPH• scavenging activity (IC_{50} µg/ml extract)	363.37 ± 1.1^{d}	143.10 ± 1.0^{b}	44.15 ± 0.02^{a}	**11.58 ± 0.3^{a}**	834.72 ± 0.6^{h}	377.36 ± 0.8^{d}	54.78 ± 0.4^{a}	48.13 ± 1.2^{a}
$O_2^{\bullet-}$ scavenging activity (IC_{50} µg/ml extract)	83.74 ± 0.1^{g}	70.05 ± 0.2^{f}	49.17 ± 0.2^{d}	**10.94 ± 0.2^{a}**	100.2 ± 0.1^{h}	95.33 ± 0.2^{h}	84.63 ± 0.1^{g}	**22.52 ± 0.3^{b}**
Nitric oxide Radical Scavenging activity (IC_{50} µg/ml extract)	992.06 ± 0.1^{g}	732.06 ± 0.2^{f}	91.88 ± 0.3^{a}	**18.86 ± 0.2^{a}**	874.13 ± 0.2^{g}	1098.9 ± 0.2^{h}	118.29 ± 0.1^{b}	95.38 ± 0.2^{a}
Lipid peroxidation (IC_{50} µg/ml)	97.2 ± 0.2^{f}	77.45 ± 0.1^{e}	38.49 ± 0.2^{c}	**10.99 ± 0.1^{a}**	137.17 ± 0.3^{h}	72.46 ± 0.1^{c}	57.07 ± 0.2^{d}	53.92 ± 0.1^{d}
Trolox equivalent antioxidant capacity (µmol trolox/ g extract)	155.2 ± 15.5^{h}	462.4 ± 5.8^{g}	1624 ± 5.8^{d}	**2642 ± 10.1^{a}**	47.2 ± 5.8^{h}	425.2 ± 10^{g}	600.7 ± 3.2^{c}	1049.6 ±10.3^{e}
Hydroxyl Radical scavenging activity[#]	7.8 ± 0.9^{h}	36.07 ± 0.3^{e}	65.84 ± 0.2^{b}	**79.97 ± 0.2^{a}**	6.61 ± 0.4^{h}	22.83 ± 0.7^{f}	36.1 ± 0.4^{e}	58.9 ± 0.2^{c}
Phosphomolybdenum assay[#]	7.45 ± 0.1^{g}	12.67 ± 0.2^{f}	34.51 ± 0.4^{c}	**47.46 ± 0.2^{a}**	6.54 ± 0.1^{g}	9.5 ± 0.3^{g}	24.67 ± 0.1^{d}	29.93 ± 0.4^{d}
Metal chelating Assay[@]	12.56 ± 0.1^{g}	21.19 ± 0.1^{e}	40.05 ± 0.1^{c}	**59.22 ± 0.1^{a}**	3.84 ± 0.1^{h}	7.67 ± 0.1^{g}	34.45 ± 0.1^{d}	46.69 ± 0.1^{b}

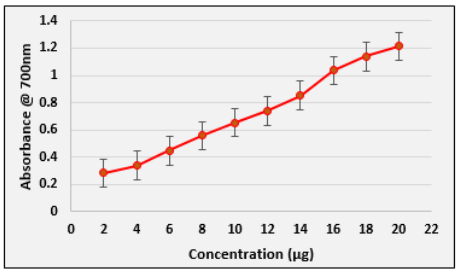
*Values are mean ± standard deviation (SD) of three independent experiments; Values not sharing a common letter in a column are significantly different (P<0.05); [@] Values expressed in mg EDTA/g extract; [#] Values expressed in mg AAE/g extract; PE-Petroleum ether; CHL-Chloroform; EA-Ethyl acetate; MeOH-Methanol; AAE-Ascorbic acid equivalent; EDTA-ethylenediaminetetraacetic acid.



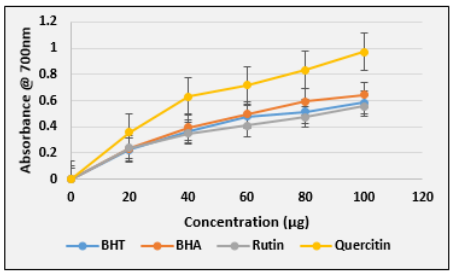
a) Petroleum ether and chloroform extracts of *T. lobata* leaf and stem



b) Ethyl acetate leaf and stem and methanol stem of *T. lobata*



c) Methanol extract of *T. lobata* leaf



d) Standard antioxidants

Fig. 2: Reductive capability of various solvent extracts of *T. lobata* leaf and stem.

In silico analysis

Ligand preparation

The ligands used in the study were obtained from the PubChem database and were purified using Biovia Discovery Studio. PyRx was used for the energy minimization and PDBQT conversion of the ligands, as shown in Fig. 3.

Protein preparation

To assess antioxidant activity, several protein targets were selected, including cytochrome P450 (CYPs), glutathione reductase (GR), lipoxygenase (LOX), NADPH oxidase, tyrosinase (TYR), and xanthine oxidase (XO). The Protein Data Bank (PDB) files of these proteins were converted to 'pdbqt' format for analysis of binding energy or affinity using molecular

docking software. Autodock Tools were used to prepare the proteins for docking, as illustrated in Fig. 4.

Drug likeness analysis

The pharmacological significance of the phytochemicals derived from *T. lobata* was evaluated based on various parameters such as ADMET, drug bioavailability, and drug likeness. Lipinski's rule of five was used to determine the characteristics of the phytochemicals/ligands. Only those phytochemicals with optimum characteristics and no observed toxicity are presented (Table 2).

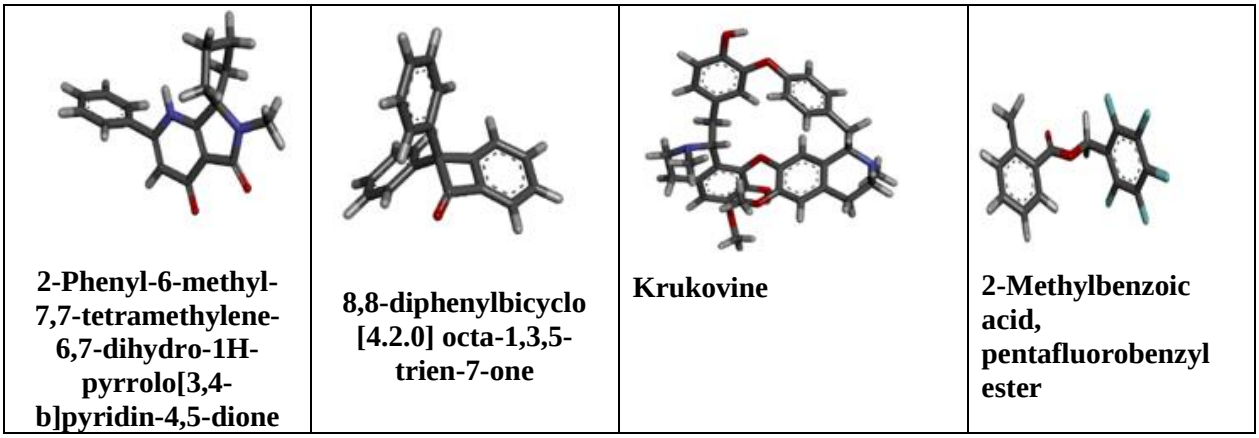


Fig. 3: Ligand structures for antioxidant molecular docking analysis

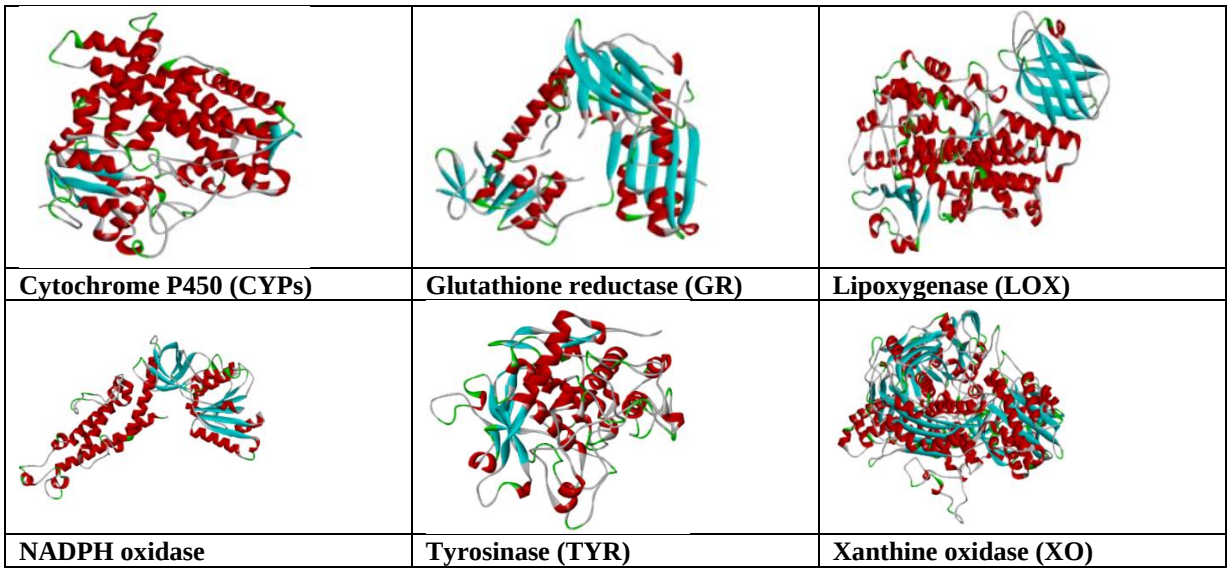


Fig. 4: Protein structures for antioxidant molecular docking analysis

Table 2: ADMET analysis of selected compounds using the SWISSADME server

Compound	Molecular Weight	H-Bond Donor	H-Bond Acceptor	TPSA	GI Absorption	BBB permeant	PAINS Alert
2-Phenyl-6-methyl-7,7-tetramethylene-6,7-dihydro-1H-pyrrolo[3,4-b]pyridin-4,5-dione	294.35	1	2	53.17	High	Yes	0
Krukovine	594.70	2	8	83.86	High	No	0
8,8-diphenylbicyclo[4.2.0] octa-1,3,5-trien-7-one	270.32	0	1	17.07	High	Yes	0
2-Methylbenzoic acid, pentafluorobenzyl ester	316.22	0	7	26.30	High	No	0

TPSA: Topological polar surface area; **GI:** gastrointestinal**; BBB:** Blood-Brain barrier**;**
PAINS: Pan Assay Interference compound

Table 3: Molecular docking of antioxidant phytocompounds of *T. lobata* against oxidant proteins

Protein	Plant Extract	Phytocompounds	Binding affinity[#]	Amino acid residue*
Cytochrome P450 (CYPs) [1OG5]	EA-L	2-Phenyl-6-methyl-7,7-tetramethylene-6,7-dihydro-1H-pyrrolo[3,4-b]pyridin-4,5-dione	-8.3	TYR A:367, ARG A:451, ILE A:96, VAL A:105, TRP A: 363, TRP A:447, GLN A:104, THR A:414, LEU A:532, ARG A:92, THR A:525, GLY A:526, LEU A:524, GLU A:460, PHE A:462, LEU A:413
	EA-S	Krukovine	-8.8	ASN A:371, LEU A:370, PHE A:4632, TYR A:367, LEU A:413, GLU A:460, ILE A:96, ARG A:92, THR A:525, ASP A:97, GLY A:95, PRO A:522
	MeOH-L	8,8-diphenylbicyclo[4.2.0] octa-1,3,5-trien-7-one	-8.7	PHE A:537, PRO A:534, LEU A:362, PRO A:536, PHE A:221, CYS A:219, ALA A:533, SER A:256, ASP A:355, ASN A:359, GLY A:253, ARG A:527, GLU A:260, ARG A:257
	MeOH-S	2-Methylbenzoic acid, pentafluorobenzyl ester	-8.3	PHE A:483, CYS A:490, VAL A:417, MET A:491, ILE A:351, VAL A:166, PHE A:167, LEU A:530, LEU A:259, THR A:531, THR A:356, ALA A:352, ALA A:420
Glutathione reductase (GR) [1XAN]	EA-L	2-Phenyl-6-methyl-7,7-tetramethylene-6,7-dihydro-1H-pyrrolo[3,4-b]pyridin-4,5-dione	-7.5	SER A:471, GLY A:446, PHE A:447, PHE A:460, ALA A:450, ASP A:459, THR A:463, VAL A:475, GLU A:472, ALA A:465, VAL A:464, ILE A:466
	EA-S	Krukovine	-8.8	VAL A:449, GLN A:445, GLU A:442, MET A: 443, SER A:471, ILE A:466, VAL A:475, PHE A:447, THR A:463, ALA A:450, ASP A:459, PHE A:460, GLY A:446
	MeOH-L	8,8-diphenylbicyclo[4.2.0]octa-1,3,5-trien-7-one	-8.8	PHE A:447, GLY A:446, MET A:443, THR A:463, VAL A:464, ALA A:465, GLU A:472, VAL A:475, ILE A:466, SER A:471, PHE A:460, ALA A:450
	MeOH-S	2-Methylbenzoic acid, pentafluorobenzyl ester	-7.3	PHE A:460, ALA A:450, VAL A:464, ALA A:465, GLU A:472, SER A:471, VAL A:475, PHE A:447, ILE A:466, THR A:463, ASP A:459, ALA A:455
Lipoxygenase (LOX) [2IUJ]	EA-L	N-Cbz-glycyl-L-tyrosine benzyl ester-	-8.8	ASP A:286, VAL A:285, LEU A:245, ARG A:247, VAL A:362, GLU A:288, LEU A:289, PHE A:287, ILE A:366, ALA A:440, ARG A:458, VAL A:244, ARG A:371, ALA A:454, SER A:448, GLN A:550, LEU A:449, THR A:546, PHE A:545, PHE A:451, TYR A:571
	EA-S	2-(3-Nitrophenyl)-1,3-bis(m-tolyl)imidazolidine	-8.5	ALA A:440, ILE A:366, THR A:367, ARG A:371, SER A:448, VAL A:244, VAL A:285, ASP A:286, LEU A:245, VAL A:362, LEU A:289, GLU A:288, ASP A:443
	MeOH-L	Dimethyl Dibenzofuran-3,4-dicarboxylate-	-7.9	ILE A:366, LEU A:245, ARG A:371, THR A:367, THR A:372, VAL A:554, LEU A:449, GLN A:550, PHE A:545, PHE A:451, TYR A:471, SER A:448, ALA A:454, VAL A:244, ARG A:458
	MeOH-S	2-Methylbenzoic acid, pentafluorobenzyl ester	-9.1	ASP A:167, TYR A:143, ARG A:139, ARG A:102, ALA A:389, PHE A:394, CYS A:100, TYR A:101, TRP A:103, HIS A:625, ASP A:171, VAL A:398, TYR A:82, GLN A:13, ARG A:402, GLU A:623, PHE A:403, ALA A:399, ILE A:168, TYR A:384
NADPH Oxidase [7D3E.1.A]	EA-L	Cyclohexane, 1,1',1",1'"-(1,6-hexane diylidene) tetrakis	-9.9	ILE A:248, GLY A:182, ILE A:244, ARG A:247, LEU A:181, LEU A:173, PHE A:174, LEU A:298, ILE A:178, TRP A:300, ARG A:250, GLN A:255, GLY A:180, PHE A:251
	EA-S	Krukovine	-9.2	PHE A:289, TRP A:300, HIS A:64, LEU A:61, TRP A:168, PHE A:167, ARG A:247, HIS

Protein	Plant Extract	Phytocompounds	Binding affinity[#]	Amino acid residue*
				A:171, HIS A:172, TYR A:243, ARG A:204, GLU A:246, ASN A:60, ARG A:250
	MeOH-L	Phthalic acid, monoamide, N,N-di cyclohexyl-, octyl ester	-8.5	LEU A:61, PRO A:302, PHE A:251, ARG A:253, ARG A:250, ARG A:247, TRP300, TRO A:168, PHE A:174, LEU A:298, GLU A:299, HIS A:171, PHE A:167, HIS A:301
	MeOH-S	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	-7.8	TRP A:168, HIS A:172, LEU A:173, ARG A:247, LEU A:181, LEU A:298, ARG A:250, GLN A:255, ILE A:178, PHE A:251, TRP A:300, PHE A:174
Tyrosinase (TYR) [3NM8.1.A]	EA-L	Queasitic acid	-8.7	GLN A:437, THR A:448, VAL A:447, ASN A:439, THR A:69, PRO A:446, VAL A:68, HIS A:100, THR A:98, CYS A:99, GLU A:451, SER A:106, CYS A:101, ARG A:114, GLY A:107, PRO A:445
	EA-S	2-Amino-3-phenyl-6-nitroindole	-8.8	ARG A:114, PRO A:115, CYS A:113, VAL A:126, ARG A:118, TRP A:117, GLY A:116, GLN A:236, ILE A:128, GLU A:232, LEU A:229, LYS A:233, TYR A:226, ARG A:230
	MeOH-L	6-Hydroxy-4,7-dimethylcoumarin	-7.8	ILE A:128, GLN A:236, GLY A:116, TRP A:117, LYS A:233, CYS A:113, PRO A:115, TYR A:226, SER A:106, THR A:112, ARG A:114, LEU A:229, ARG A:230
	MeOH-S	1H-Cyclopenta[5,6]pyrido[3,4-d]pyridazine-1,4,5-trione, 2,3,6,7,8,9-hexahydro-	-9.3	PRO A:115, LYS A:233, LEU A:229, GLU A:232, GLN A:236, ILE A:128, PRO A:242, VAL A:126, ARG A:118, GLY A:116, TRP A:117, ARG A:114, CYS A:113
Xanthine oxidase (XO) [3AX7.1.A]	EA-L	N-Cbz-glycyl-L-tyrosine benzyl ester	-9.2	VAL A:342, ALA A:338, GLY A:47, GLU A:45, LEU A:74, GLY A:46, ILE A:266, PHE A:337, GLY A:350, ALA A:346, SER A:347, GLY A:260, ASN A:261, VAL A:258, VAL259, LEU A:257, ILE A:264, GLU A:263, THR A:262, THR A:354, ASN A:351, ILE A:358, ILE A:431, ASP A:430, ASP A:429, SER A:1226
	EA-S	Olean-13(18)-ene	-10.7	LYS A:269, GLN A:680, GLU A:677, ARG A:599, MET A:827, ARG A:32, LEU A:41, ARG A:31, PRO A:676, LEU A:27, ALA A:28, ASP A:829THR A:25, GLU A:23, MET A:268, GLY A:265
	MeOH-L	Phthalic acid, pentafluoro phenyl propyl ester	-9.4	LEU A:257, VAL A:259, VAL A:258, THR A:354, ILE A:264, GLU A:263, GLY A:350, ASN A:351, ASN A:261, THR A:262, GLY A:260, ALA A:346, ALA A:302, SER A:347, GLY A:249, ILE A:353, ALA A:301, LEU A:287, LEU A:404, ILE A:403
	MeOH-S	Hydroxy codeinone	-9	ASP A:430, PHE A:337, ASP A:429, ALA A:338, GLY A:47, GLU A:45, GLY A:46, VAL A:342, THR A:262, ASN A:351, ALA A:432, GLU A:263, ILE A:358, SER A:359, THR A:354, SER A:356, ASP A:360, LYS A:360, ILE A:431

Molecular docking analysis

The selected ligands were energy minimized and converted into the 'pdbqt' file format. The PyRx tool was utilized to estimate the binding affinities of all the ligands. Following the docking process, the docked structures were saved and visualized using the Biovia Discovery Studio tool. The receptor-ligand interactions of all the docked structures were analyzed in a 2D diagram. Considering antioxidant activity, krukovine was predicted with higher binding affinity against CYPs (-8.8 kcal/mol) and GR -8.8 kcal/mol) whereas, for proteins LOX, NADPH oxidase, TYR, and XO, higher binding affinities were noted in N-Cbz-glycyl-L-tyrosine benzylester- (-8.8 kcal/mol), Cyclohexane, 1,1',1",1'"-(1,6-hexanediylidene) tetrakis

(-9.9 kcal/mol), 1H-Cyclopenta[5,6] pyrido [3,4-d]pyridazine -1,4,5-trione, 2,3,6,7,8,9 -hexahydro- (-9.3 kcal/mol) and Olean-13(18) -ene (-10.7 kcal/mol) respectively. Ligand and protein interactions such as Van der Waals, conventional hydrogen bonds, carbon-hydrogen bonds, Pi-alkyl, Pi-Sigma, alkyl, halogen, amide-Pi-stacked, Pi-Carbon, Pi-Pi-stacked, Pi-Pi-T-shaped, and Pi-anion were suggested. Many of the compounds were predicted with conventional hydrogen and carbon-hydrogen bonds with the target (Table 3).

DISCUSSION

Free radicals, highly reactive molecules generated as byproducts of normal metabolic processes or introduced from exogenous sources like pollution, radiation, or specific chemicals, exert detrimental effects on cellular integrity and tissue homeostasis. Unpaired electrons within free radicals render them intrinsically unstable, driving their avid reactivity with vital cellular constituents such as DNA, proteins, and lipids (16). The ensuing cascade of molecular interactions culminates in oxidative stress, a state marked by an imbalance between the production of free radicals and the body's antioxidant defenses. Oxidative stress engenders deleterious consequences, compromising cellular architecture, perturbing physiological processes, and conferring susceptibility to a diverse spectrum of maladies, encompassing cardiovascular disorders, oncogenesis, neuro-degenerative pathologies, diabetes mellitus, inflammatory ailments, and the process of aging (18). Antioxidants, conversely, represent substances capable of quenching free radicals and mitigating their deleterious repercussions. Their functionality hinges upon the donation of an electron, a process facilitating stabilization of the unpaired electrons within free radicals, while remaining inert themselves. This process, termed scavenging, constitutes the neutralization of free radicals. Antioxidants assume a pivotal role in preserving cellular homeostasis and safeguarding the organism against afflictions stemming from oxidative stress. The advantages of antioxidants within the biological milieu are multifaceted. Primarily, they serve to shield cellular frameworks, encompassing DNA, proteins, and lipids, from oxidative harm. Through this defensive mechanism, antioxidants confer a reduced susceptibility to the onset of chronic disorders, notably encompassing cardiovascular ailments and malignancies. Secondly, antioxidants contribute to the dampening of inflammatory processes manifesting in the body. Although inflammation denotes a natural response to injury or infection, its chronicity precipitates the development of sundry maladies. By modulating the inflammatory response, antioxidants exhibit a potential to assuage inflammation. Lastly, antioxidants potentially assume a role in fortifying the immune system, augmenting its capability to combat infections, and bolstering overall wellness. Antioxidants can be procured from an array of origins, encompassing dietary sources and supplementary interventions. Prominent dietary reservoirs of antioxidants comprise fruits, vegetables, whole grains, nuts, seeds, and legumes. Plants, having been duly acknowledged as principal reservoirs of antioxidants, have garnered significant attention. Within this purview, *Trichosanthes lobata*, colloquially recognized as pointed gourd or patola, emerges as a noteworthy exemplar. *T. lobata*, a creeping vine ubiquitously distributed across tropical and subtropical regions of Asia, has traditionally found application in medicinal practices owing to its multifarious pharmacological properties. Notably, this botanical entity is renowned for its antioxidant potential, propelling its significance in therapeutic contexts (19-21).

T. lobata harbors a diverse repertoire of bioactive constituents that substantiate its antioxidant prowess. Among these constituents, phenolic compounds, flavonoids, and various other phytochemicals prominently feature. Empirical investigations have substantiated the antioxidant efficacy of *T. lobata* extracts, elucidating their adeptness in free radical scavenging (22). The antioxidant potential of *T. lobata* is attributed to its capacity to impede lipid peroxidation, safeguard DNA against oxidative detriment, and augment the functionality of endogenous antioxidant enzymes. Cyclooxygenase-2 (COX-2), aldose reductase, lipoxygenase, and xanthine oxidase are a cohort of enzymatic catalysts that occupy pivotal roles in a multitude of physiological processes. Nonetheless, aberrant upregulation or dysregulation of these enzymes can precipitate the emergence of free radicals and oxidative stress within the organism. Efficacious inhibition of these enzymes has evinced a propensity for curtailing the production of free radicals, thereby ameliorating the pernicious ramifications commonly associated with oxidative stress. COX-2, an enzyme intricately involved in the biosynthesis of prostaglandins, serves as a lipid mediator integral to processes of inflammation and nociception. While COX-2 serves as an indispensable contributor to normative inflammatory responses, its hyperactivation can engender the generation of free radicals and propagate an inflammatory milieu. Deliberate suppression of COX-2 activity can manifest as an efficacious approach toward mitigating the generation of free radicals, concomitantly abating the inflammatory cascade and averting oxidative harm (23). Aldose reductase, an enzyme germane to the metabolic processing of glucose, assumes a pivotal role in numerous physiological contexts. Pathologies characterized by elevated aldose reductase activity, such as diabetes mellitus, can incite augmented production of free radicals. These deleterious free radicals inflict oxidative stress upon cellular

constituents, thereby eliciting perturbations within the cellular milieu. Suppression of aldose reductase activity has been evidenced to efficaciously reduce the generation of free radicals, thereby endowing cellular and tissue protection against the oxidative detriment that typifies diabetes-related conditions.

Lipoxygenases, constituting an enzyme family instrumental in the oxygenation of polyunsaturated fatty acids, facilitate the generation of diverse bioactive molecules. However, lipoxygenase activity can also precipitate the emergence of free radicals, particularly in the context of inflammatory processes. Targeted inhibition of lipoxygenase activity duly curtails the production of free radicals, thus exerting a diminutive influence upon oxidative stress and its concomitant deleterious effects upon the architectural integrity of cellular structures. Xanthine oxidase, an enzyme intimately implicated in purine metabolism, partakes in the generation of reactive oxygen species (ROS) as byproducts during normal metabolic flux. Excessive ROS production arising from exaggerated xanthine oxidase activity instigates oxidative stress, thereby conferring predisposition to tissue damage. Deliberate inhibition of xanthine oxidase activity confers a decline in ROS levels, attenuating oxidative stress and conferring protection upon cellular entities against the encumbrances of oxidative damage.

The inhibition of these enzymes offers a viable stratagem for curbing the production of free radicals and mitigating the deleterious consequences associated with oxidative stress (24). It was noticed that the methanolic extract of *Trichosanthes lobata* exhibited better antioxidant activity in both *in vitro* and *in silico* analysis.

CONCLUSION

The current investigation on *T. lobata* evidenced its nutritious quantity which satisfies the daily dietary requirement. It also attributes to the presence of diverse functional groups containing significant phytocompounds from flavonoids, alkaloids, terpenoids, phenols, steroids, *etc*. such as quinone, morphinane, thiophene, lupeol, sitosterol, serine, and many more. The interaction of phytocompounds specifically, krukovine, Lup-20(29)-en-3-one, and Olean-13(18)-ene to the specific protein targets for respective ailments were predicted through molecular docking. Methanol leaf extract evidenced the best antioxidant and anti-diabetic activities in various *in vitro* assays. The present research indicates that *T. lobata* is a plant that contains a variety of secondary phytochemicals, and the observed antioxidant activity is likely due to the presence of one or more of these compounds working individually or in combination.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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