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Invitro and *Insilico* analysis of antioxidant activity of selected medicinal plants

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Abstract

Medicinal plants are the important essential sources given by the god for all the living creatures. The usage of the medicinal plants are increased rapidly day by day. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. The present study deals with the three different medicinal plants for phytochemical analysis and molecular docking studies. The analysis was undergone and which showed the results like carbohydrates, flavonoids, alkaloids, and the molecular docking studies shows the antioxidant compound present in all the selected samples.

Key words: Medicinal plants, phytochemical analysis, Molecular docking, compounds, and antioxidant activity**.**

Introduction

Plants play a vital role in our lives more than animals mainly due to their extraordinary array of diverse classes of biochemical with a variety of biological activities. The usage of the medicinal plant are increased rapidly day by day. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a battery of detoxifying proteins or preventing the generation of reactive oxygen species. In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases, (Rashmi and Linu Mathew, 2012).

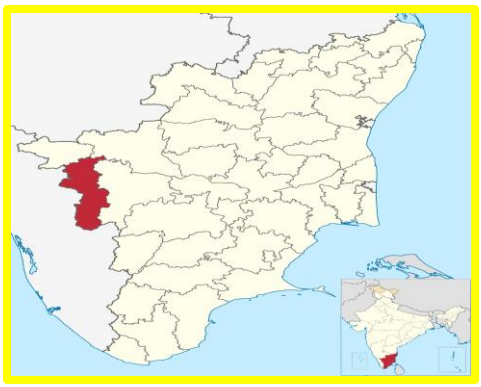
The main of the study is to go through with the morphological and medicinal properties of the selected medicinal plants. To conduct phytochemical screening for the evaluation of compounds in selected medicinal plants like *Justicia tranquebariensis* L.f., *Adhatoda vasica* Linn, and *Eclipta prostrata*, L., and to identify the primary metabolites in the methanolic leaf extract of the selected samples. The antioxidant properties also present in the selected medicinal plants through analyzing the Molecular Docking studies.

Materials and Methods

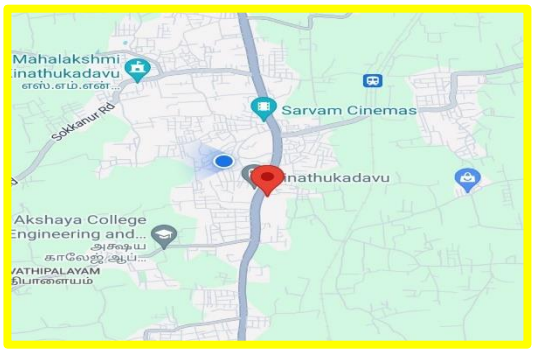
Study area

Tamil Nadu lies in the southernmost part of the Indian peninsula. Coimbatore is the city in Tamilnadu, South India. It is the capital city Kongunadu region and is often referred to as the Manchester of south India.

Kinathukadavu is situated in the district of Coimbatore, which has a pleasant climate due to the presence of forests to the north and cool winds blowing through the Palghat gap in the Western Ghats.



A



B

Fig-1: **A)** Study Area **B**) Location map

Collection of the selected samples

For the present study the samples were collected and washed with running tap water to remove adhering dust and then dried under the shade. Then the dried samples were powdered with the help of a Pulverizer. The powdered samples were stored in an airtight container for further analysis.

Sample-1 Systematic Position:

Division : Plantae
Class : Dicotyledonous
Order : Lamiales
Family : Acanthaceae
Genus : *Justica*
Species : *J. tranquebariensis* L.f.

A

B

Fig-2: A) Habit of *J. tranquebariensis* L.f. **B)** Powder of *J. tranquebariensis* L.f.

Plant description

Justicia tranquebariensis L.f.is a small annual herb common to tropical countries. It is distributed throughout the hotter parts of India and Australia, often found in waste places along the rodesides.It is usually erect, slender-stemmed; spreading up to 80 cm tall, though sometimes it can be seen lying down. The plant is an annual broad-leaved herb that has a hairy stem with branches from the base to the top. The leaves are opposite, elliptical, oblong, or oblong- lanceolate with a faintly toothed margin and darker on the upper surface. Flowers are small, sexual, solitary, numerous and crowded together in axillary cymes about l cm in diameter. The fruits are yellow, three-celled capsules with wrinkled seeds. The stem and leaves reduce a white or milky juice that is frequently seen occupying open waste spices, honks of watercourses, grasslands, road sides, and pathway. *Justicia tranquebariensis* L.f. Is widely used as a traditional medicine herb in all the tropical countries (Begum *et al.*, 2011).

Uses

It is a popular herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility, and venereal diseases.

Sample-2 *Adhatoda vasica*, Linn.

Systematic Position:

Kingdom : Plantae
Class : Dicotyledonous
Order : Lamiales
Family : Acanthaceae
Genus : *Adhatoda*
Species : *A. vasica*, Linn.

A

B

Fig-3: A) Habit of *A. vasica*, Linn. **B)** Powder of *A. vasica*, Linn.,

Plant description

Adhatoda vasica Linn, is a shrub with 10-20 lance-shaped leaves 8-9 centimeters in length by four wide. They are oppositely arranged, smooth-edged, and borne on short petioles. When dry they are of a dull brownish-green color. They are bitter- tasting. When a leaf is cleared with chloral hydrate and examined microscopically the oval stomata can be seen. They are surrounded by two crescent-shaped cells at right angles to the ostiole. The epidermis bears simple one- to three-celled warty hairs, and small glandular hairs. Cystoliths occur beneath the epidermis of the underside of the blade, (Wantkhem Sandhya chanu and Kanambala sarangthem, 2014).

Uses

The leaves are containing phytochemicals such as alkaloids, tannins, saponins, phenols and flavonoids. The most important is vasicine, and a quinazoline alkaloid.

Sample - 3 *Eclipta prostrata*, L.,

Kingdom : Plantae
Class : Angiosperms
Order : Asterales
Family : Asteraceae
Genus : *Eclipta*
Species : *E. prostrata*,L.,

A **B**

Fig-3: A) Habit of *E. prostrata.*L. **B)** Powder of *E. prostrata.*L.,

Plant description

It is widely distributed throughout India, Nepal, China, Thailand, Bangladesh, and Brazil, In India it is widely used as a cholagogue and deobstruent in hepatic enlargement. Plant has cylindrical, grayish roots. Solid, circular, purplish stems with white fine hairs 0.8m. Leaves arranged in opposite pairs, hairy in two-sided, lanceolate, serrated 2–12.5 cm long, 5-35 mm wide. The solitary flower heads are 6-8 mm (0.24 -0.31 in) in diameter, with white florets. This species grows commonly in moist places in warm temperate to tropical areas worldwide (Shipam Sinha and Richa Raghuvanshi, 2016).

Uses:

Contains a wide range of active phytoconstituents, which includes coumestan derivatives, triterpene saponins, steroidal saponins, triterpene, steroids, steroidal alkaloids, flavonoids, phenolic acids, thiophene derivatives and many other compounds.

I. **Preliminary phytochemical analysis - (Malathi *et al*., 2018.)**

Qualitative phytochemical tests are performed as preliminary tests.

For the preliminary phytochemical screening of methanolic leaf extracts of selected samples were analyzed by the standard methods and showed the presence of various phytochemical constituents such as saponins, phenols, alkaloids, proteins, tannins, carbohydrates and terpenoids.

Test of saponins

2 ml of extract was shaken vigorously with 5 ml distilled water to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

Test for phenols

To 1 ml extract, add 5 ml distilled water followed by drops of 10% ferric chloride, the formation of blue or black color indicates the presence of phenolic groups.

Test for alkaloids

1 ml of extract in a test tube was treated with a few drops of Mayer's reagent Creamy white precipitate indicating the presence of alkaloids.

Test for protein

To 1 ml extract, add 2 ml water followed by a few drops of conc. HNO3. The formation of yellow color indicates the presence of protein.

Test for tannins

To 2ml extract, 1 ml of distilled water and 1-2 drops of ferric chloride solution was added and observed for brownish green or a blue-black coloration.

Test for flavonoids

A few drops of 1% NH3 solution was added to 2 ml of extract in a test tube. A yellow coloration was observed for the flavonoids.

Test for carbohydrates and reducing sugars

5-8 drops of Fehling solution were added to 2 ml extract. The mixture was heated in a boiling water bath for 5 minutes. A brick red precipitate shows the presence of reducing sugars.

Test for Quinone

2ml extract was mixed with 2 ml of chloroform and 1 ml of 10% ammonia solution was added.
The presence of a pink, red or violet color indicates the anthraquinones.

Test for terpenoids

2ml extract was mixed with 2 ml chloroform in a test tube. To this, 3 ml cone. H2SO4 was carefully added along the wall of the test tube to form a layer. An interface with a reddish-brown coloration the presence of terpenoids.

Test for glycosides

1 ml of 50% H2SO4 was added to the 2 ml of extract in a boiling tube. The mixture was heated in a boiling water bath for 5 minutes. 10 ml of Fehling's solution was added and boiled. A brick red precipitate indicated the presence of glycosides.

II. Molecular Docking- (Tomasz J. Guzik *et al.*, 2006)

The 2D structures of the compounds were retrieved from PubChem (Kim *et al.*, 2023) in SMILES format. The Smiles format was translated into 3D structure PDB format using online Smiles Translator tool. The Molecular Weight, Hydrogen Bond Donors, Hydrogen Bond Acceptors and Log P, the Lipinski parameters along with Drug Likeness Score were calculated using MuleSoft Online Software that can be accessed at, the receptor for the docking was retrieved from Protein Data Bank (Berman *et al*, 2000). Molecular Docking of the identified receptor and synthesized ligands was performed using Autodesk Tools (ADT) 1.5.6 (Morris *et al.*, 2009) by employing the following method:

Preparation of Receptor and Ligand files

Autodock entails both the receptor and ligand in PDBQT format for assessing the binding affinity between them. PDBQT format restrains the atomic coordinates, partial charges and atom types. Initially, the receptor file in PDB format obtained from Protein Databank was accessed in Autodock Workspace. The water molecules in the receptor file were removed and implicit Hydrogen atoms were added. Finally, partial charges were added and the receptor file was saved in PDBQT format. Similarly, the ligand files in PDB format was retrieved by Autodock and saved in PDBQT format.

Preparation of Grid and Dock Parameter files

Autogrid 4.2 program in ADT was used to perform the grid computation. The grips maps with a dimension of 60X60X60 and spacing of 0.375 Aº were centered along the ligand binding site. The receptor and ligand files in PDBQT format along with the grid maps were saved as the grid parameter file to execute the Autogrid program. After the Autogrid calculation, Autodock parameter file was created with the receptor, ligand and selection of Autodock parameters.

Docking

Docking was performed using Lamarckian Genetic Algorithm with 10 independent runs per ligand with an initial population of 150 randomly placed ligand on the receptor binding site. A maximum of $2.5X10^5$ evaluations on the energy will be carried out for $27X10^3$ generations with a mutation rate of 0.02 and a cross over rate of 0.80. The local-energy-minimization algorithm was limited to 100 steps for 6% of the population. To explore the conformational space of ligands, the overall translation steps was set to 0.2 Å, and the overall rotation and torsion rotation step were set to 5 in the docking studies. The auto dock 4.0 program in ADT was executed and the docking scores were reported using binding free energy energies in kcal/mol.

Molecular Interactions Visualization using PyMOL

The bound complex with the receptor and ligand was visualized using PyMOL molecular Visualization Software (Schrodinger and DeLano, 2020). PyMOL is a user-sponsored molecular visualization system on an open-source foundation, maintained and distributed by Schrödinger. PyMOL, a cross-platform molecular graphics tool, has been widely used for three-dimensional (3D) visualization of proteins, nucleic acids, small molecules, electron densities, surfaces, and trajectories. It is also capable of editing molecules, ray tracing, and making movies.

Results and Discussions

I. Phytochemical Analysis of the sample (1) *Justicia tranquebariensis* L.f. contains Alkaloids, Phenols, Tannins, Flavonoids, Carbohydrate, Steroids, Glycosides and Protein, sample (2) *Adhatoda vasica*, Linn., results with the presence of Alkaloids, carbohydrate, protein, flavonoids and terpenoids and the sample (3) *Eclipta prostrata*, L., reveals the presence of Alkaloids, Phenols, Tannins, Flavonoids, Carbohydrate, Steroids, terpenoids and Protein.

S. No	Compounds	*Justicia tranquebariensis* L.f.	*Adhatoda vasica,* Linn.,	*Eclipta prostrata*, Linn.,
1.	Carbohydrate	+ +	+	+ +
2.	Proteins	-	+ +	+
3.	Reducing sugars	-	-	-
4.	Steroids	+ +	-	+ +
5.	Glycosides	+ +	-	-
6.	Flavonoids	+ +	+ +	+ +
7.	Alkaloids	+ +	+ +	+ +
8.	Tannins	+	-	+ +
9.	Saponins	-	-	+ +
10.	Terpenoids	-	+ +	+ +

Table - 1 Phytochemical Analysis of the selected medicinal plant
++ Present + Partial present – Absent

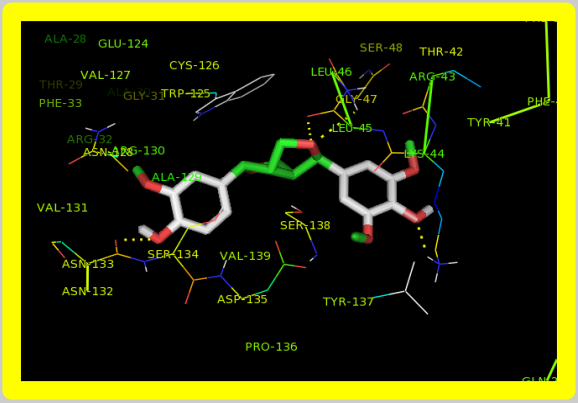
II. Molecular Docking

Sample - 1 *Justicia tranquebariensis* L.f

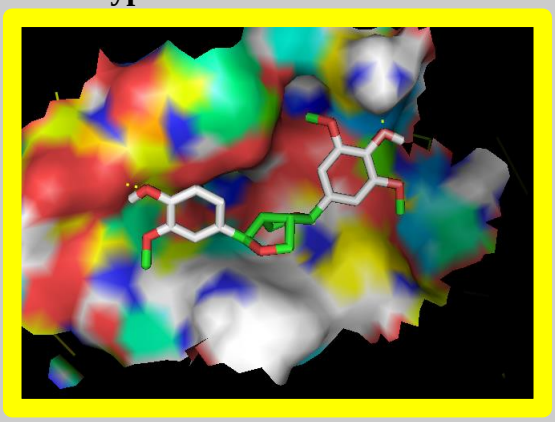
Potential target for anti-oxidant activity is NADPH oxidase (Nox enzymes), that play a central role because they can regulate other enzymatic sources of ROS (Tomasz and David, 2006). Hence the Drug Target was chosen as NADPH oxidase and the structure was downloaded from PDB with PDB Id, 7u8g.
The binding energy between Isolariciresinol and NADPH oxidase, was -4.78 kcal/mol with a ligand Efficiency of -0.18 kcal/mol. 314.02 µM of Isolariciresinol is required to acquire half maximum Inhibition in NADPH oxidase. There are 2 Hydrogen bonds formed between Leucine 45 and Glycine 47 of NADPH oxidase and Oxygen atoms of Isolariciresinol. The binding energy between 4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4- c]furan-6-yl]-2,6-dimethoxyphenol and NADPH oxidase, was -5.98 kcal/mol with a ligand Efficiency of -0.21 kcal/mol. 41.48 µM of **4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3- methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-2,6-dimethoxyphenol** is required to acquire half maximum Inhibition in NADPH oxidase. There are 2 Hydrogen bonds formed between Asparagine 133 and Lysine 33 of NADPH oxidase and Hydrogen and Oxygen atoms of 4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4- c]furan-6-yl]-2,6-dimethoxyphenol respectively**.**

The Docking of the Phytocompounds was compared with the Standard Ascorbic acid and the binding energy between Ascorbic acid and NADPH oxidase, was -4.64 kcal/mol with a ligand Efficiency of -0.39 kcal/mol. 397.52 µM of Ascorbic acid is required to acquire half maximum Inhibition in NADPH oxidase. There are four Hydrogen bonds formed between O of Arginine 43 and Oxygen of Ascorbic acid and another Hydrogen bond between Glycine 47 and Oxygen atom of Ascorbic acid. Among the compounds that were docked both the Compounds, Isolariciresinol and 4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4- c]furan-6-yl]-2,6-dimethoxyphenol showed better affinity than the Standard Ascorbic acid due to its least binding energy. Also, the IC50 value 4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3- methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-2,6-dimethoxyphenol was 41.48 µM which is much less than the Standard Ascorbic Acid as well as Isolariciresinol, thus can be a potential candidate for anti-oxidant activity

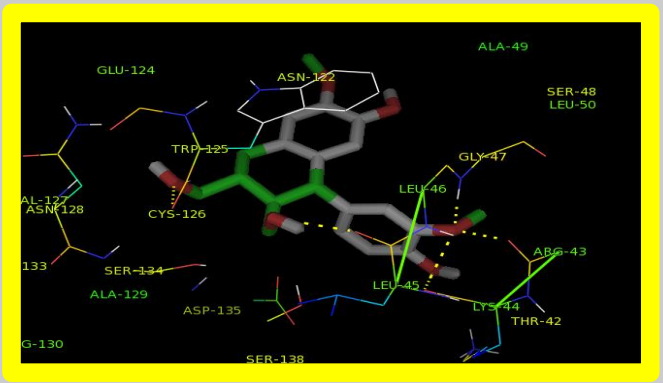
Docked conformation between 4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-2,6-dimethoxyphenol And NADPH oxidase



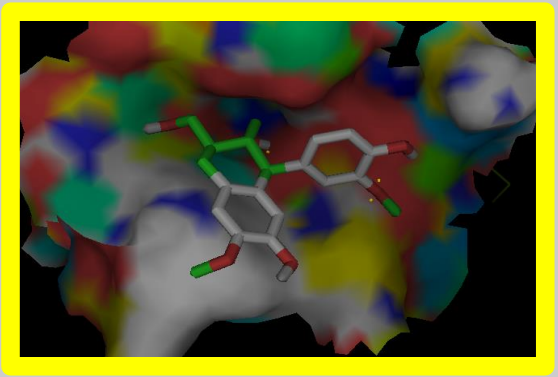
Surface representation of Docked conformation between 4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-2,6-dimethoxyphenoland NADPH oxidas



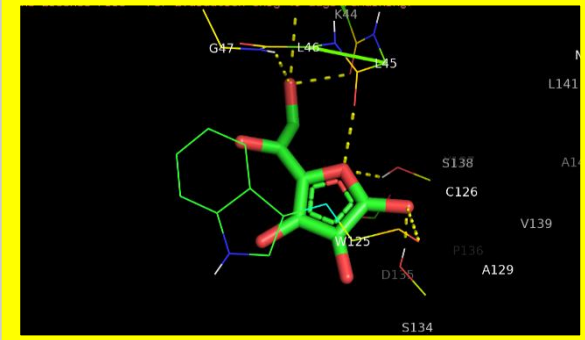
Docked conformation between Isolariciresinol and NADPH oxidase



Surface representation of Docked conformation between Isolariciresinol and NADPH oxidase



Docked Conformation of Ascorbic Acid and NADPH Oxidase



Surface representation of Docked Conformation of Ascorbic Acid and NADPH Oxidase

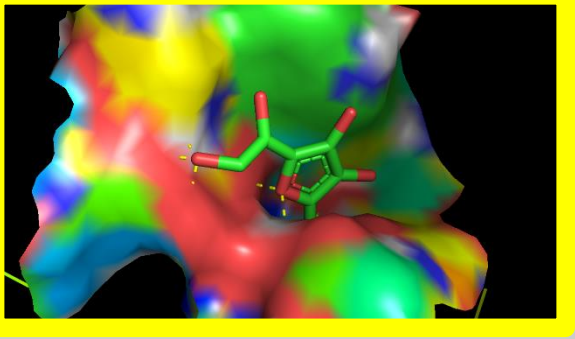


Table - 2 Anti-Oxidant Activity of *Justicia tranquebariensis* L.f.

S.No	Compound	Binding energy (KJ/mol)	Ligand efficiency (KJ/mol)	Inhibitory constant	No. Of hydrogen bonds	Hydrogen bond
1.	Isolariciresinol	-4.78	-0.18	314.02 µM	2	Lig:H::Leu45:O Gly47:N::Lig:O
2.	4-[(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-2,6-dimethoxyphenol	-5.98	-0.21	41.48 µM	2	Lig:H::Asn133:O Lys33:H::Lig:O
3.	Ascorbic acid	-4.64	-0.39	397.52 µM	4	Arg43:O::LigO Gly47:NH::Lig:O Ser134:HG::Lig:O Leu45:O::Lig:O

Sample - 2 *Adhatoda vasica* Linn,

Potential target for anti-oxidant activity is NADPH oxidase (Nox enzymes), that play a central role because they can regulate other enzymatic sources of ROS (Tomasz and David, 2006). Hence the Drug Target was chosen as NADPH oxidase and the structure was downloaded from PDB with PDB Id, 7u8g.

The binding energy between Luteolin and NADPH oxidase, was -5.73 kcal/mol with a ligand Efficiency of -0.27 kcal/mol. 62.85 µM of Luteolin is required to acquire half maximum Inhibition in NADPH oxidase. There are three Hydrogen bonds formed between Aspartic acid 135, Serine 138 and Lysine 44 of NADPH oxidase and Oxygen/Hydrogen atoms of Luteolin.

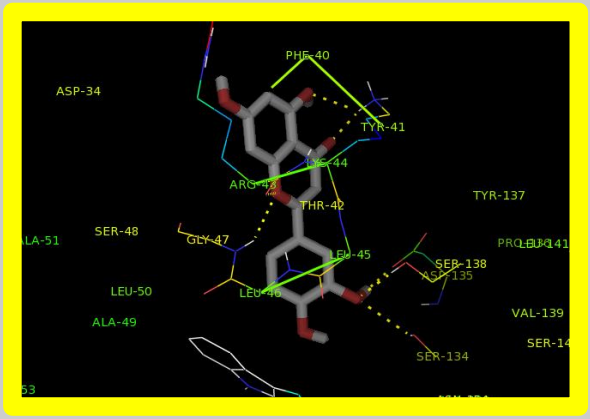
The binding energy between **Kaempferol** and NADPH oxidase, was -5.86 kcal/mol with a ligand Efficiency of -0.28 kcal/mol. 50.26 µM of **Kaempferol** is required to acquire half maximum Inhibition in NADPH oxidase. There are 4 Hydrogen bonds formed between Aspartic acid 135, Lysine 44, Asparagine 133 and Serine 138 of NADPH oxidase and Oxygen atoms of **Kaempferol**.

The Docking of the Phytocompounds was compared with the Standard Ascorbic acid and the binding energy

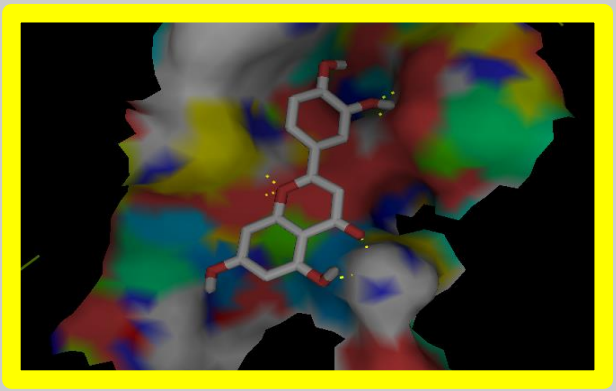
between Ascorbic acid and NADPH oxidase, was -4.64 kcal/mol with a ligand Efficiency of -0.39 kcal/mol. 397.52 µM of Ascorbic acid is required to acquire half maximum Inhibition in NADPH oxidase. There are four Hydrogen bonds formed between O of Arginine 43 and Oxygen of Ascorbic acid and another Hydrogen bond between Glycine 47 and Oxygen atom of Ascorbic acid. The third Hydrogen bond is formed between Serine 134 of NADPH Oxidase and Oxygen of the Standard Ascorbic acid and the last hydrogen bond between Leucine 45 and Oxygen of Ligand.

Among the compounds that were docked both the Compounds, Luteolin and **Kaempferol** showed better affinity than the Standard Ascorbic acid due to its least binding energy. Also, the IC50 value **Kaempferol** was 50.26 µM which is much less than the Standard Ascorbic Acid as well as Luteolin, thus can be a potential candidate for anti-oxidant activity.

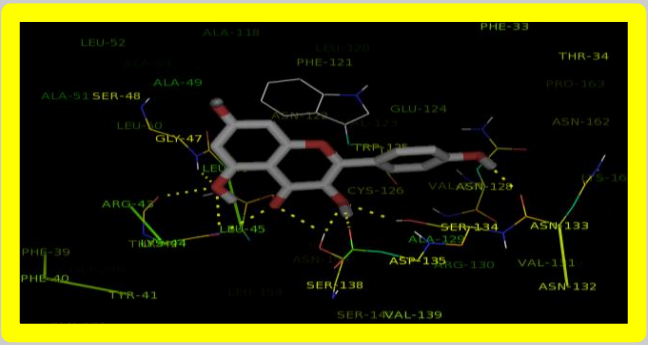
Docked conformation between Luteolin and NADPH oxidase



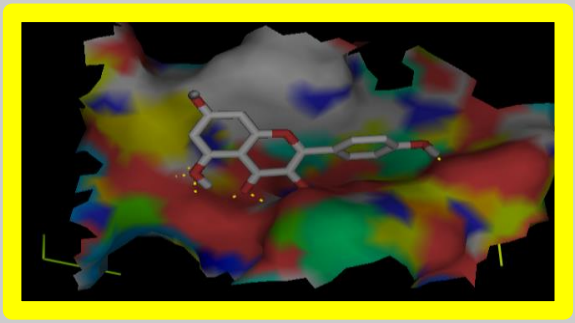
Surface representation of Docked conformation between Luteolin and NADPH oxidase



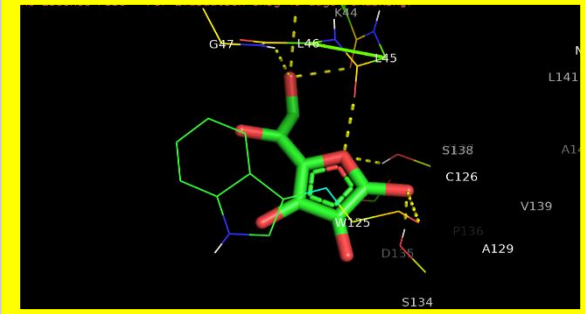
Docked conformation between Kaempferol and NADPH oxidase



Surface representation of Docked conformation between Kaempferol and NADPH oxidase



Docked Conformation of Ascorbic Acid and NADPH Oxidase



Surface representation of Docked Conformation of Ascorbic Acid and NADPH Oxidase

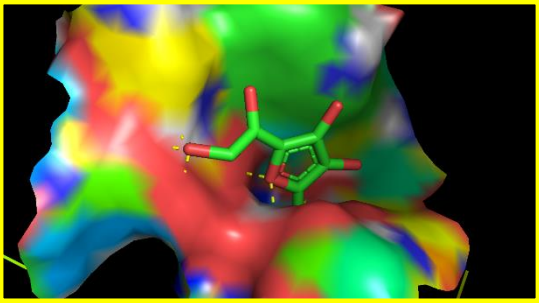


Table - 3 Anti-oxidant Activity of *Adhatoda vasica* Linn,

S. No	Compound	Binding energy (KJ/mol)	Ligand efficiency (KJ/mol)	Inhibitory constant	No. Of hydrogen bonds	Hydrogen bond
1.	Luteolin	-5.73	-0.27	62.85 µM	3	Lig:H::Asp135:O Ser138:H::Lig:O Lys44:H::Lig:O
2.	Kaempferol	-5.86	-0.28	50.26 µM	4	Lig:H::Asp135:O Lig:H::Lys44:O Lig:H::Asn133:O Ser138:H::Lig:O
3.	Ascorbic acid	-4.64	-0.39	397.52 µM	4	Arg43:O::Lig:O Gly47:NH::Lig:O Ser134:HG::Lig:O Leu45:O::Lig:O

Sample - 3 *Eclipta prostrata*.L

Potential target for anti-oxidant activity is NADPH oxidase (Nox enzymes), that play a central role because they can regulate other enzymatic sources of ROS (Tomasz and David, 2006). Hence the Drug Target was chosen as NADPH oxidase and the structure was downloaded from PDB with PDB Id, 7u8g.

The binding energy between **Diosmetin** and NADPH oxidase, was -5.96 kcal/mol with a ligand Efficiency of -0.27 kcal/mol. 43.02 µM of Diosmetin is required to acquire half maximum Inhibition in NADPH oxidase. There are three Hydrogen bonds formed between Lysine 44, Tryptophan 125 and Serine 134 of NADPH oxidase and Oxygen atoms of Diosmetin.

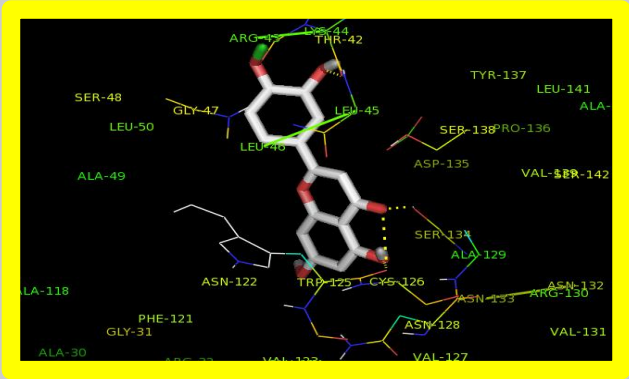
The binding energy between alpha-Terthienylmethanol and NADPH oxidase, was -5.66 kcal/mol with a ligand Efficiency of -0.33 kcal/mol. 71.44 µM of alpha-Terthienylmethanol is required to acquire half maximum Inhibition in NADPH oxidase. There is a Hydrogen bond formed between Asparagine 133 of NADPH oxidase and Oxygen atom of alpha- Terthienylmethanol.

The Docking of the Phytocompounds was compared with the Standard Ascorbic acid and the binding energy between Ascorbic acid and NADPH oxidase, was -4.64 kcal/mol with a ligand Efficiency of -0.39 kcal/mol. 397.52 µM of Ascorbic acid is required to acquire half maximum Inhibition in NADPH oxidase. There are four Hydrogen bonds formed between O of Arginine 43 and Oxygen of Ascorbic acid and another Hydrogen bond between Glycine 47 and Oxygen atom of Ascorbic acid. The third Hydrogen bond is formed between Serine 134 of NADPH Oxidase and Oxygen of the Standard Ascorbic acid and the last hydrogen bond between Leucine 45 and Oxygen of Ligand.

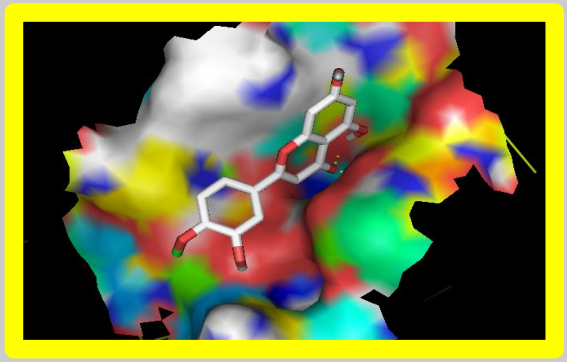
Among the compounds that were docked both the Compounds, **Diosmetin** and alpha- Terthienylmethanol showed better affinity than the Standard Ascorbic acid due to its least binding energy. Also, the IC50 value Diosmetin was 43.02 µM which is much less than the Standard Ascorbic Acid as well as alpha-Terthienylmethanol, thus can

be a potential candidate for anti- oxidant activity.

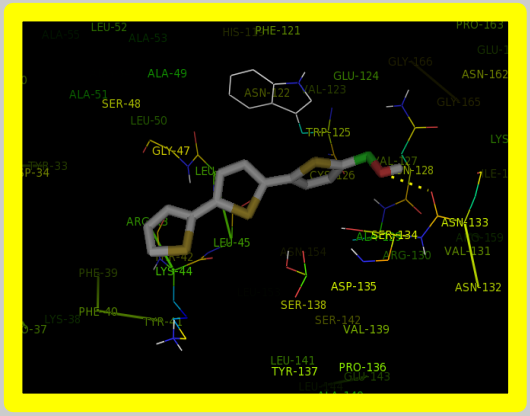
Docked conformation between Diosmetin and NADPH oxidase



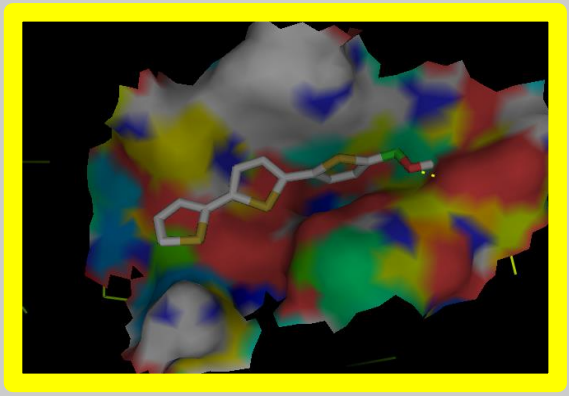
Surface representation of Docked conformation between Diosmetin and NADPH oxidase



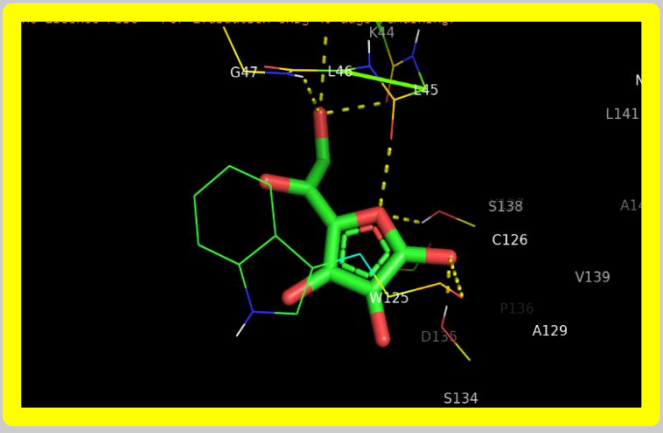
Docked conformation between alpha-Terthienylmethanol and NADPH oxidase



Surface representation of Docked conformation between alpha-Terthienylmethanol and NADPH oxidase



Docked Conformation of Ascorbic Acid and NADPH Oxidase



Surface representation of Docked Conformation of Ascorbic Acid and NADPH Oxidase

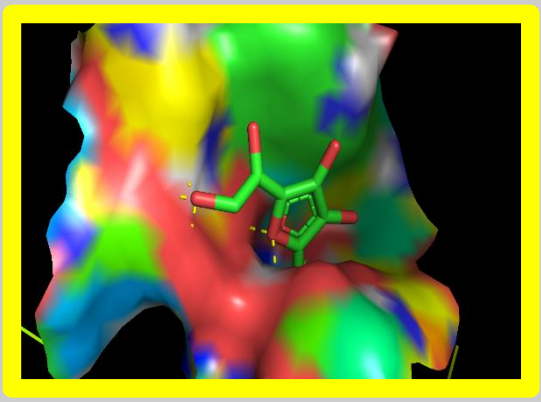


Table -2 Anti-oxidant Activity of *Eclipta prostrata*, L.,

S. No	Compound	Binding energy (KJ/mol)	Ligand efficiency (KJ/mol)	Inhibitory constant	No of hydrogen bonds	Hydrogen bond
1.	Diosmetin	-5.96	-0.27	43.02 µM	3	Lig:O::Lys44:O Lig:H::Trp125:O Ser134:H::Lig:O
2.	alpha-Terthienylmethanol	-5.66	-0.33	71.44 µM	1	Lig:H::Asn133:O
3.	Ascorbic acid	-4.64	-0.39	397.52 µM	4	Arg43:O::Lig:O Gly47:NH::Lig:O Ser134:HG::Lig:O Leu45:O::Lig:O

Summary and Conclusion

All the selected samples have the activities such as antibacterial, anticancer, antifungal, antioxidant, and ant proliferative activities. As the result of phytochemical analysis of the selected samples (*Justicia tranquebariensis* L.f., *Adhatoda vasica* Linn and *Eclipta prostrata*.L) *Eclipta prostrata*.L., have more number of compounds among the selected samples. And the molecular docking studies of the selected sample shows the different compounds like *Justicia tranquebariensis* L.f., contains Isolariciresinol, 4- [(3R,3aS,6R,6aS)-3-(4-hydroxy-3-methoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c] furan-6-yl]-2,6-dimethoxyphenol and Ascorbic acid. *Justicia adhathoda*,Linn., contains Luteolin, Kaempferol and Ascorbic acid. *Eclipta prostrata*.L contains Diosmetin, alpha-Terthienylmethanol and Ascorbic acid. Alkaloids directly act on the Central Nervous System in the human body. The compound tannins is useful in the therapy of diseases related to the oxidative stress such as colorectal cancer and heart and liver damage. Carbohydrate acts as an energy source helps in control blood glucose, insulin metabolism and helps in fermentation. Glycolysis helps in the sodium potassium metabolism. Protein reduces the appetite and gives strength to the bones and muscles and lowers the blood pressure. Flavonoids contains ant oxidative activity and good for heart diseases. Steroids will reduce the swelling and inflammation in our human body. Reducing sugar is good in weight gain. Terpenoids compounds are enrich in anti-inflammatory, anti-diabetic and used as a diuretic. Saponins decrease blood lipids, lower cancer risks and lower blood sugar level.

Among these compounds the selected medicinal plants contain carbohydrates, flavonoids, alkaloids in all the three plants. Hence these plants are used as a folk medicine for various diseases like fever, anemia, cold, cough and digestive diseases.

References

Alka Sharma and Ashwani Kumar, (2018). Analysis of Antimicrobial activity of *Justicia adhathoda* Linn World *Journal Pharmaceutical Science*, (5), 1332-1342.

Akilandeswari.S, Manimaran.S, Valarmathi.R, Karpagam Kumara.S and Sundari.V, (2001). Phytochemical Observation on the Leaf of *Justicia tranquebariensis*. L.F, *National Library of Medicine*, 22557013, 60-61.

Balaji karuppaiah, Perumal saraswathi, Prabhu.K, Melani rajendran and Shaila Samuel (2019) Preliminary Phytochemical Screening of *Eclipta prostrate (L)*, World journal of pharmaceutical research, 8(3), 783-789.

Begum.M.S, Ilyas.M, and Burkanudeen.A, (2011). Protective and curative effects of *Justicia tranquebariensis (Linn)* leaves in acetaminophen induced hepatotoxicity, International Journal of Pharmaceutical and Biological Archives, (2), 0976-3333, 989-995.

Bharathi D, Rajalakshmi S and PT kalaichelven, (2018) Studies of Biological synthetic of sliver nanoparticles by using leaf extract of *Justicia adhathoda*, Linn, Journal of Phytochemical (7), 391-395.

Dhankhar Sandeep, Kaur Ramanjeet, Ruhil S, Balhara M, Dhankhar Seema and Chhillar AK, (2011). Studies of A Review of *Justicia adhathoda* Linn ., Potential source of natural medicine *Africa journal of plant science*, 5(11), 620-627.

Hari V, Jothishwari D and Sesha Maheswarmma K, (2021). Evaluation Of Antitumor Activity Of *Justicia tranquebariensis L.F* Natural Volatiles & amp; Essential Oils Journal, 8(6), 2148-9637, 5038-5048.

Jothishwari.D, Hari.V, Naveena.N, Swetha.S and Ananda Deepak, (2021). Computer Aided Perspective Study of *Justecia tranquebariensis* on Rabies Virus, *Journal of Advances in Bio- pharmaceutics and Pharmacovigilance*, 3(1).

Jyoti rani, Manish kapoor and Rupinder kaur , (2020) In-vitro phytochemical screening of *Eclipta prostrata (L.)* Leaves extracts, Plant archives, (20), 0972-5210, 2055-2060.

Kannadas. S, Pathirana.R N and Ratnasooriya, (2020) In-vitro investigation of anti- inflammatory activity and evaluation of phytochemical profile of *Eclipta prostrata (L.)*, Journal of pharmacognosy and phytochemistry, (9), 2349-8234, 831-835.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2023). PubChem 2023 update. Nucleic Acids Res., 51(D1), 1373 - 1380.

Krishnamoorthi.R,(2015). Phytochemical Screening And Antioxidant Activity Of *Justicia tranquebariensis* and *Bauhinia racemosa*, International Journal Of Pharmacognosy, 7(2), 2348- 3962, 362-367.

Krishnamoorthi R and Radha Bai V, (2015) Phytochemical Analysis And Antioxidant property of *Justicia tranquebariensis*, International Journal Of Pharmacognosy, 10(8), 0975- 8232, 254-258

Kumeshini Sugalingam , Kumar Ganesan and Baojun Xu,(2018) Protective Effect Of Aqueous Extract From The Leaves Of *Justicia tranquebariesis* Against Thioacetamide-Induced Oxidative Stress And Hepatic Fibrosis .

Lalita Singh, Reena Antil, Dheeraj Kumar and Pushpa Dahiya, (2019). Phytochemical analysis and In-vitro assays for antimicrobial and antioxidant activity of Bhringraj herb *Eclipta prostrata (L.), Journal of Pharmacognosy and Phytochemistry* , 8(3) , 4527-4533.

Malathi R, Kaviyarasan D and Chandrashekhar S, (2018) Analysis of preliminary phytochemical analysis of *Justicia adhathoda,* Linn. Leaves extract using different solvents. *International journal of pharmacology and drug analysis*, (7), 186-190.

Pugazhenthen thangaraju , and Sree Sudha T Y,(2021) Evaluation of the role of *Justicia adhathoda,* Linn . As prophylaxis for covid -19- Analysis Based on Docking, Journal of infections disorders -drug target, 21(8), 1-7.

Rashmi P A, Reshma John and Linu Mathew,(2012) Analysis of Isolation and characterization of vasicine from in vitro culture of *Justicia adhathoda,* Lin, Indian journal of pharmacology and Biotechnology ,(3), 58-64.

Risfa M Samanudeen, Banukie N Jayasuriya, Liyanage Dona Ashanthi Menuka Arawwawala ,Priyani Hettiarachchi and Sugandhika Suresh.T ,(2024) Pharmacognostical studies of *Eclipta prostrata (L.)* grown in sri lanka Boletin latinoamericano y del caribe de plantas medicinales y aromáticas , (23), 0717-7917, 248-256.

Saritha.B and Brindha.P, (2013) Review on Ethnopharmacognosy of *Justicia tranquebariensis L.*, A Traditional Siddha Drug, International Journal of Pharmtech Research, (5), 0974-4304, 467-474,

Sameera Begum and Rajasekhar .S,(2023) Pharmacological Screening Of Antidiabetic Activity Of Ethanolic Extracts Of *Justicia tranquebarensis* On Streptozocin Induced Rats, World Journal Of Current Medical and Pharmaceuticals Research , 6(5), 2582-0222.

Schrödinger3] L.,&Delano, W. (2020). PyMOL. Retrieved from http://www.pymol.org/pymol

Shikha Sharma , Richa and Harsimran , (2017) Phytochemical and anatomical screening of *Eclipta prostrate (L)* an important medicinal herb from Chandigarh , Journal of medicinal plants studies , (5) , 2320-3862 .

Shipam sinha and Richa raghuwanshi, (2016) the phytochemical screening and antioxidant potential of *Eclipta prostrata (L)*, International journal of pharmacy and pharmaceutical sciences, 8(3), 256-260.

Thiravia Doss Dons and Sebastian Soosai Raj, (2016) Spectral And Antibacterial Studies On Some Ethnobotanically Important Medicinal Plants Used By Thottianaickans Of Semmalai, Indo American Journal of Pharmaceutical Research, 7(5) , 2617-2625.

Tomasz J. Guzik, David G. Harrison, (2006) Vascular NADPH oxidases as drug targets for novel antioxidant strategies, Drug Discovery Today, 11(11–12), 1359- 6446, 524-533.

Usha Gutti , Jaswant Kumar Komati and Aneesh ,(2006) Studies of *Justicia adhathoda,* Linn . Induces Megakaryocyte different through Mitochondrial Ros Generation, Journal of Phytomedicine, (43), 135-139.

Velpandian.V, Elangovan.S, Naansi Agnes. L and Mohamed Musthafa. M,(2014) Clinical Evaluation Of *Justicia tranquebariensis L.* In The Management of Bronchial Asthma, American Journal of Phytomedicine and Clinical Therapeutics, 2321 – 2748, 1103-1111,

Wantkhem Sandhya chanu and Kanambala sarangthem, (2014.) Analysis of Phytochemical constituent of *Justicia adhathoda,* Linn. Found in Manipur, India journal of plant science, 3(2), 19-20.

Wipawan Pukumpuang, Sunee Chansakaow and Yingmanee Tragoolpua (2014), Antioxidant activity, phenolic compound content and phytochemical constituents of *Eclipta prostrata (L.),* (41), 568-576.

Zafar alam khan, Salaj khare and Dubey.B.K,(2020) Comparative extraction phytochemical screening and biological activities of *Eclipta prostrata* extract ,journal of drug delivery and therapeutics ,(10), 2250-1177.