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In Silico and in Vitro Evaluation of Antihyperlipidemic and Antioxidant Activity of *Vigna Mungo* Seed Extract

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Abstract:

Vigna mungo is a legume which provide significant amount of nutrients. *Vigna mungo* is one of the important pulse crop in India. It is rich in protein content and traditionally used for treating various disease conditions. The present investigation is conducted to evaluate the potential of *Vigna mungo* seeds against hyperlipidemia. These compounds were analysed for its activity by molecular docking. The ethanolic extract of both seeds were subjected to qualitative analysis to determine the presence of phytochemicals followed by TLC and HPTLC analysis for the identification of bio constituents. Total phenolic content(TPC) and Total flavonoid content(TFC) of the seed extracts was determined. *In vitro* antioxidant activity was performed by DPPH assay method and IC_{50} value was determined. *In vitro* antihyperlipidemic activity using lipid peroxidation assay method was performed. Ethanolic extract of *Vigna mungo* seed extracts show the presence of alkaloids, flavonoids, glycosides, phenolic compounds, proteins and terpenoids.

Keywords: *Vigna mungo*, HPTLC, In-silico, Docking, Lipid peroxidation.

INTRODUCTION

Vigna mungo is commonly known as Black gram or Urad Dal. It belongs to the family, Fabaceae (Leguminosae), which is a family of flowering plants commonly known as the legume or pea family. Herbal extracts are substances prepared from parts of herbal raw material, usually by using a different solvent. The extraction process is often designed to maximize one specific part of the plant's chemical compounds, many of which have therapeutic effects. *Vigna mungo* or Black gram is an erect, fast growing, annual, herbaceous legume reaching 30-100 cm in height. It is having a well-developed taproot and its stems are widely branched from the base. On occasion it has a twinning habit and is mainly pubescent. The leaves are trifoliate with ovate leaflets 4-10 cm long and 2-7 cm wide. The inflorescence is borne at the extremity of long peduncle and bears yellow, small, papilionaceous flowers. The fruit is a cylindrical, erect pod. The pod is hairy and has a short-hooked beak.

Also it includes 4-10 ellipsoid black or mottled seeds[2,3]. *Vigna mungo* can be used for different purposes like improving digestion, promotes heart health, supports skin health, enhances energy levels, and promotes muscle health. They contain high concentration of proteins and fibres and can help to reduce the overall calorie intake, leading to weight loss. Docking is a "lock-and-key" concept, in that the "lock" is the protein, and the "key" is the ligand. The ligand and the protein alter their conformation to yield the best fit and is thus known as "induced - fit". It is also a valuable tool in drug discovery since it saves time and money. It may also be used to repurpose medicinal plants for various diseases[2]. Docking can be combined with traditional knowledge of medicinal plants to help select plants for bioactivity evaluation studies.

MATERIALS AND METHODS

COLLECTION OF *VIGNA MUNGO* SEEDS

The *Vigna mungo* plants were widely cultivated along the boundaries of paddy fields in Kerala and also in various South Indian states. The seeds are available usually in fresh form. Seeds for study were collected from the local market near Palakkad, Kerala.

Figure 1:*Vigna mungo* plant

AUTHENTICATION OF PLANT

The authentication of the plant *Vigna mungo* (L.) Huth. was done by Dr. Sherief, Scientist F, Botanical Survey of India, Southern region, Tamilnadu Agricultural University, Coimbatore, Tamilnadu.

PREPARATION OF SEED EXTRACT

In Soxhlet Extraction,

After the collection of dried seeds of *Vigna mungo* it is ground into powder. Coarse were separated by sieving using sieve no:10 and weighed accurately. Weighed materials were rolled in a filter paper and is kept inside the condenser of the Soxhlet apparatus. Extraction was carried out with aid of70% ethanol at room temperature for 72 hours. The filtrate of ethanolic solutions was evaporated under reduced pressure at room temperature.

Preparation of test solution

Seed extracts is diluted to suitable concentration with the aid of ethanol.

Pleliminary phytochemical screening for carbohydrates, alkaloids, saponins, steroids, terpenoids, tannins, flavonoids,phenols, cardiac glycosides, proteins was done.

ESTIMATION OF TOTAL FLAVONOID CONTENT

Aluminium chloride colorimetric assay method is used according to *Zhishen et al.*1ml of diluted extracts were added to test tube containing 4ml of double distilled water. After 5min, 0.3ml of 5%$NaNO_2$ were added to the test tube followed bt the addition of 0.3ml of 10% $AlCl_3$. Add 2ml of 1M NaOH was added and the total volume is made upto 10ml with double distilled water. The blank solution is prepared using doubled distilled water instead of the test sample. The obtained solution is mixed thoroughly and the absorbance is measured at 510nm against the blank solution. Total flavonoid content of the sample were expressed as mg catechin equivalent per gram of the extract.

ESTIMATION OF TOTAL PHENOLIC CONTENT

Folin- Ciocalteau reagent were used for the determination of total phenolic content using Gallic acid as standard. 1ml of diluted extracts were added to 50ml volumetric flask containing 1ml of Folin-Ciocalteau reagent diluted in a ratio of 1:2 and add 2ml of 20% Na_2CO_3 solution and the volume is made upto 50ml. The mixture was allowed to stand for 50 min. The obtained mixture was centrifuged at 6000rpm for 10 min. The absorbance of the supernatant solution was measured at 730nm using Shimadzu UV- Visible spectrophotometer against blank solution. The obtained phenolic content was multiplied by the dilution factor and the results were expressed as mg Gallic acid equivalent per gram of extract.

THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS

Ethanolic extracts of the seed was separated on a silica gel thin layer aluminium plates of 3mm thickness which is pre coated. The extracts were spotted on the plate manually
using capillary tube. The solvent system used for the separation of flavonoid is ethyl acetate: formic acid: glacial acetic acid: water(100:11:11:26). After separation the spots were identified and retention factor(R_f) is calculated using the formula:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) ANALYSIS

HPTLC SAMPLE PREPARATION

- Smalll quantity of seed extracts were dissolved in methanol in a centrifuge tube.
- It was then sonicated for 15min and centrifuged for 5min at 5000RPM.
- Resultant supernatent solution was used for further study.

HPTLC METHOD

- In the analysis, Silica gel 60F 254 Precoated plates(100×100mm) were used with ethyl acetate: formic acid: acetic acid: water (100:11:11:26) as mobile phase.
- Samples were spotted in the precoated HPTLC Plate and the solvent front position is 70mm.
- The HPTLC plates were scanned at 254 and 366nm.

MOLECULAR DOCKING

1. Data collection of receptor proteins and ligand compounds

The first step in molecular docking was the determination of protein receptors and ligand compounds. In this research process, proteins such as adrenergic receptor, HMG CoA reductase, Human- C Reactive protein, MAPK proteins of specific codes 2YCW, 1HW9, 1BO9, 4DLI as protein receptors was used. These protein receptors were obtained by downloading the protein structure in PDB format from Protein data Bank website and the 3D structure of ligand was downloaded from the PubChem website.

2. Download of receptor proteins and ligand compounds

The proteins were downloaded through the Protein Data Bank website (https://www.rcsb.org/pdb). Open the website, then in search menu, enter the protein code to be downloaded (2YCW:Adrenergic receptor, 1HW9: HMG CoA reductase, 1B09: Human-C-Reactive protein, 4DLI: MAPK). The protein file was opened until detailed information of the protein appears, then Download File was selected. The selected file is in PDB format.

3. Protein separation with accompanying components

The protein structure downloaded from Protein Data Bank is a complex structure that contains several components, including native ligands and heteroatoms. Before initiating the molecular docking process, all the components in the protein structure are removed leaving one protein molecule used as a receptor when running docking. Molecular docking only requires one protein molecule as receptor. Other compounds need to be removed by clicking on the option to be deleted(including proteins with symbols for the letters B,C,D,E,F,G and H, heteroatoms, active sites, and ligand group) them click the 'X' option in turn. The remaining protein molecules are receptors that will be used in the docking process.

4. Receptor preparation

In receptor preparation, the first step is opening PyRx software. Protein molecules are enterd into the software by selecting the 'File' menu, followed by clicking the 'Load Molecules' command. The selected molecule is a protein file separated with itsaccompanying the components. Select the 'AutoDock' and click on the 'Make Macromolecule' command to change the receptor format from '.pdb' to '.pdbqt'.

5. Ligand preparation.
6. Running molecular docking using PyRx.
7. Visualization of docking results using Biovia Discovery Studio

IN VITRO ANTIOXIDANT ACTIVITY

The antioxidant activity of *Vigna mungo* was performed by DPPH radical scavenging assay method.

DPPH Radical Scavenging Assay Method

The stable 2, 2- Diphenyl-1-1-picrylhydrayzl (DPPH) radical shows maximum absorbance at 517nm which impart purple colour. The odd electron which reduces at 517nm turning purple to yellow when becomes paired with hydrogen. The disappearance of its violet colour confirm the radical scavenging activity. IC_{50} is the ability

of sample to scavenge 50% of the free radical at corresponding concentration. Low IC_{50} values depicts high antioxidant activity of the sample[8,9].

Stock solution of test and standard 1000µg/ml (10mg in 10ml ethanol), DPPH 200µg/ml (10mg DPPH in 50ml ethanol) was prepared. Various concentration of 20, 40, 60, 80, 100µg/ml test solution were taken. Test solution is prepared in 2:2 ratio of test solution and DPPH was taken and incubate at dark condition for 30min. Control of ratio 2:2 of 2ml ethanol and DPPH was taken. Blank used is 4ml ethanol. Absorbance was measured at 517nm using UV spectrophotometer.

IN VITRO ANTI HYPERLIPIDEMIC ACTIVITY

The antihyperlipidemic activity of *Vigna mungo* was performed by TBARS assay method.

Preparation of liver homogenate

Freshly collected fatty liver of chicken was excised and washed with 0.9%NaCl solution. Tissue homogenate was prepared in a ratio of 1gm wet tissue to 10 times(w/v) 0.05M ice cold phosphate buffer and homogenised by using Teflon homogeniser. This prepared homogenate was used for estimation of Thiobarbituric acid Reactive Substance.

TBARS Assay method: The lipid peroxidation of *Vigna mungo* seed extracts were determined using TBARS assay method. 0.25ml of liver homogenate was mixed with 0.1ml Tris HCl buffer, 0.05ml of 0.1mM Ascorbic acid, 0.05ml 4mM $FeCl_2$ solution and 0.05ml of extracts. The extracts were tested at 5 different concentrations (4 - 64µg/ml). The mixture was incubated at 37°c for 1hr and then 1.5ml 0.8%w/v 2-Thiobarbituric acid, 1.5ml 20%acetic acid, and 0.2ml 8.1%w/v sodium dodecyl sulphate were added to the reaction mixture. Then the mixture was made upto 4ml with distilled water and heated at 95^{O}c for 60min. Cooled with tap water, 1ml of distilled water and 5ml of mixture of n-butanol and pyridine (15:1v/v)were added. Mixture was shaken vigorously. Absorbance was measured at 532nm using a spectrophotometer and Ascorbic acid as standard[12].

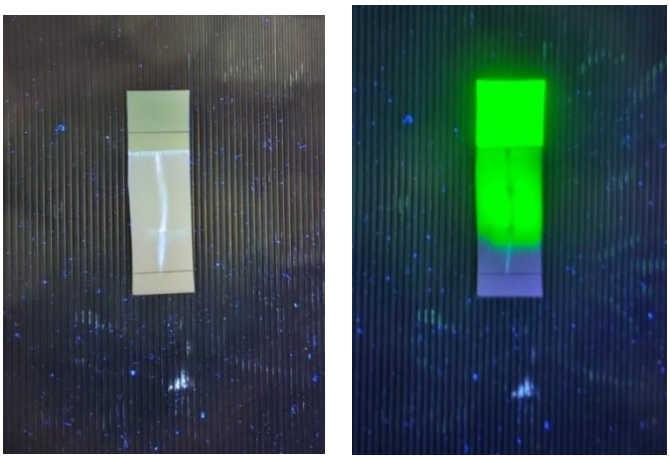
RESULTS & DISCUSSION

Vigna mungo or urad dal is commonly used legumes in daily life. Although it has been proven for its various activities including Antidiabetics, anti bacterial, antimicrobial, Promoting the skin health etc. Herbal extracts are being more popular now a days for the various health benefits. Hence it has replaced the use of synthetic medicines. Herbal products have gained popularity in International Commerce, as it has a promising side effect free health outcome.

Total flavonoid content of seed extract is 173.16±6.72mgQAE/g

Total Phenolic Content of seed extracts is 55.27±1.14mgGAE/g

THIN LAYER CHROMATOGRAPHIC ANALYSIS

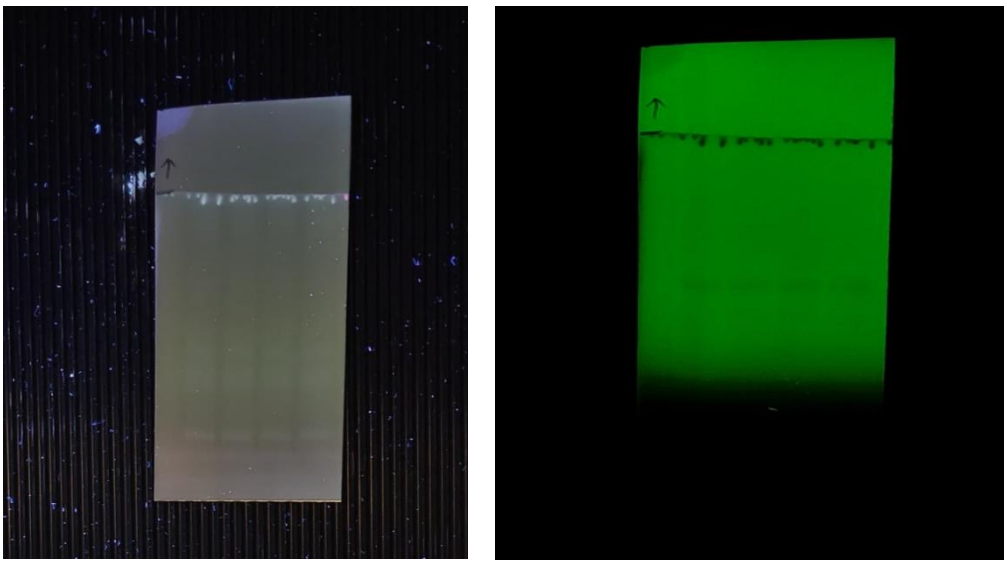


Absorbance @ 366nm Absorbance @ 254nm

Figure 2: Development of the solvent front in TLC plate (*Vigna mungo*)

R_f value of *Vigna mungo* (flavonoids)=3.7/5= 0.74

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

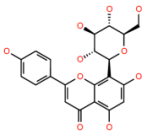
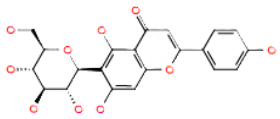
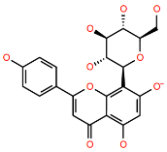
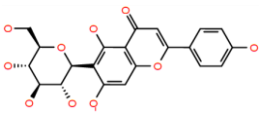


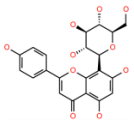
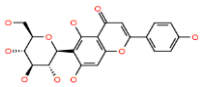
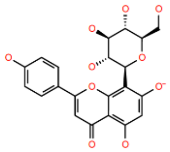
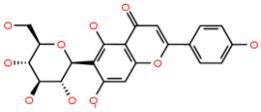
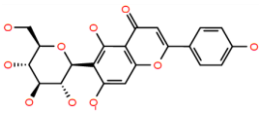
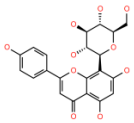
Absorbance @ 366nm Absorbance @ 254nm

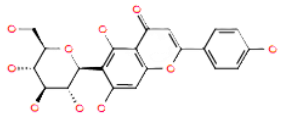
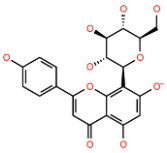
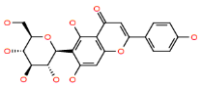
Figure 3: Development of HPTLC plate of seed extracts

R_f value of *Vigna mungo* = 3.2/7 = 0.45

MOLECULAR DOCKING OF *VIGNA MUNGO*

SL NO	**RECEPTOR**	***VIGNA MUNGO***
1	2YCW: β-1 Adrenergic Receptor	1. Vitexin  Score: -11.083
		2. Isovitexin  Score: -10.679
		3. Vitexin  Score: -9.325
		4. Isovitexin  Score: -9.106
2	1CX2: Cox-2	**NIL**

3	1HW9: HMG CoA Reductase	1. Vitexin  Score: -6.059
		2. Isovitexin  Score: -3.504
		3. Vitexin  Score: -3.019
		4. Isovitexin  Score: -1.728
4	108A: Angiotensin Converting Enzyme	1. Isovitexin  Score: -10.07
		2. Vitexin  Score: -8.959

		3. Isovitexin  Score: -8.569 4. Vitexin  Score: -7.843
5	1B09: Human C- Reactive Protein	**NIL**
6	4DLI: Mitogen Activated Protein Kinase	**NIL**
		1. Isovitexin  Score: -10.601

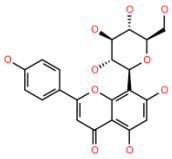
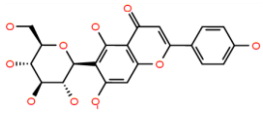
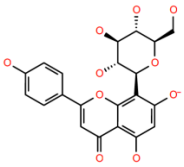
7	4YAY: Human Angiotensin Receptor	2. Vitexin  Score: -9.804
		3. Isovitexin  Score: -7.45
		4. Vitexin  Score: -6.843

Table 1: Docking score of various flavonoids of *Vigna mungo*

Molecular docking was performed for identifying the binding affinity of the flavonoids present in *Vigna mungo* seeds. Major flavonoids present in *Vigna mungo* were Vitexin and Isovitexin. Proteins were downloaded from the protein data bank having PDB ID 2YCW, 1CX2, 1B09, 1HW9, 108A, 4DLI, 4YAY. Docking was done using PyRx software and the dock score were founded.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH ASSAY

In DPPH method, IC_{50} value of *Vigna mungo* was found to be **30.121µg/ml**. IC_{50} value of standard ascorbic acid was found to be **92.826µg/ml**. Hence it is proven that *Vigna mungo* seed extracts shown antioxidant property.

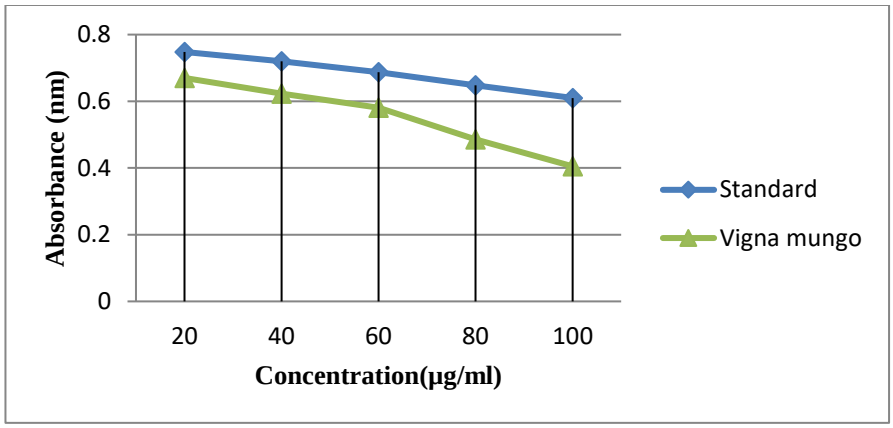


Figure 4: Concentration VS Absorbance curve of DPPH assay

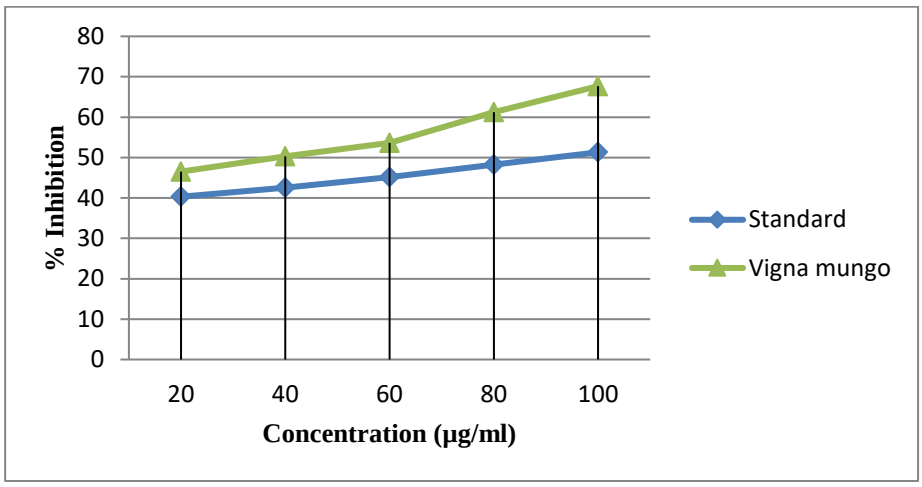


Figure 5: Concentration VS % Inhibition curve of DPPH assay

TBARS ASSAY

The thiobarbituric acid reactive substance (TBARS) assay is a method for measuring lipid peroxidation that can be used to study the effects of antihyperlipidemic drugs. The TBARS assay measures the amount of malondialdehyde (MDA), a product of lipid oxidation that reacts with thiobarbituric acid (TBA) to form a red-pink complex. When the TBARs assay was performed for *Vigna mungo* seed extract, shows the presence of antihyperlipidemic activity.

Teflon Homogenizer: Take one lobe of the fatty chicken liver and wash with 0.9% NaCl solution. Tissue homogenate was prepared in a ratio of 1g wet tissue to 10 times(w/v) 0.05M ice cold phosphate buffer (pH-7.5)

and homogenized by using Teflon homogenizer. The homogenate was used for the estimation of Thiobarbituric Acid Reactive Substance (TBARS) assay.

Figure 6: Teflon homogenizer

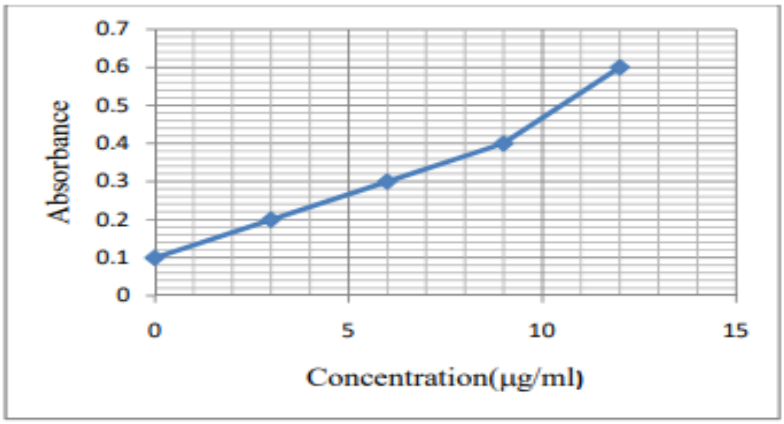


Figure 7: TBARS ASSAY

DISCUSSION

Vigna mungo commonly used traditionally in folk medicine to treat a variety of ailments. It can be used for various purposes like improving digestion, promotes heart health, supports skin health, enhances energy levels, promotes muscle health. It contains high concentration of proteins and fibres and can help to reduce the overall calorie intake leading to weight loss.

Vigna mungo is a rich source of flavanoids and phenolic contents. Flavonoids play an important role to control the growth of toxin-producing bacteria in plants. Flavonoids like Luteolin, glucoluteolin, quercetin and isoquercetin may also be attributed to antioxidant and antihyperlipidemic activity for other plant extracts have been reported. Major flavonoids present in *Vigna mungo* were Vitexin and Isovitexin. Various proteins were downloaded from protein data bank and were subjected to dock and their docking score was determined.

When the DPPH assay was performed for *Vigna mungo* seed extract, shows the presence of antioxidant activity. The thiobarbituric acid reactive substance (TBARS) assay is a method for measuring lipid peroxidation that can be used to study the effects of antihyperlipidemic drugs.

SUMMARY AND CONCLUSION

Molecular docking was performed for identifying the binding affinity of the flavonoids present in *Vigna mungo* seeds. Major flavonoids present in *Vigna mungo* were Vitexin and Isovitexin. Proteins were downloaded from the protein data bank .Docking was done using PyRx software and the dock score were founded. For the protein receptor 2YCW (β-1 Adrenergic Receptor), the dock score, shown that flavonoids, Vitexin has more binding affinity. Stimulation of β1-ARs in the kidney leads to increased water and salt retention, which increases blood pressure and blood volume. The binding affinity of constituents towards β-1 Adrenergic Receptor shows antihypertensive activity. The antioxidant activity of *Vigna mungo* was determined by DPPH assay method. The IC 50 value of *Vigna mungo* was found to be 30.121µg/ml .Lower IC50 value indicates higher antioxidant activity. Hence it has proven that *Vigna mungo* seeds has greater antioxidant potential for free radical scavenging activity.

REFERENCES

1. Mohammed Rageeb Mohammed Usman, Barhate S. D. Estimation of Phytoconstituents and Evaluation of Anti-inflammatory activity of Vigna Mungo Linn. Seeds. Int. J. Res. Pharm. Sci. 2012 Mar. 15 [cited 2025 Jan. 8];3(1):106-12.

2. Arockianathan PM, Rajalakshmi K, Nagappan P. Proximate composition, phytochemicals, minerals and antioxidant activities of Vigna mungo L. seed coat. Bioinformation. 2019; 15(8):579.

3. Mehta N, Rao P, Saini R. A review on metabolites and pharmaceutical potential of food legume crop mung bean (Vigna radiata L. Wilczek). Biotechnologia. 2021; 102(4):425.

4. Patel AK. A Molecular Docking Study of Indirubin against Hyperlipidemia. In Biological Forum–An International Journal 2023 (Vol. 15, No. 2, pp. 246-255).

5. Noor Nailis Sa'adah et.al;Molecular Docking of Anthocyanin Compound as Anti-Hyperlipidemia Against PPARα, HMG-CoA Reductase and ACAT Proteins; Advances in Biological Sciences Research; 2 May 2022.

6. Hill MF, Bordoni B. Hyperlipidemia. In: StatPearls. Treasure Island (FL): StatPearls Publishing 2023 Aug 8.

7. Jing, Y.-S.; Ma, Y.-F.; Pan, F.-B.; Li, M.-S.; Zheng, Y.-G.; Wu, L.-F.; Zhang, D.-S. An Insight into Antihyperlipidemic Effects of Polysaccharides from Natural Resources. Molecules 2022, 27, 1903.

8. Dov Lichtenberg, Ilya Pinchuk; Oxidative stress, the term and the concept; Biochemical and Biophysical Research Communications; Volume 461, Issue 3; 2015; Pages 441-444.

9. Michael A. Chen, MD, PhD, Associate Professor of Medicine, Division of Cardiology,Harborview Medical Center, University of Washington Medical School, Seattle.

10. Arul Amuthan, Bharti Chogtu, K.L. Bairy, Sudhakar, MungliPrakash,Evaluation of diuretic activity of Amaranthus spinosus Linn. aqueous extract in Wistar rats, Journal of Ethnopharmacology,Volume 140, Issue 2,2012,Pages 424-427.

11. Anti Hypertensive Activity of the Ethanolic Extract of Lantana camara leaves on high salt loaded wistar albino rats, Matta, Vijay Kumar, Pasala Praveen Kumar, Netala Silvia, Pandrinki Satish, and Konduri Prasad , Pharmacognosy Journal, 01/2015, Volume 7, Issue 5, p.289-295,(2015).

12. Vallejo-Vaz AJ, Robertson M, Catapano AL, Watts GF, Kastelein JJ, Packard CJ, Ford I, Ray KK. Low-Density Lipoprotein Cholesterol Lowering for the Primary Prevention of Cardiovascular

Disease Among Men With Primary Elevations of Low-Density Lipoprotein Cholesterol Level. 2017 Nov14;136(20):1878-1891.

13. Methods for testing antioxidant activity,critical review the analyst, Michael Antolovich, Paul D. Prenzler, Emilios Patsalides, Suzanne McDonald and Kevin Robards, 23rd November 2001,page no:183-198.

14. Peters SA, Den Ruijter HM, Bots ML, Moons KG. Improvements in risk stratification for the occurrence of cardiovascular disease by imaging subclinical atherosclerosis: a systematic review. Heart. 2012 Feb 1;98(3):177-84.

15. Supasatyankul B, Saisriyoot M, Klinkesorn U, Rattanaporn K, Sae-Tan S. Extraction of phenolic and flavonoid compounds from mung bean (Vigna radiata L.) seed coat by pressurized liquid extraction. Molecules. 2022 Mar 24;27(7):2085.