Research Article

In Silico and *In Vitro* Analysis of Red Kidney Beans (*Phaseolus vulgaris*) and Black-Eyed Beans (*Vigna unguiculata*) and Their Phytochemical Analysis

Afshan Kaleem*, Iqra Noor, Roheena Abdullah and Mehwish Iqtedar

Department of Biotechnology, Lahore College for Women University, Lahore.

Abstract | Beans and legumes are an excellent source of protein, minerals, dietary fiber, carbohydrates and bioactive compounds. They serve as medicinal plants and are used as common food in developing countries. The objective of the current study was to analyze the various biological characteristics of *Phaseolus vulgaris* and *Vigna unguiculata* in order to identify new therapeutic leads for specific disorders (colon cancer and memory improvement in Alzheimer's disease). Phytochemical testing showed the presence of various bioactive compounds including flavonoids, steroids, carbohydrates, proteins, glycosides, saponins, phenols etc. Antibacterial analysis of aqueous extracts of *Phaseolus vulgaris* and *Vigna unguiculata* were done by using the disc diffusion method, which showed that both extracts have potential antibacterial activity against *Staphylococcus aureus* and *Escherichia coli,* though *Phaseolus vulgaris* has higher antibacterial activity than *Vigna unguiculata*. FTIR and i*n silico* analysis showed that the aqueous extracts of both *Vigna unguiculata* and *Phaseolus vulgaris* contain multiple phytochemicals with good binding capacities and a considerable number of hydrogen bonds and hydrophobic interactions. *Phaseolus vulgaris* contains the plant pigment rutin, which showed good docking results with lowest docking energy of –13.461 Kcal/mol against the caspase-3 protein. This protein is an apoptotic marker in colon cancer that prevents colon cancer cells from multiplying by triggering apoptosis, achieved by cell cycle arrest and activation of the caspase protein. While in the case of *Vigna unguiculata*, Beta-amyloid (APLP1 and APP) proteins were docked with various bioactive compounds showing it have beneficial effects on Alzheimer's disease treatment and hence can become a potential drug for memory improvement.

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***Correspondence** | Afshan Kaleem, Department of Biotechnology, Lahore College for Women University, Lahore; **Email:** afshan.kaleem@lcwu.edu.pk
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Keywords | *Phaseolus vulgaris, Vigna unguiculata*, Disc diffusion method, FTIR analysis, Caspase-3 protein, apoptosis, Beta-amyloid protein

Introduction

Red kidney beans (*Phaseolus vulgaris*) are herbaceous plants belonging to the family *Fabaceae*. These are significant food crops on account of both their nutritive value and their financial advantages. Kidney beans are vital component of human nutrition as they are a rich source of protein, containing approximately 20% to 25% protein, and carbohydrates, which make up 50% to 60% of their nutritional content. In

addition, they are also excellent sources of vitamins, minerals, and unsaturated fatty acids (Hayat *et al.*, 2014). While Black-eyed bean (*Vigna unguiculata*) is a plant which belongs to *Fabaceae*. High nutritious values of black-eyed beans make them popular all over the world (Chandrasekaran *et al.*, 2015).

The main difference between red kidney beans and black-eyed beans lies in their shape, flavor and size but they also differ in toxicity. Red kidney beans are large in size while black eyed beans are smaller in size. Red kidney beans have meaty, slightly sweet and nutty taste while Black-eyed beans have dense creamy taste (Gyrisco *et al.*, 1959).

Dry beans, including types like red kidney and black-eyed beans, consist of a variety of advantageous phytochemicals and antioxidants, including coumestrol, phytate, isoflavones, lecithin, phytosterols etc. Studies show, consumption of these beans decreases the chance of various diseases like cancer, aging, cardiovascular diseases, obesity, colon cancer etc. (Djordjevic *et al.*, 2011).

Beans show anti-microbial potential against many important food-borne pathogens. However, antimicrobial effect is associated with phytochemicals present in beans, vegetables such as polyphenols, lectins, peptides, flavonoids and Tannis. Red kidney beans and black-eyed beans exhibit antimicrobial properties against various types of bacteria for example *Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi, Klebsiella pneumoniae, Escherichia coli,* which are Gram-positive and Gram-negative respectively, as well as some fungi such as *Aspergillus fuinigatus, Rhizopus stolonifer* and *Mucor mucedo* (Olajide, 2020). In beans, by using different bioinformatics tools, different modifications are done to overcome the factors which affect their yield like climatic condition, occurrence of insect pest (Bello *et al.*, 2014).

The objective of the current study was to analyze the various biological characteristics of *Phaseolus vulgaris* and *Vigna unguiculata* to identify new therapeutic leads for specific disorders (colon cancer and memory improvement in Alzheimer's disease).

Materials and Methods

Sample collection

Red kidney and black-eyed beans were purchased from the local market of Lahore, Pakistan

Extract preparation

Both bean samples were dried and grounded to fine powder and aqueous extracts were prepared. The sample was dissolved in distilled water. The solution was homogenized on the magnetic stirrer for 25-30 minutes, then filtrated and stored at 4°C (Hang *et al.*, 1980).

Qualitative analysis

Different qualitative test for detection of phytochemicals were performed including alkaloid, flavonoids, carbohydrates, proteins, saponins, steroids, cardiac glycosides, tannins, terpenoids, phenol and quinones (Tripathi *et al.*, 2017; Pascale *et al.*, 2018; Jaya *et al.*, 2019).

Anti-microbial activity

Anti-microbial activity was performed using the disk diffusion method by Roy *et al.* (2020).

FTIR analysis

Both aqueous extract samples were run on the Fourier transform infrared (FTIR) to analyze their bioactive compounds (Akinpelu *et al.*, 2017). Peaks were matched with FTIR spectra retrieved from the PuBChem database (https://pubchem.ncbi.nlm.nih.gov/) and ligands, which were determined from the FTIR spectra, were downloaded from the PDBeChem software (https://www.ebi.ac.uk/pdbe-srv/pdbechem/).

Bioinformatics investigation

Protein docking was performed by using the Galaxy web server (https://galaxy.seoklab.org/). For docking, protein of interest was downloaded from PDB (Protein Data Bank). Beta amyloid protein (APLP1 (Uniprot ID: P51693), APP (Uniprot ID: P05067)) and Caspase-3 (PDB ID: 1NME) were downloaded from PDB and their charges were removed using the Pymol software.

Results and Discussion

Antibacterial activity of Vigna unguiculata and Phaseolus vulgaris

Antibacterial activity of *Vigna unguiculata* and *Phaseolus vulgaris* was evaluated against *Staphylococcus aureus* and *Escherichia coli*. Both extracts showed anti-bacterial activity against *Staphylococcus aureus* and

Escherichia coli. The maximum zone of inhibition was reported in both gram-negative and gram-positive strains by *Phaseolus vulgaris* (Figure 1).

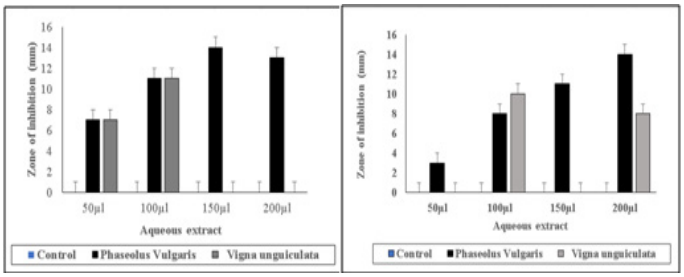

Figure 1: *(a) Antibacterial activity of aqueous extracts of Phaseolus Vulgaris and Vigna unguiculata against Staphylococcus aureus (b) Antibacterial activity of aqueous extracts of Phaseolus Vulgaris and Vigna unguiculata against Escherichia coli.*

FTIR analysis

FTIR analysis of *Vigna unguiculata* (Black-eyed beans) and *Phaseolus vulgaris* (red kidney beans) showed the presence various functional groups like hydroxyl, carbonyl, phenol, alkane, alcohol etc. However, peaks matched from PuBChem software at 3334.92 and 3317.56, 1153.43, 709.80 and 1639.49 indicating rutin, kaempferol, benzoic acid and naringenin bioactive compounds (Figure 2).

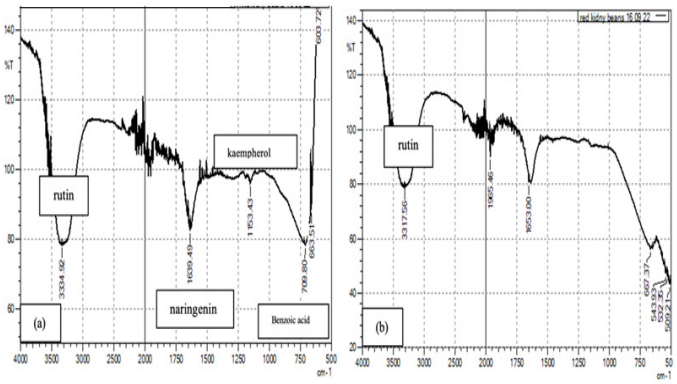

Figure 2: *FTIR spectrum of (a) Vigna unguiculata (b) Phaseolus vulgaris.*

Bioinformatic analysis of Vigna unguiculata and Phaseolus vulgaris

Different detected bioactive compounds (ligand) were docked with respective proteins. Rutin, Naringenin, Benzoic acid and kaempferol were docked with both APLP1 and APP proteins, which were determined in *Vigna unguiculata*, (Tables 2-4) (Figures 3-6), while in the case of *Phaseolus vulgaris*, rutin was docked with Caspase-3 (Table 5) (Figure 6). The binding with various amino acids were found to consist of different hydrogen bonding (HB) and hydrophobic interactions (HI). However, kaempferol did not bind with any protein.

Table 1: *Qualitative phytochemical analysis of extracts.*

Phytochemicals	*Phaseolus vulgaris*	*Vigna unguiculata*
	Aqueous extract	Aqueous extract
Alkaloids	+	+
Carbohydrates	+	+
Proteins	+	+
Flavonoids	+	+
Steroid	+	+
Saponins	+	+
Phenol	+	+
Quinones	+	+
Tannins	-	+
Cardiac glycoside	+	+
Terpenoids	+	+
Phenol	+	+
Saponins	+	+

Table 2: *Binding of different ligands with APLP1 (285-499) protein.*

Ligand	APLP1 amino acid	Types of bonds	Length of the bond	Ligand amino acid binding sites
Rutin	Arg 540	1: HB	3.13	NH2-O8
		2: HB	3.29	NH1-O8
	Pro 541	HI		
	Leu 547	HI		
Narin-genin	Arg 480	HB	3.02	NE-O6
	Gln 486	HB	3.00	NE2-O8
	Leu 547	HI		
	His 548	HI		
	Gln 483	HI		
	Glu 519	HI		
	Lle 518	HI		
Benzoic acid	Glu 519	HB	2.87	OE2-O5
	Gln 486	HI		

Table 3: *Binding of different ligands with APLP1 (290-495) protein.*

Ligand	APLP1 amino acid	Types of bonds	Length of the bond	Ligand amino acid binding sites
Rutin	Asn 461	HB	3.20	OD1-O36
	Glu 494	1: HB	3.32	OE1-O25
		2: HB	2.87	OE1-O27
	Leu 464	HI		
	Leu 485	HI		
	Ser 493	HI		
Naringenin	Arg 478	HB	3.26	NH2-O10
Benzoic acid	Arg 478	HB	3.28	NH1-O5
	Ala 474	HI		
	Asp 468	HI		

Table 4: *Binding of different ligands with APP protein.*

Ligand	APP amino acid	Types of bonds	Length of the bond	Ligand amino acid binding sites
Rutin (Model 6)	Arg 42	1: HB	3.08	NH1-O16
		2: HB	2.99	NH1-O4
	Asn 44	HB	2.82	ND2-O14
	Arg 20	HB	2.95	NH2-O16
	Asp 46	1: HB	2.73	OD1-O14
		2: HB	3.05	OD1-O16
		3: HB	3.18	OD2-O20
	Thr 47	HB	2.89	OG1-O28
Naringenin (Model 5)	Glu 10	HB	3.23	OE1-O8
	Arg 42	HI		
Benzoic acid (Model 3)	Thr 26	HB	3.27	OG1-O5
	Asp 24	HB	3.04	OD2-O5
	Tyr 22	HI		
	Val 25	HI		
	Ser 6	HI		

Table 5: *Binding of ligand with caspase-3 protein.*

Ligand	Caspase-3 amino acid	Types of bonds	Length of the bond	Ligand amino acid binding sites
Rutin	Lys 271	HB	3.24	NZ-O36
	Lys 186	1: HB	3.11	NZ-O34
		2: HB	3.07	NZ-O38
		3: HB	2.91	NZ-O40
	Leu 194	HI		
	Leu 269	HI		
	Leu 273	HI		
	Lle 187	HI		
	His 277	HI		
	Val 189	HI		

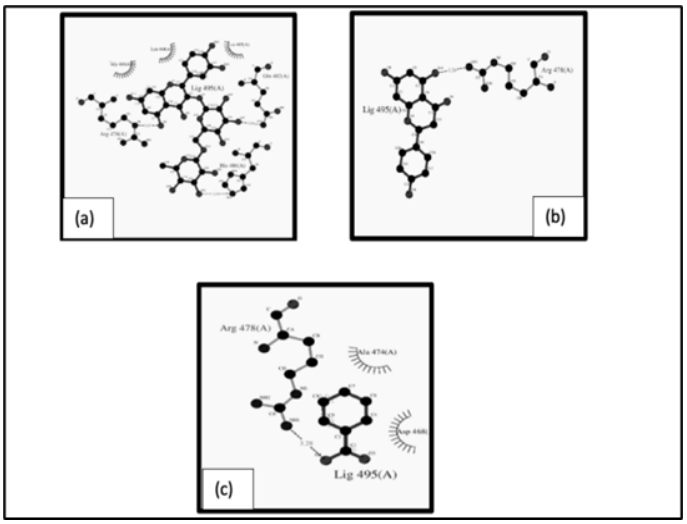

Figure 3: *Docking results of (a) rutin (b) naringenin (c) benzoic acid with APLP1 (290-495).*

Both in developing and developed nations, the tendency of diabetes, obesity, cardiovascular illnesses and cancer are rising. It is anticipated that both the diet and nutrition would be crucial in preventing certain illness problems. Beans and other legumes are an excellent source of protein, minerals, dietary fiber, carbohydrates, and bioactive compounds. They also serve as medicinal plants and are used as common food in developing countries. In current study various experiments were done in order to evaluate biological characteristics of the beans (*Phaseolus vulgaris* and *Vigna unguiculata*) and their therapeutic effect on specific diseases (colon cancer and memory improvement in Alzheimer's disease).

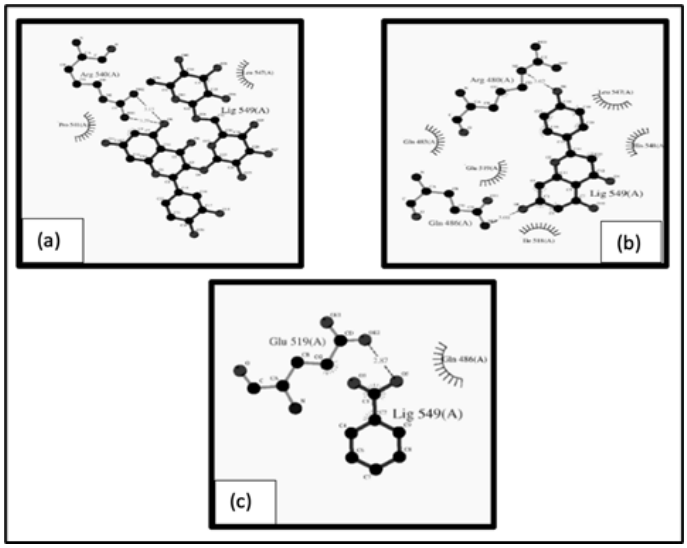

Figure 4: *Docking results of (a) rutin (b) naringenin (c) benzoic acid with APLP1 (285-499).*

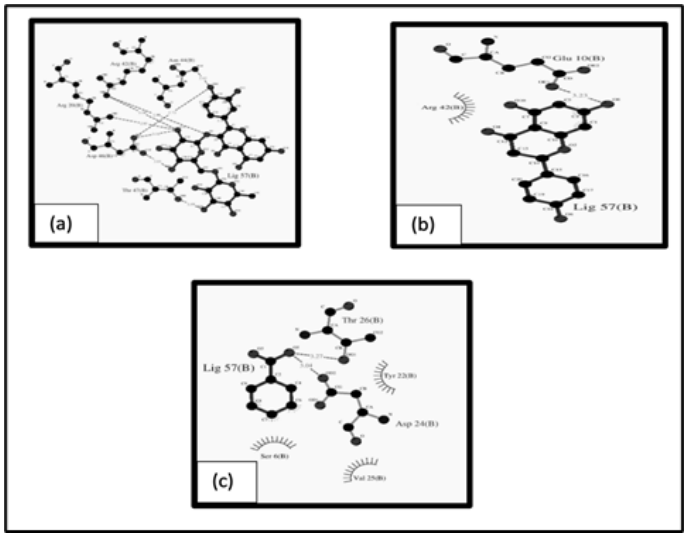

Figure 5: *Docking results of (a) rutin (b) naringenin (c) benzoic acid with APP.*

Qualitative analysis of aqueous extracts of *Phaseolus vulgaris* and *Vigna unguiculata* showed that both extracts contain alkaloids, steroids, phenols, carbohydrates, proteins, terpenoids, glycosides, saponins, quinones, tannins, flavonoids. All these

results were in agreement with the results with the work reported in the literature (Luka *et al.*, 2013; Tripathi *et al.*, 2017; Jaya *et al.*, 2019). However, tannin was not found in *Phaseolus vulgaris*, which contradicts the results in literature (Jaya *et al.*, 2019).

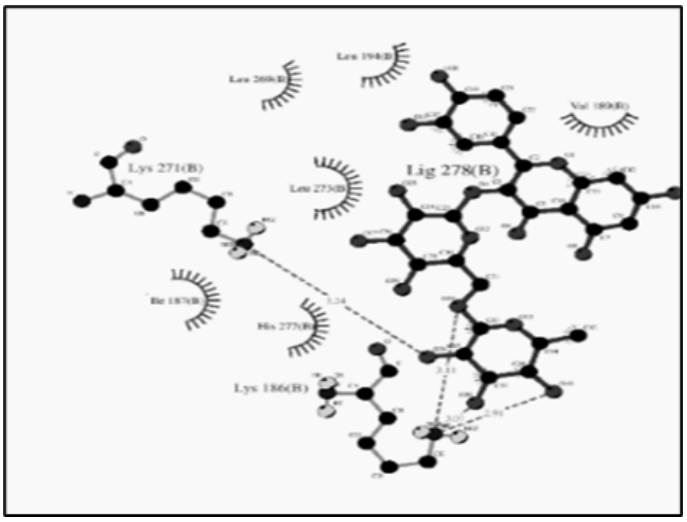

Figure 6: *Docking results of rutin with Caspase-3.*

Aqueous extract of *Phaseolus vulgaris* and *Vigna unguiculata*, both showed anti-bacterial activity against *Staphylococcus aureus* and *Escherichia*. The maximum zone of inhibition was reported in both Gram-Negative and Gram-Positive strains by aqueous extract of *Phaseolus* vulgaris. However, in *Phaseolus vulgaris* seeds, lectin which is active phytohemaglutinin is responsible for the antibacterial activity, while in the case of *Vigna unguiculata* the bioactive component, globulins, has shown comparable antibacterial efficacy (Chidebelu *et al.*, 2019).

The FTIR of *Vigna unguiculata*, peaks obtained in the area of 3334.92 refer to O-H stretching, peak at 1639 refers to C=C, were similar with the working of Akinpelu *et al.* (2017). While in the case of *Phaseolus vulgaris*, peak obtained in the area of 1653 cm^{-1} this was similar with the results of Liu *et al.* (2013).

FTIR analysis of *Phaseolus vulgaris* confirms the presence of rutin, which was docked with the protein Caspase-3, an apoptotic marker in colon cancer and binds to various amino acids through hydrogen bonding like Lys 271, Lys 186. These results showed good docking with lowest docking energy of –13.461 Kcal/mol. Rutin have anti-cancerous and anti-proliferative properties, and it prevents colon cancer cells from multiplying by triggering apoptosis, which is achieved by cell cycle arrest and activation of the Caspase protein. Furthermore, Caspase-3 expression was enhanced after treatment with rutin (Jayameena *et al.*, 2018).

FTIR analysis of *Vigna unguiculata* confirms the presence of rutin, kaempferol, benzoic acid and naringenin. These bioactive compounds were docked with beta amyloid protein (APLP1 and APP). These bioactive compounds bind with various amino acids through hydrogen bonding like Arg 540 (Rutin), Arg 480, Gln 486 (Naringenin) and Arg 478 (Benzoic acid) in the case of APLP1 (285-499) as shown in Table 3. Similarly, Asn 461, Glu 494 (Rutin), Arg 478 (Naringenin) and Arg 478 (Benzoic acid) in APLP1 (290-495) (Table 4). All these interactions take place in the E2 domain, the collagen binding region and the compositional bias region which include acidic and basic residues. In the case of APP, different bioactive compounds form bond with amnio acids like Arg 42, Asn 44, Arg 20, Asp 46, Thr 47 (Rutin), Glu 10 (Naringenin) and Thr 26, Asp 24 (Benzoic acid) as shown in Table 5. Rutin and naringenin interaction take place in the GFLD subdomain and E1 domain.

Rutin has potential to reduce oxidative stress, to prevent Aβ aggregation and cytotoxicity and proinflammatory cytokines. Oral administration has shown to significantly reduce interleukin IL-1 and IL-6 levels in the brain and alleviated memory problems in AD transgenic mice (Xu *et al.*, 2014). Moreover, administration of naringenin may lead to an enhanced spatial learning and memory in a rat model of Alzheimer's disease through controlling the PI3K/AKT/GSK-3 pathway (Nouri *et al.*, 2019).

The studies by Aduema (2016, 2019) also indicate that consumption of these beans improves learning and memory. The determined bioactive compounds of *Vigna unguiculata* have beneficial effects on Alzheimer's disease treatment and hence can become a potential drug for memory improvement.

Conclusions and Recommendations

In conclusion, the current study suggests that *Phaseolus vulgaris* and *Vigna unguiculata* are excellent source of proteins, carbohydrates and various bioactive compounds. They have good antimicrobial activity which make them potent agent against several microbes. FTIR analysis indicated the presence of several functional groups. *Phaseolus vulgaris* contain rutin, a glycoside of the flavonoid quercetin and

rutinose, that showed good docking results with lowest docking energy of –13.461 Kcal/mol against Caspase-3 protein, which is an apoptotic marker in colon cancer. It might serve as a standard therapy for colon cancer. *Vigna unguiculata* contains rutin, naringenin and benzoic acid, which showed good docking activity against Beta-amyloid (APLP1 and APP) protein for memory improvement in Alzheimer's disease. These results suggest that *Vigna unguiculata* could be beneficial and may act as a therapeutic agent in Alzheimer's disease and hence could become a potential drug for memory improvement.

Author's Contribution

Afshan Kaleem: Conceptualisation, formal analysis, administration, writing, review and editing.
Iqra Noor: Conceptualisation, data curation, formal analysis and writing.
Roheena Abdullah: Conceptualisation, administration, review and editing.
Mehwish Iqtedar: Data curation, administration, review and editing.

Conflict of interest
The authors have declared no conflict of interest.

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