South African Journal of Botany 177 (2025) 225–236

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Antibacterial and anti-breast cancer activities, GC–MS profiling, molecular docking and pharmacokinetic studies of nutritious white kidney beans

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ARTICLE INFO

Article History:
Received 3 July 2024
Revised 13 November 2024
Accepted 23 November 2024
Available online xxx

Edited by: N. Ngobese

Keywords:
White kidney beans
Uro-pathogens
Er-α
Cytotoxic
Drug likeliness

ABSTRACT

White kidney beans (*Phaseolus vulgaris* L.), a type of common bean, are very noteworthy due to their distinct biological activity and great nutritional value. This study aimed to assess the effectiveness of bean extracts in eliminating bacterial uropathogens, finding the active components of beans, and the potential of their chloroform extract to combat the protein Er-α found in breast cancer cells. Bean extracts with acetone, chloroform, and ethyl acetate showed higher antibacterial action than other extracts against *Pseudomonas aeruginosa* (MIC, 64 μg/ml), while methanol and acetone extracts were more effective against *Klebsiella pneumoniae* (MIC, 8 μg/ml), and methanol extract was more inhibitory to *Escherichia coli* (MIC, 16 μg/ml). The chloroform extract was found to be cytotoxic in MCF-7 cells, with a CC_{50} value of 3.849 μg/ml based on the MTT experiment. The GC–MS analysis of chloroform extract revealed 17 compounds, with lupeol, 8-pentadecanol, and 2-butoxyethyl oleate being key elements. Molecular docking investigations show that lupeol has a binding affinity of −10.5 Kcal/mol and a stronger interaction with Er-α than 8-pentadecanol and 2-butoxyethyl oleate. The *in silico* pharmacokinetic and toxicological properties of chosen substances were evaluated using Swiss ADME and admetSAR, and lupeol was found to be satisfactory. Lupeol exhibited a higher Pa score (0.799) for breast cancer prevention than doxorubicin, 2-butoxyethyl oleate, or 8-pentadecanol. The current study indicated the antibacterial importance of methanol and chloroform extracts of *P. vulgaris* seeds as well as the importance of lupeol from chloroform extract as a potential treatment for breast cancer.

1. Introduction

Breast cancer (BC) is the top-listed cause of morbidity and mortality in women. As per the 2020 global statistics, BC ranked as the number one malignant tumor in the world (Sung et al., 2021). Unfortunately, more than 2.1 lakh cases of BC have been reported from India in 2022, which are projected to reach 2,32,832 in 2025 (SathishKumar et al., 2022). In addition to BC, urinary tract infections (UTIs) are more prevalent in women (Vasudevan, 2014). Uropathogenic *E. coli* and *Klebsiella* sp. contribute 75–95 % of total UTI cases. The emergence of multidrug-resistant uropathogenic bacteria, including *E. coli,* has become a major challenge in treatment (Mohapatra et al., 2022), suggesting a need to search for alternative bioactive molecules.

Phaseolus vulgaris (L), or white kidney beans (WKB), are nutritionally rich edible legumes in the Fabaceae family. They are commonly consumed worldwide in their immature, green, or mature, dried form. The WKBs are usually pale, creamy, or white in color with a mild earthy and nutty taste. They provide an excellent source of proteins, minerals, vitamins, dietary fibers, and carbohydrates (Ganesan and Xu, 2017). The presence of these human health-related components and their associated biological activities have been studied in the last few years and gained worldwide attention (Hayat et al., 2014). The studies also suggested the benefits of regular consumption of beans in reducing the risk of cardiovascular diseases, diabetes, and obesity (Ganesan and Xu, 2017; Hayat et al., 2014; Rodríguez et al., 2022). In addition to these benefits, the role of beans in the prevention of different cancers, including prostate (Kolonel et al., 2000), colon (Bernardi et al., 2023), and breast cancer (Thompson et al., 2012), has been indicated earlier. The *in vitro* antiproliferative activity of trypsin isoinhibitors from *P. vulgaris* beans against leukemia L1210 and lymphoma MBL2 is also reported (Sun et al., 2010).

Abbreviations: Er-α, Estrogen receptor-α; ATCC, American Type Culture Collection; mg/ml, Milligrams per milliliter; %, Percentage; μl, Microliter; m/z, Mass per charge number of ions; NIST, National Institute of Standards and Technology; 3D, Three-Dimensional; PDB, Protein databank database; TPSA, Topological polar surface area; BBB, Blood-brain-barrier; Pg-P, P-Glycoprotein; CYP, Cytochrome P; KP, Permeation coefficient; PAINS, Pan assay interference; μg/ml, Micrograms per milliliter; ADMET, Absorption, Distribution, Metabolism, Excretion and Toxicity; M.F., Molecular formula

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https://doi.org/10.1016/j.sajb.2024.11.033
0254-6299/

WKBs contain a high phenolic content, which includes phenolic acids, flavones, flavanones, isoflavonoids, and chalcones. These compounds support beans' biological activities, which include antioxidant, anti-inflammatory, and hypolipidemic properties. Because white kidney beans possess a high protein content, numerous studies have identified biological activities related with these proteins. El-Saadony et al. (2021) demonstrated that the bioactive peptides in WKB have antibacterial and antioxidant activity against spoilage-causing bacteria. Similarly, Roy et al. (2020) also documented the antimicrobial properties of bean proteins against *E. coli* and *Pseudomonas aeruginosa*. The high flavonoid content found in plants or beans is known to inhibit or attenuate the beginning, progression, and spread of cancer (Newman and Cragg, 2007). As elevated levels of oxidative stress and inflammation are major risk factors for cancer development, beans rich in antioxidant and anti-inflammatory properties can play a crucial role in reducing the risk of different cancers (Kumar et al., 2017). Although WKBs are widely studied as a popular nutraceutical with anti-obesity and anti-diabetic properties, the studies highlighting the impact of WKBs in preventing cancer are underdeveloped, indicating a need for more in-depth studies (Nchanji and Ageyo, 2021; Bernardi et al., 2023). In this work, we investigated the antibacterial effect of WKB extracts on bacterial strains isolated from urinary tract-infected patients and their anticancer activity against the breast cancer cell line MCF-7. Molecular docking, ADME analysis, and toxicity parameter evaluation were performed to better understand the mechanism of interaction of WKB constituents with the breast cancer marker protein estrogen receptor-α (Er-α).

2. Materials and methods

Ethanol, methanol, acetone, ethyl acetate, chloroform, and dimethyl sulfoxide (DMSO) were purchased from Thermo Fisher Scientific, and MTT was purchased from Sigma-Aldrich. All media and media ingredients were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai (M.S.), India.

2.1. Collection and processing of the sample

The dried seeds of *Phaseolus vulgaris* (PVS) were purchased from a local market of the Nanded district (19°06′43″N 77°17′20″E) of Marathwada region, India. The seeds were washed two to three times using deionized water to remove any adhered dirt particles and dried completely at 40 °C for 24 h. The dried seeds were finely powdered using a mechanical grinder and used for the preparation of extracts.

2.2. Preparation of extract

10 g of powdered bean sample was mixed individually with ethanol (PVSE), methanol (PVSM), acetone (PVSA), ethyl acetate (PVSEA), and chloroform (PVSC) in a 1:10 ratio and subjected to extraction by the maceration method (Ingle et al., 2017). The individual solvent mixtures were kept under shaking conditions of 120 rpm at 30 °C for 24 hrs. After the completion of the shaking period, the resultant mixtures were filtered, and the filtrate was considered a primary extract. The leftover residues were processed similarly to get secondary, and tertiary extracts. All the extracts were pooled together and evaporated to dryness using rotavapor. The yield of each solvent extract (%) was calculated by using the following formula (Abbas et al., 2021).

$$Yield\ (\%) = \frac{X}{Y} \times 100$$

Where X and Y are the weights (g) of solvent-free plant extract and dried plant material respectively.

100 mg of individual extracts were diluted in 100 ml DMSO (10 %) to get a final concentration of 1 mg/mL

2.3. Test organisms

Three clinical bacteria were recovered from urinary tract-infected patients admitted to Uro-care hospitals in Nanded. A total of 23 urine samples were collected from urinary tract-infected patients admitted to Uro-care hospitals in Nanded in sealed glass containers, before starting any antibiotic treatment. The urine samples were inoculated (0.1 ml) individually into sterile petri plates containing MacConkey's agar and Cetrimide agar media. The plates were incubated at 37 °C for 24 hrs and after incubation, three well-isolated and morphologically distinct colonies (M1 and M2 grown on MacConkey's agar and C1 on cetrimide agar media) were selected, sub-cultured for purification, transferred on nutrient agar slants, and maintained at 4 °C till further use. These bacteria were identified using a VITEK compact machine. Based on morphological, motility, Gram's nature, and biochemical characteristics, the isolates are *E. Coli* (M1), *Klebsiella pneumoniae* (M2), and *Pseudomonas aeruginosa* (C1).

2.4. Antibacterial activity of extracts

The antibacterial effect of PVSE, PVSM, PVSA, PVSEA, and PVSC extracts on test bacteria was determined by an agar-well diffusion assay (Bhosale et al., 2018). The test organisms were cultivated separately in nutrient broth for 24 h at 37 °C, and the cell density was adjusted to 0.5 McFarland turbidity standards. Then bacterial inoculums were spread individually on the surface of sterile Muller-Hinton agar plates. The wells (5 mm) were punctured on agar surface medium, and the extracts (100 μg/ml) were loaded separately into the wells. The plates were incubated at 37 °C for 24 h after the diffusion of extracts at 4 °C for 30 min. The extracts showing the presence of inhibition zones around wells were selected, and the zone sizes were measured to identify effective antibacterial extracts. Gentamycin (10 μg/ml) and chloramphenicol (30 μg/ml) were used as standard antibiotics. DMSO (10 %) and sterile distilled water were used as a solvent and negative control, respectively. The test was carried out in triplicate, and the results were recorded as the mean ± standard deviation.

2.5. MIC determination

The bacterial suspensions corresponding to the 0.5 McFarland standard (OD 600 = 0.1) were prepared in nutrient broth. As indicated by Mogana et al. (2020), and according to the Clinical and Laboratory Standards Institute (CLSI, 2006) guidelines, broth microdilution methods were used to determine minimum inhibitory concentration (MIC) values for studied extracts. The different concentrations of PVSC extract were added individually to wells of a microtiter plate containing 100 μl of bacterial suspension to achieve final concentrations between 0.25 μg/ml and 256 μg/ml. The plate was incubated at 37 °C for 24 h. The lowest concentration at which no visible growth was observed was considered the MIC of the extract.

2.6. Anticancer activity of extracts

The human breast adenocarcinoma (McF-7) cell line was procured from ATCC (American Type Culture Collection). The stock cells were cultured in Dulbecco's modified eagle (DMEM) medium supplemented with 10 % inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 μg/ml) at 37 °C and in the presence of 5 % CO_2 until confluent growth was observed. The cells were dissociated using 0.05 % trypsin and centrifuged at 100 rpm for 5 min. The supernatant was discarded, and the cell pellet was resuspended in 2 ml of DMEM media. The viability of cells was checked, and a cell suspension corresponding to 5.0 X 10^5 cells/ml was prepared. The DMSM, FBS, Penstrep, and trypsin used in the assay were procured from Invitrogen. The 96-well microtiter plate was

prelabeled, and 100 μl of cell suspension was added to each well. The plate was incubated at 37 °C with 5 % CO_2 for 24 hrs. After incubation, 100 μl of each of PVSE, PVSM, PVSA, PVSEA, and PVSC extract (0.3125 to 10 μg/ml) were added in a predesignated well and incubated for 24 hrs. Doxorubicin (1.5625 to 100 μM) was used as a standard drug; the medium without cells was the medium control, and the medium with cells without extract or standard drug was kept as a negative control. The cytotoxicity effect of extracts was studied using the 3 (4, 5 dimethyl-thiazol-2−41), 2, 5-diphenyl tetrazolium bromide (MTT) assay. MTT was prepared in phosphate-buffered saline (5 mg/10 ml) and 100 μl of it was added to each well. The plate was incubated for 4 h at 37 °C in the presence of 5 % CO_2. The supernatant was removed and 100 μl of DMSO was added. The plate was gently shaken to solubilize the formazan crystals. The absorbance was measured using the microplate reader at 590 nm using a multimode plate reader (Spectramax i3X). The percentage cell viability was calculated using the following formula (Chan et al., 2015):

$$\text{Cell viability } (\%) = \frac{\mathbf{a}-\mathbf{b}}{\mathbf{c}-\mathbf{b}}\ X\ 100$$

Where a, b, and c were average absorbances of cells treated with individual PVS extracts, mean absorbance of the blank medium, and mean absorbance of cells in the absence of PVS extracts respectively.

The 50 % cytotoxic concentration (CC_{50}) of the extract or test drug was determined from the dose-response curves (plotted between cell viability v/s concentration of extract) using Graph Pad Prison 5.0 software (Graph Pad, San Diego, CA, USA).

2.7. GC–MS analysis of PVSC

Gas Chromatography–Mass spectroscopy (GC–MS) analysis of chloroform extract of *P. vulgaris* seeds was performed using a GC–MS (Model TQ 80 50) equipped with a DBSMS silica capillary column (30 X 0.25 mm ID X 0.25 μm length), helium as carrier gas, and electron ionization mode. The flow rate was adjusted to 1.0 ml/min. The initial temperature for sample injection was set at 250 °C and the oven temperature increased from 50 °C to 180 °C at 10 °C/min rise and was finally set to 250 °C for 5 min. The 1 μl of the sample was injected in split mode, and a scanning range of 20–700 *m/z* with a scan time of 0.5 s per scan was used. The complete run time was 48 min. The relative percentage indicating the amount of each component was calculated using the National Institute of Standards and Technology (NIST) library in 2014 (Srikalyani and Ilango, 2020).

2.8. FTIR analysis of PVSC

The functional groups present in the PVSC extract were identified by employing FTIR analysis by the KBr pellet method. The spectra were obtained on the FTIR spectrophotometer (Shimadzu) in the spectral range of 4000–400 cm^{-1}.

2.9. Molecular docking studies

The GC–MS analysis of the PVSC extract identified three principal phyto-compounds: 8-pentadecanol, lupeol, and 2- butoxyethyloleate. To define the potency correlation of results observed for *in vitro* cytotoxic activity with the structure of the phyto-compounds, an attempt was made to investigate our findings by using an in *silico* molecular docking approach. The 3D structures of selected phyto-compounds and the standard anticancer drug doxorubicin used in this study were obtained from the energy-minimized PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) (accessed on January 27, 2024) in SDF format. The 3D structure of the target protein estrogen receptor alpha (ER-α) with Uniport ID (2 IOG) was retrieved from the protein databank database (PDB) (http://www.rcsb.org) (accessed on 27 January 2024) (PDB). The docking studies for ER-α and three selected compounds were carried out using AutoDock 4.2 (Huey et al., 2007; Morris et al., 1998). To achieve optimal docking conformations, the x, y, and z dimensions were adjusted to $40 \times 40 \times 40$, and the favorable docking conformations were obtained by positioning the grid box at the center. The grid file was saved as a (.gpf) file and ran with autogrid. The Lamarckian genetic method was then used to calculate docking using a default run count of nine. The final docked data including information on binding residues, binding energy, and inhibition constant was extracted in (.dlg) format. The structures illustrating ligand-protein interactions were viewed using PyMol V 0.99 (http://www.pymol.org) (accessed on January 27, 2024).

2.10. In silico *study of physiological and pharmacokinetic properties of phytocompounds*

The Swiss web server www.swissadme.ch (Diana et al., 2017; accessed on January 27, 2024) was used for computing the adsorption, distribution, metabolism, and excretion (ADME) properties of 8-pentadecanol and lupeol (Mostofa et al., 2023) and comparing them with the properties of the standard drug doxorubicin. The parameters considered for study were molecular weight, number of rotational bonds, topological polar surface area (TPSA A2), aqueous solubility (Log S by ESOL method), polarity (Log P octanol; water), gastrointestinal tract (GI) absorption, blood-brain-barrier (BBB) permeant inhibition of CYP450 isoforms, skin permeation coefficient (Kp) value, Lipinski's rule of 5, Veber's rule, and Pan assay interference (PAINS) alert. The SMILE format of each compound was uploaded to the web software, and the obtained results were recorded manually.

2.11. In silico *studies on toxicological properties of phytocompounds*

The toxicological properties of selected phytoconstituents were studied using the online tool admetSAR 3.0 (http://lmmd.Ecust.Edu.in/admetsar1/predict/arrow/↑) (accessed on 27 January 2024). The parameters considered for this study were Ames toxicity, carcinogenic properties, acute oral toxicity, and rat acute toxicity (Filimonov et al., 2014).

2.12. In silico *studies on the prediction of activity spectra for phytocompounds*

The selected compounds from the PVSC extract were assessed for their activity spectrum prediction in terms of anticancer and other biological activities using the prediction of activity spectra for substances (PASS) online platform (http://www.way2drug.com/Passonline/↑) (version 2022, accessed on January 27, 2024).

2.13. Statistical analysis

Every experiment pertaining to the antibacterial and anticancer properties was conducted in triplicate, with the outcomes being reported as the mean ± standard deviation. To determine the CC_{50} values, dose-response curves were analyzed using linear regression. The program Graph Pad Prison 5.0 (Graph Pad, San Diego, CA, USA) was used to calculate the regression coefficients, mean, and standard deviation values.

3. Results

The extraction of phytoconstituents from PVS in organic solvents showed variable yield. Chloroform, ethanol, and acetone extracts showed 22.0, 13.7, and 13.6 % yield of the dried sample respectively-while with ethyl acetate, and methanol it was recorded 10.6, and 9 % respectively. Table 1 displays the impact of various PVS seed extracts on test microorganisms. The growth of *E. Coli* was decreased by all extracts; however, the presence of PVSM showed the greatest

Table 1
Antibacterial activity of WKB extracts against bacterial uropathogens.

WKB extract (100 μg/mL)	Test organism / Zone of inhibition (mm)[a]		
	E. coli	*K. pneumoniae*	*Ps. aeruginosa*
Ethanol	25 ± 0.05	19 ± 0.31	0
Methanol	32 ± 0.023	28 ± 0.069	0
Acetone	13 ± 0.126	24 ± 0.28	10 ± 0.01
Chloroform	16 ± 0.082	15 ± 0.044	12 ± 0.0169
Ethyl acetate	10 ± 0.5	17 ± 0.012	10 ± 0.09
Gentamicin (**10μg/mL**)	17 ± 0.02	0	32 ± 0.17
Chloramphenicol (**30μg/mL**)	24 ± 0.035	0	16 ± 0.082

[a] Each value of the zone of inhibition activity is presented as mean ± standard deviation (n = 3).

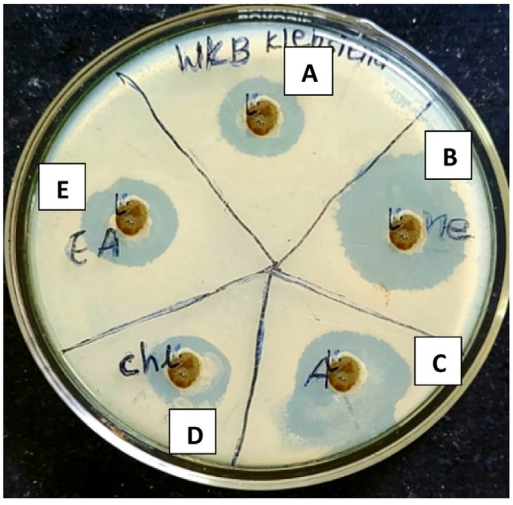


Fig. 1. Antibacterial activity of PVS extracts (100μg/mL) against *K. pneumoniae* by agar well diffusion assay. A: Ethanol extract, B: Methanol extract, C: Acetone extract, D: Chloroform extract, E: ethyl acetate extract.

inhibition, which was then followed by PVSE, PVSC, and PVSA. The least amount of *E. Coli* growth inhibition was seen when PVSEA was present. Only three extracts (PVSA, PVSC, and PVSEA) were able to suppress *Ps. aeruginosa* growth, with PVSC extract exhibiting the greatest inhibition (12 mm). When compared to the results obtained with gentamycin and chloramphenicol, *E. coli* showed a stronger inhibitory response to PVSE and PVSM extracts. All PVS extracts inhibited the growth of the *K. pneumoniae* strain, with the PVSM extract causing the most inhibition, followed by the PVSA and PVSE extracts (Fig. 1). However, growth was not inhibited in the presence of both standard antibiotics.

The MIC values for *E. coli* varied from 16 μg/mL to 64 μg/ml. The MIC value for PVSM extract against *E. coli* was 16 μg/ml, PVSE, PVSA, and PVSC extracts had MIC values of 32 μg/ml, while the MIC value for PVSEA extract was 64 μg/ml. The MIC values for *K. pneumoniae* varied from 8 μg/mL to 32 μg/mL, whereas *Ps. aeruginosa* had values ranging from 64 μg/mL to 128 μg/ml (Fig. 2)

The cytotoxicity activity of PVS extracts against the MCF-7 cell line was examined by MTT assay at varying concentrations (0.3125, 6.25, 1.25, 2.5, 5.0, and 10.0 μg/ml) to determine the CC_{50} value. The MCF-7 viable count was decreased in a dose-dependent manner in the presence of PVS extracts at the studied concentrations. The PVSC extract effectively reduced viable cell count followed by PVSEA and PVSE extracts. The CC_{50} values of 8.446 μg/ml, 9.539 μg/ml, 15.188 μg/ml, 3.849 μg/ml, 6.800 μg/ml, and 30.034 μM were found for PVSE, PVSM, PVSA, PVSC, PVSEA extracts, and the standard anti-cancer drug, doxorubicin, respectively (Table 2). The effects of varying concentrations of PVSC extract and doxorubicin on the viability of MCF-7 cells are shown in Fig.3. As PVSC extract showed a higher inhibitory effect on the viability of MCF-7 cells, it was selected further. The size and morphology of MCF-7 cells were altered, typically at higher concentrations (Fig. 4).

Among all PVS extracts, PVSC extract was more effective against MCF-7 cells (based on the lowest CC_{50} value) and also showed a promising antibacterial effect; hence, it was further selected for GC–MS analysis. The GC–MS chromatogram of PVSC extract revealed the presence of 17 compounds, whose peak area varied from 0.10 to 38.74 percent. The peaks representing prominent phytoconstituents of the extract are displayed in Fig. 5. The elemental analysis and molecular details of these compounds are given in table 3. Of these chemical compounds, 8-pentadecanol (38.74 %), lupeol (16.38 %), and 2-butoxyethyl oleate (14.08 %) were identified as principal components of PVSC extract and selected for further studies. The GC–MS spectrum of each of the principal phyto-compounds selected from the chloroform extract of PVS is shown in the Fig. 6a–c. The 2D structures of selected phyto-compounds derived from the NIST Chemistry WebBook are shown in Table 4.

Fig. 7 shows the findings of the FTIR examination of the PVSC extract. The distinctive infrared absorption frequencies of functional groups were taken into account while interpreting the spectra (http://www2.ups.edu/faculty/havson/spectroscopy/IR/IRfrequencies.html). While the strong peak at 3300 cm^{-1} showed the presence of phenolic -OH group, the IR peak at 1660 cm^{-1} signified the C=C group corresponding to high lipidic content in PV seeds. The peak at 1050 cm^{-1} indicated the presence of the alcoholic -OH group.

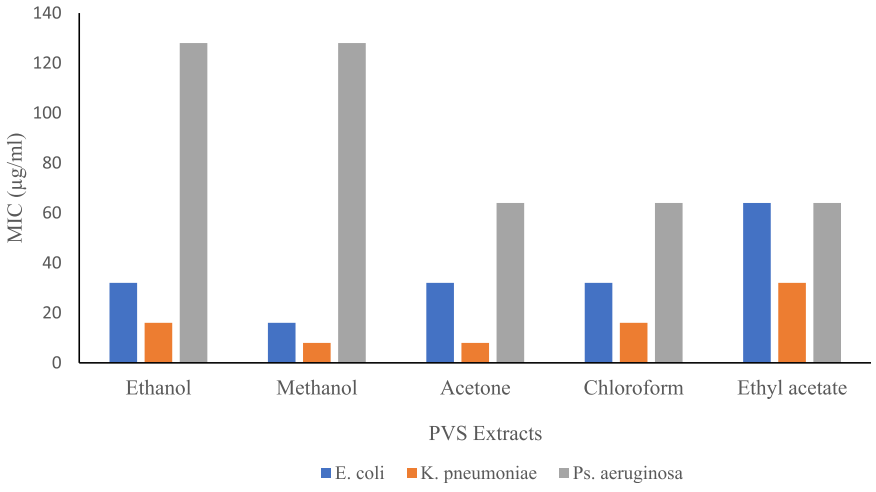


Fig. 2. MICs of PVS extracts for growth inhibition of test bacteria by broth microdilution methods.

Table 2
Cytotoxic effect of WKB extracts against MCF-7 cells.

Sample	Test conc (μg/ml)	Absorbance at 590 nm	% Inhibition	CC_{50}
PVSE	0.3125	0.685	8.05	8.446
	0.625	0.653	12.34	
	1.25	0.619	16.91	
	2.5	0.578	22.41	
	5	0.503	32.48	
	10	0.317	57.44	
PVSM	0.3125	0.721	3.22	9.539
	0.625	0.687	7.78	
	1.25	0.625	16.10	
	2.5	0.543	27.11	
	5	0.482	35.30	
	10	0.389	47.78	
PVSA	0.3125	0.731	1.87	15.188
	0.625	0.702	5.77	
	1.25	0.684	8.18	
	2.5	0.641	13.95	
	5	0.596	20.0	
	10	0.498	33.15	
PVSC	0.3125	0.668	10.34	3.849
	0.625	0.584	21.61	
	1.25	0.468	37.18	
	2.5	0.386	48.19	
	5	0.216	71.01	
	10	0.101	86.44	
PVSEA	0.3125	0.711	4.56	6.800
	0.625	0.643	13.69	
	1.25	0.566	24.02	
	2.5	0.442	40.67	
	5	0.386	48.18	
	10	0.299	59.86	
Doxorubicin (μM)	1.5625	0.685	8.05	33.034
	3.125	0.598	19.73	
	6.25	0.505	32.21	
	12.5	0.425	42.95	
	25	0.345	53.69	
	50	0.157	78.93	
	100	0.078	89.93	
Control	0	0.745	0.00	0.00

In terms of binding energies, the binding affinities of lupeol, 8-Pentadecanol, and 2-butoxyethyl oleate were −10.5 Kcal/mol, −6.5 Kcal/mol, and −8.5 Kcal/mol for ER -α, respectively (Table 5). The compounds that exhibited the highest binding affinity for ER-α were lupeol, 8-pentadecanol, and 2-butoxyethyl oleate. For 8-pentadecanol, lupeol, and 2-butoxyethyl oleate, the inhibition constant (Ki) values were found to be 0.99748134873, 0.99573456365, and 0.99670765608, respectively. Fig. 8a–d show the interaction details of doxorubicin, 8- pentadecanol, 2-butoxyethyl oleate, and lupeol with the Er-α active site, respectively. Mostly through Vander Waals contacts, the 8-pentadecanol and lupeol interacted with the amino acid residues of Er-α. Er-α reacted with the standard medication doxorubicin through hydrogen bonding with the residues Arg 394 and Glu 353. Table 5 further shows that Met 388, Glu 419, Met 343, Leu 536, Asp 351, Thr 347, Leu 384, and Leu 349 are engaged in hydrophobic interactions.

Using a Swiss online server, the physicochemical and pharmacokinetic characteristics of the three phytoconstituents of PVSC extract and the reference medication doxorubicin were examined. Table 6a displays the comparison of the phytoconstituents and ADME characteristics with doxorubicin. Lipinski's rule of five criteria showed that conventional doxorubicin violates three parameters, lupeol, 8- pentadecanol, and 2- butoxyethyl oleate only one (Table 6b). While 8-pentadecanol has a high GI absorption level, doxorubicin, lupeol, and 2-butoxyethyl oleate have low amounts. According to the ESOL method's solubility results, doxorubicin is a highly soluble drug, 8-pentadecanol is moderately soluble, and lupeol and 2-butoxyethyl oleate are weakly soluble. Lupeol complied with both Veber's and Lipinski's laws, while doxorubicin did not meet either requirement. Except for 8-pentadecanol, none of the chosen drugs were BBB permeant; doxorubicin and lupeol did not inhibit all CYP 450 isoforms, while 8-pentadecanol and 2-butoxyethyl oleate inhibited both CYP 450 enzymes.

Table 7 presents the findings of the toxicological characteristics of each chosen substance as determined by the Admet SAR online server. Other than 8-pentadecanol, all of the phytoconstituents in the PVSC extract were non-carcinogens, and none of them were Ames hazardous. Level III acute oral toxicity group was indicated for all of them. Doxorubicin, the typical medication, is classified as having class III acute oral toxicity, is Ames hazardous, and is not carcinogenic. Based on the ADME and toxicological characteristics of phyto-compounds, it was found that lupeol is the most potent cytotoxic compound compared to doxorubicin, 8-pentadecanol, and 2-butoxyethyl oleate.

The results of the PASS online server's analysis of the biological potential spectrum of the phyto-compounds and doxorubicin are provided in tables S1, S2, S3, and S4 (supplementary data). The anti-neoplastic activity of lupeol against breast cancer was measured by Pa values of 0.799, which were higher than the similar Pa values of doxorubicin (0.372). Pa values for the anticancer properties of 2-butoxyethyl oleate and 8-pentadecanol were lower than those of lupeol.

4. Discussion

Common bacterial infections, or UTIs, affect women more often than males. A condition that is projected to have affected 500 million people globally has been identified, and half of all females are predicted to be impacted at some point in their lives (Medina and Castillo, 2019; Cek et al., 2014). The overuse of broad-spectrum antibiotics during the treatment of UTI resulted in the emergence of drug-resistant pathogens, making treatment more challenging (Cheeseman et al., 2017).

Apart from the difficulties in managing infections brought on by drug-resistant bacterial strains, the growing incidence of breast cancer in females is garnering global attention because of the high death rate linked to it and the effectiveness of current chemotherapeutic treatments. The last few decades have seen the identification of the pharmacological potential of plant-based therapies over synthetic medications (Pan et al., 2013). Research has shown that plants provide a safer and non-poisonous source of antibacterial and anticancer chemicals. Additionally, the use of traditional medicinal plants in public health development programs was highlighted in the WHO traditional-medicine-strategy launch program (WHO, 2013). While several traditional plants are being investigated for their anticancer properties, only a few of these have undergone thorough scientific or clinical examination. *P. vulgaris*, a member of the Fabaceae family, has been used historically as a dietary supplement for human consumption all over the world. The seeds are abundant in phenolic compounds, enzyme inhibitors, and essential minerals. The seeds are extensively researched for their antibacterial and hypolipidemic properties in addition to their status as a nutraceutical food (Kimothi and Dhaliwal, 2020). This is the reason it was chosen for the current study.

The antibacterial and anticancer activity of several solvent extracts of *P. vulgaris* seeds has been examined in the current investigation. The PVSC extract was chosen for additional research due to its efficacious anticancer potential. Compared to *Pseudomonas aeruginosa*, the antibacterial properties of all extracts showed more promise against *E. coli* and *Klebsiella pneumoniae*. According to Harbarth et al. (2015), uropathogenic *E. coli* is the primary cause of UTIs, accounting for 65–75 % of cases reported globally. When tested against *E. coli*, the antibacterial activity of PVSM and PVSE extracts at 100 μg/ml was higher than that of the common antibacterial medications, gentamycin (10 μg/ml) and chloramphenicol (30 μg/ml). PVS

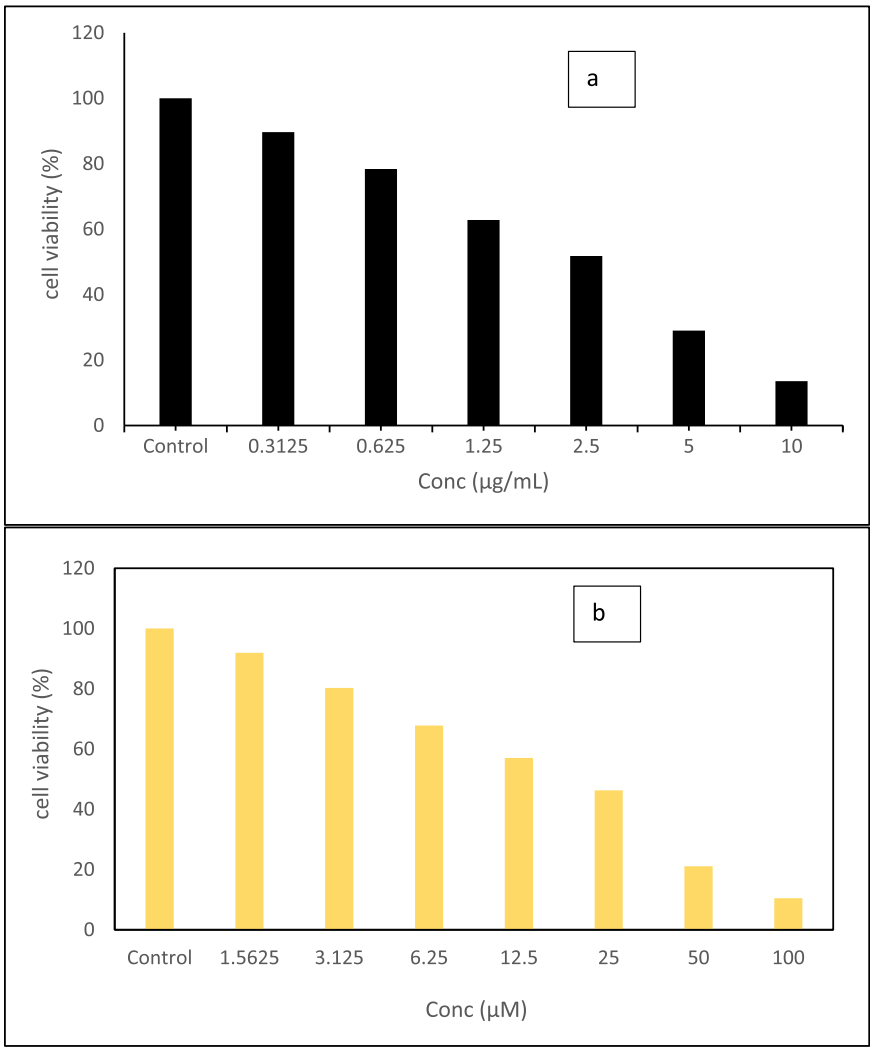


Fig. 3. Effect of varying concentrations of PVSC (a) and doxorubicin (b) on the viability of MCF-7 cells after 24 h incubation.

extracts demonstrated antibacterial activity against *K. pneumoniae*, which demonstrated resistance to the doses of chloramphenicol and gentamycin under investigation. Extracts from PVSE and PVSM seemed to have little effect on the *Ps. aeruginosa* strain, although extracts from other sources showed some inhibitory activity. The kind and concentration of phytoconstituents in extracts may be the cause of the variance in activity. Immense public health issues are raised globally by drug-resistant pathogenic microorganisms, which represent a major hazard. As per the World Health Organization, we are nearing the "post-antibiotic era," whereby we must confront obstacles including the ineffectiveness of current chemotherapy drugs' side effects and a substantial financial strain for illness treatment (Harbarth et al., 2015). The PVS extract to which the test microorganisms demonstrated sensitivity, could be a highlight in this

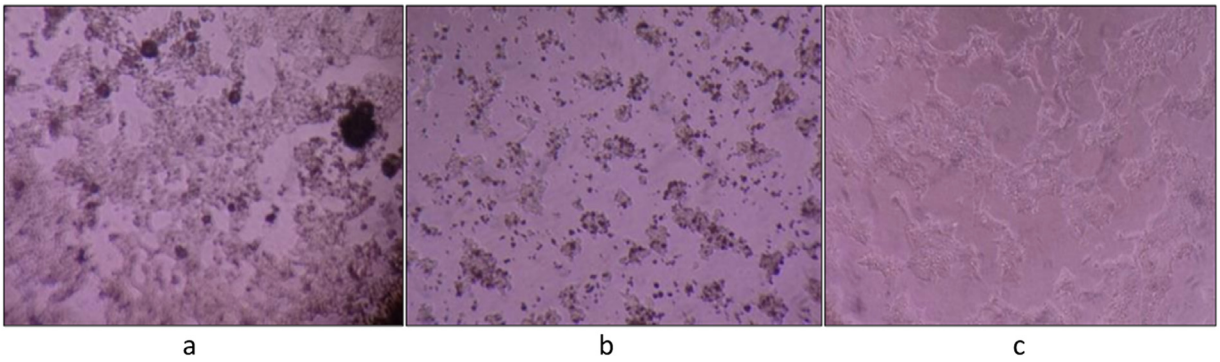


Fig. 4. Cytotoxic effect of a: PVSC extract (10μg/ml), b: Doxorubicin (100 μM) against MCF-7 cells in comparison with control (c).

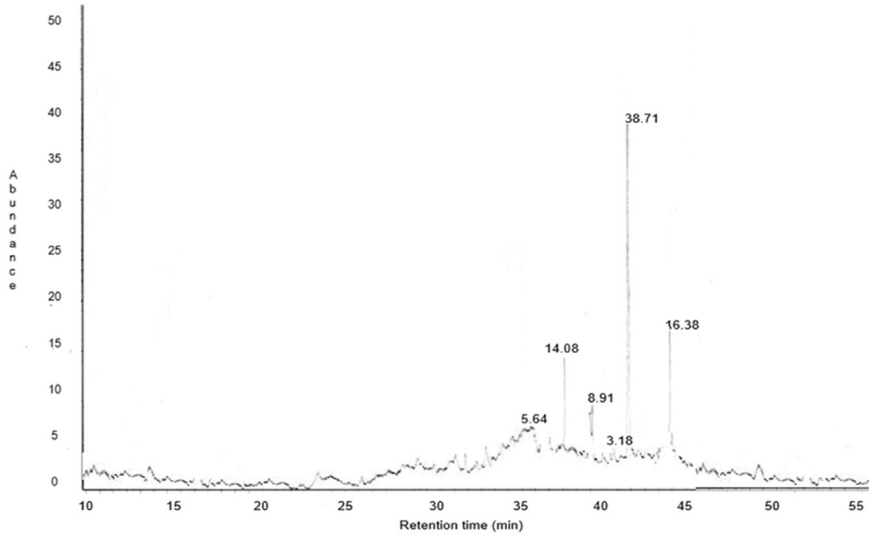


Fig. 5. GC–MS chromatogram of PVSC extract.

sense. Further research on these extracts may lead to the development of naturally occurring bioactive compounds that combat bacteria resistant to gentamycin and chloramphenicol. White kidney beans are high in lectins, phytic acid, proteins, polyphenols, and galacto-oligosaccharides. These chemicals were extracted from P. vulgaris seeds and examined for their biological activity, as previously reported (Diaz-Batalla et al., 2006). Roy et al. (2020) discovered the antioxidant and antibacterial properties of protein hydrolysates from *P. vulgaris* seeds. The seeds pepsin hydrolysate DPH-1 had an antibacterial effect on *E. coli* (20.26 mm), while papain hydrolysate DPH-2 prevented the development of *Ps. aeruginosa* (19.23 mm). In addition to previous studies (Abbas et al., 2020; Atallah et al., 2021) that described the antimicrobial potential of 7S and 11S globulins isolated from different sources of beans, Sitohy et al. (2024) reported on the antibacterial properties of methylated and native seed proteins from hexane extracts of *Phaseolus vulgaris* against *Staphylococcus pasteuri, S. pyogens, K. pneumoniae, E. coli,* and *Ps. aeruginosa*. The present study adds to the library of natural bioactive compounds from beans, and the bioactive elements of PVS extracts might be explored further to treat infections by uropathogenic bacteria.

The potential of PVSC extract in the development of natural antiproliferative medicines was also suggested by its cytotoxic effect on MCF-7 cell proliferation. People have been using everyday foods to prevent or combat cancer since ancient times. There have been reports of chemoprotective effects from bitter gourd, apples, turmeric, grapes, and other berries (Bai et al., 2016). In addition to being a functional food or nutritional supplement, *P. vulgaris* seeds have been shown to have antimutagenic, antioxidant, antidiabetic, and cardioprotective properties (Chatterjee et al., 2018; Mojica et al., 2017; Monk et al., 2016; Frassinetti et al., 2015). While the chemical components found in *P. vulgaris* seeds have been shown to have antidiabetic properties, research on white kidney beans' anticancer potential is less widely known. Our investigation found that PVS extracts decrease MCF-7 cell viability in a dose-dependent manner, with PVSC extract having the lowest CC_{50} of 3.826 μg/mL. Although there is insufficient data about the WKB's antitumoral activity on diverse cell lines, an increasing number of studies have revealed the cytotoxic effects of components from various bean extracts. El-keiy et al. (2019) showed that saponins derived from soybean seeds had anti-tumor action against a human colon cancer cell line, which is related with apoptosis triggered by caspase-9 activation. Previous research has shown that alpha galactooligosaccharides from soybeans and verbascoside from *Vicia faba* have cytotoxic action against breast cancer cells (Chappuis et al., 2017; Tiwari et al., 2019). GC–MS

Table 3
GC MS elution profile and structural details of phyto-compounds of PVSC extract.

Sr. No	Name	RT	Peak Area (%)	Molecular formula	Molecular weight (g/mol)
1	Diethyl Phthalate	25.540	0.81	$C_{12}H_{14}O$	222.24
2	Penta ethylene glycol mono dodecyl ether	31.859	0.96	$C_{22}H_{46}O_6$	406.6
3	Octadecanoic acid, 3-hydroxy-2-tetradecyl-, methyl ester, (2R,3R)-	34.191	0.10	$C_{33}H_{66}O_3$	510.9
4	n-Hexadecanoic acid	35.342	5.20	$C_{16}H_{32}O_2$	256.4
5	2-Butoxyethyl oleate	37.791	14.08	$C_{24}H_{46}O_3$	382.6
6	Oleic Acid	38.407	8.91	$C_{18}H_{34}O_2$	282.5
7	Hexadecanoic acid, 2-hydroxyethyl ester	39.701	5.64	$C_{18}H_{36}O_3$	300.5
8	Hexadecanoic acid, oxydi-2,1-ethanediyl ester	39.954	1.36	$C_{36}H_{70}O_5$	582.9
9	Glycidyl palmitate	40.477	0.68	$C_{19}H_{36}O_3$	312.5
10	Hexanedioic acid, bis(2-ethylhexyl) ester	41.923	0.62	$C_{22}H_{42}O_4$	370.6
11	Oleoyl chloride	42.521	3.18	$C_{18}H_{33}ClO$	300.9
12	8-Pentadecanol	42.714	38.74	$C_{15}H_{32}O$	228.41
13	2H-Pyran-2-one, tetrahydro-6-nonyl-	42.761	1.28	$C_{14}H_{26}O_2$	226.35
14	Methyl 5,11,14-eicosatrienoate	43.448	0.39	$C_{21}H_{36}O_2$	320.5
15	Glycidyl oleate	43.523	0.65	$C_{21}H_{38}O_3$	338.5
16	Lupeol	44.340	16.38	$C_{30}H_{50}O$	426.7
17	Bis(2-ethylhexyl) phthalate	44.739	0.77	$C_{24}H_{38}O_4$	390.6

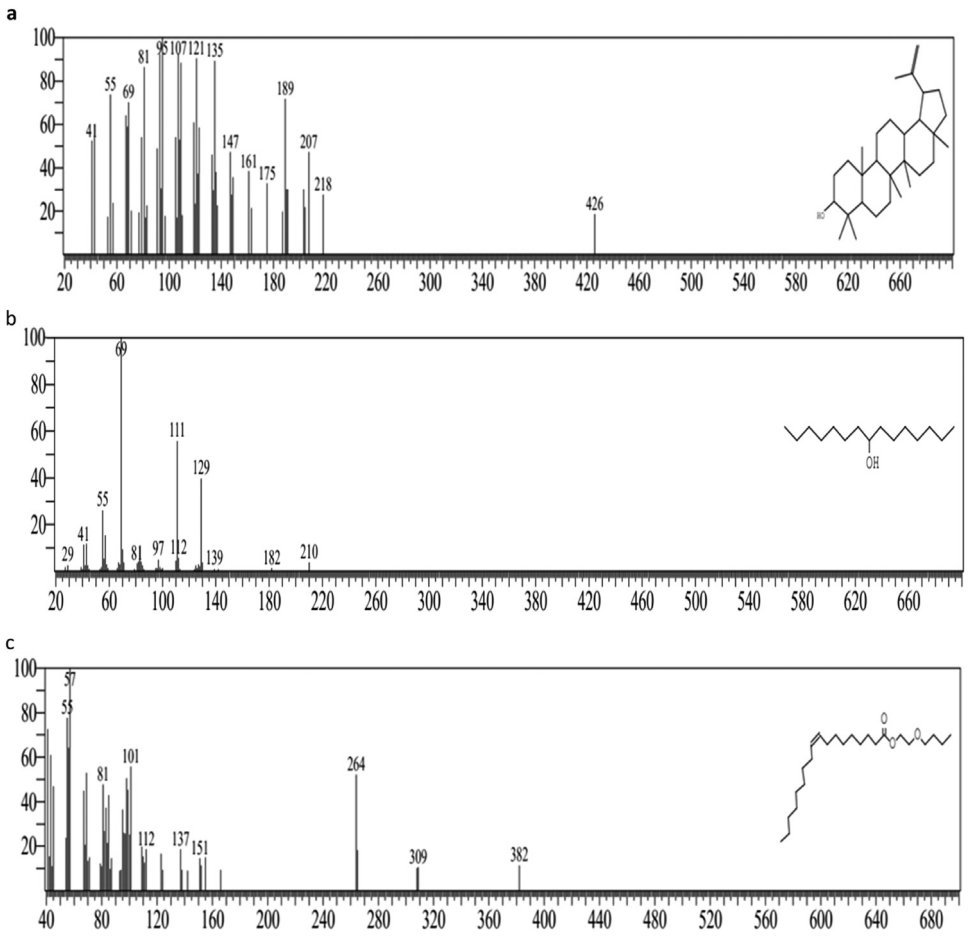
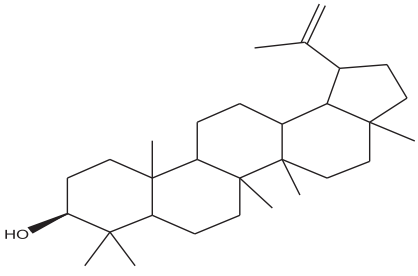
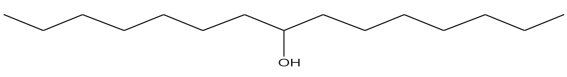
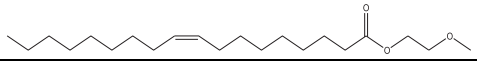


Fig. 6. GC–MS chromatogram and fragmentation pattern of most prominent identified compounds in PVSC extract (a) lupeol, (b) 8 pentadecanol, and (c) 2- butoxyethyloleate.

analysis was used to determine the active ingredients that gave PVSC extract its anticancer properties. The major components of the extract were found to be 2- butoxyethyl oleate, lupeol, and 8- pentadecanol. The docking results indicated that all three components were reactive with the target protein Er-α. Lupeol has a higher binding affinity (−10.5 Kcal/mol) for Er-α compared to doxorubicin, 2-butoxyethyl oleate, and 8-pentadecanol. Lupeol and its derivatives have binding energies ranging from −9.95 Kcal/mole to −12.24 Kcal/mole, with inhibition constant values of 0.00107 −0.05085 μM, as previously described (Pratama and Sotoma, 2018). As a result, the binding energies and inhibition constants of lupeol found in this study are consistent with previous findings. It has been discovered

Table 4
2D structures of selected phyto-compounds from PVSC extract.

Name of the phytocompound	2D structure of Phytocompound
Lupeol	
8-Pentadecanol	
2-Butoxyethyl oleate	

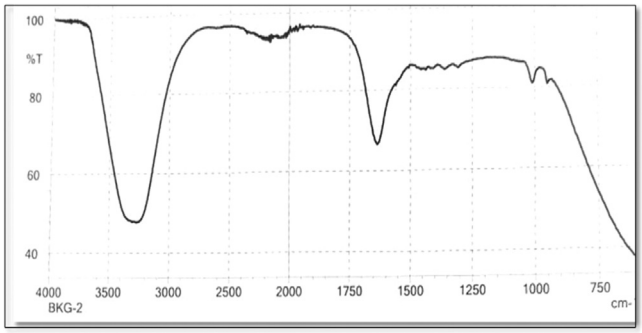


Fig. 7. FTIR spectrum of PVSC extract.

that Er-α plays a crucial role in the development of estrogen-positive breast cancer, accounting for 70 % of cases. It leads to the activation of downstream signalling pathways for estrogen formation and contributes to drug resistance, epithelial-mesenchymal transition (Tian et al., 2019), and metastasis in breast cancer (Han et al., 2018). Thus, lupeol's high affinity and low inhibitory concentrations towards Er-α suggest a potential role in the development of an anticancer drug.

Compared to doxorubicin, the phyto-compounds that were chosen also met a greater number of the criteria for the ADME criteria in terms of pharmacokinetic and physiochemical measures. According to Xu et al. (2012), AMES toxicity reveals a compound's potential for mutagenesis. Whereas doxorubicin demonstrated AMES toxicity, all three components of the PVSC extract were AMES-nontoxic. The non-toxic character of phyto-compounds in PVSC extract was deduced through toxicological examination of a subset of components. Since lupeol was discovered to have the strongest interaction with Er-α, its anticancer potential was investigated using the PASS online software. The outcomes were compared to activity scores for doxorubicin, 2-butoxyethyl oleate, and 8-pentadecanol. The anticancer potential was predicted by taking into account antineoplastic properties. The results are shown by the PASS online server as activity (Pa) or inactivity (Pi) scores, which show whether biological activity is present or not. Lupeol demonstrated antitumor activity against the majority of tumors when compared to other antineoplastic agents. Its Pa score of 0.799 against breast cancer was higher than that of doxorubicin (0.372) and higher than Pi score values. 2 butoxy ethyl oleate (0.495) and 8- pentadecanol (0.502) had related Pa scores for antineoplastic activities that were higher than doxorubicin and lower than lupeol.

This research suggested that lupeol would be a more effective anticancer agent. Based on its chemical makeup, lupeol is a pentacyclic triterpenoid with previously reported benefits including anti-inflammatory, antibacterial, anti-angiogenic, antidiabetic, and wound healing (Siddique et al., 2011). Pitchai et al. (2014) observed that lupeol was harmless to human cells and that it had an anticancer impact on MCF-7 cells with an IC_{50} value of 80 μM. Apart from its impact on MCF-7 cells, lupeol has also been documented to exhibit activity against Au 27 cells, which are lung cancer cells (He et al., 2018), human cervical cancer (HeLa) cells (Prasad et al., 2018), and HCT116 and SW620 cells, which are colorectal malignant cells (Jiang et al., 2021)

5. Conclusions

The current study evaluates the bioactive potential of nutrient-dense white kidney beans in terms of antibacterial and anti-breast cancer activity. The methanol extract of PV seeds was more efficient

Table 5
Details of molecular interactions of selected compounds with Er-α protein.

Target protein	Ligand	Binding affinity (Kcal/mol)	Ki (μM)	Hydrogen bonding pair	Residue in hydrophobic interaction or van der Waals constant
Estrogen receptor alpha (ER-α)	Lupeol	−10.5	0.99593456365	-	Gly 366, His 474
	8-Pentadecanol	−6.5	0.99748134873	-	Pro 325, His 356, Glu 323, Glu 353, Lys 449, Ile 386, Phe 445, Gly 390, Arg 349
	2-Butoxyethyl oleate	−8.5	0.99670765608	Glu 353	Ile 3236, Pro 325, Pro 324
	Doxorubicin	−9.6	0.99628238102	Glu 353, Arg 394	Met 388, Glu 419, Met 343, Leu 536, Asp 351, Thr 347, Leu 384, Leu 349

Table 6a
Physicochemical and pharmacokinetic properties of compounds.

Pharmacokinetic property	Phytocompound			
	Lupeol	8-Pentadecanol	2- Butoxyethyl oleate	Doxorubicin
Formula	$C_{30}H_{50}O$	$C_{15}H_{32}O$	$C_{24}H_{46}O_3$	$C_{27}H_{29}NO_{11}$
No. of heavy atoms	31	16	27	39
No. of rotatable bonds	1	12	22	5
TPSA	20.23 A^2	20.23 A^2	35.53 A^2	206.07 A^2
Log S (ESOL)	−8.64	−4.52	−6.24	−3.91
Solubility	Poor	Moderate	Poor	Soluble
GI absorption	Low	High	Low	Low
BBB Permeant	No	Yes	No	No
P.gp substrate	No	No	No	Yes
CYP 1A2 Inhibitor	No	Yes	Yes	No
CYP2C19 Inhibitor	No	No	No	No
CYP2C9 Inhibitor	No	No	No	No
CYP2D6 Inhibitor	No	No	No	No
CYP3AG Inhibitor	No	No	Yes	No
Log KP (Skin permeant)	−1.90 cm/s	−3.12 cm/s	−2.46	−8.71 cm/s
Veber's rule	Yes	No	No	No
Bioavailability Score	0.55	0.55	−9.55	0.17
PAINS alert	0	0	0	1

Table 6b
Drug likeliness analysis of selected compounds based on Lipinski's rule of five.

Name of the phytocompound	Molecular weight < 500	HBA < 10	HBD ≤ 5	Log P ≤ 5	MR 40 - 130	Lipinski Violation ≤ 1
Lupeol	426.72	1	1	4.68	135.14	1
8 Pentadecanol	228.41	1	1	4.19	75.38	1
2- Butoxy ethyl oleate	382.62	3	0	5.98	119.38	1
Doxorubicin	543.52	12	6	2.16	132.66	3

HBA: Hydrogen Bond Acceptor, HBD: Hydrogen Bond Donor, MR: Molar Refractivity.

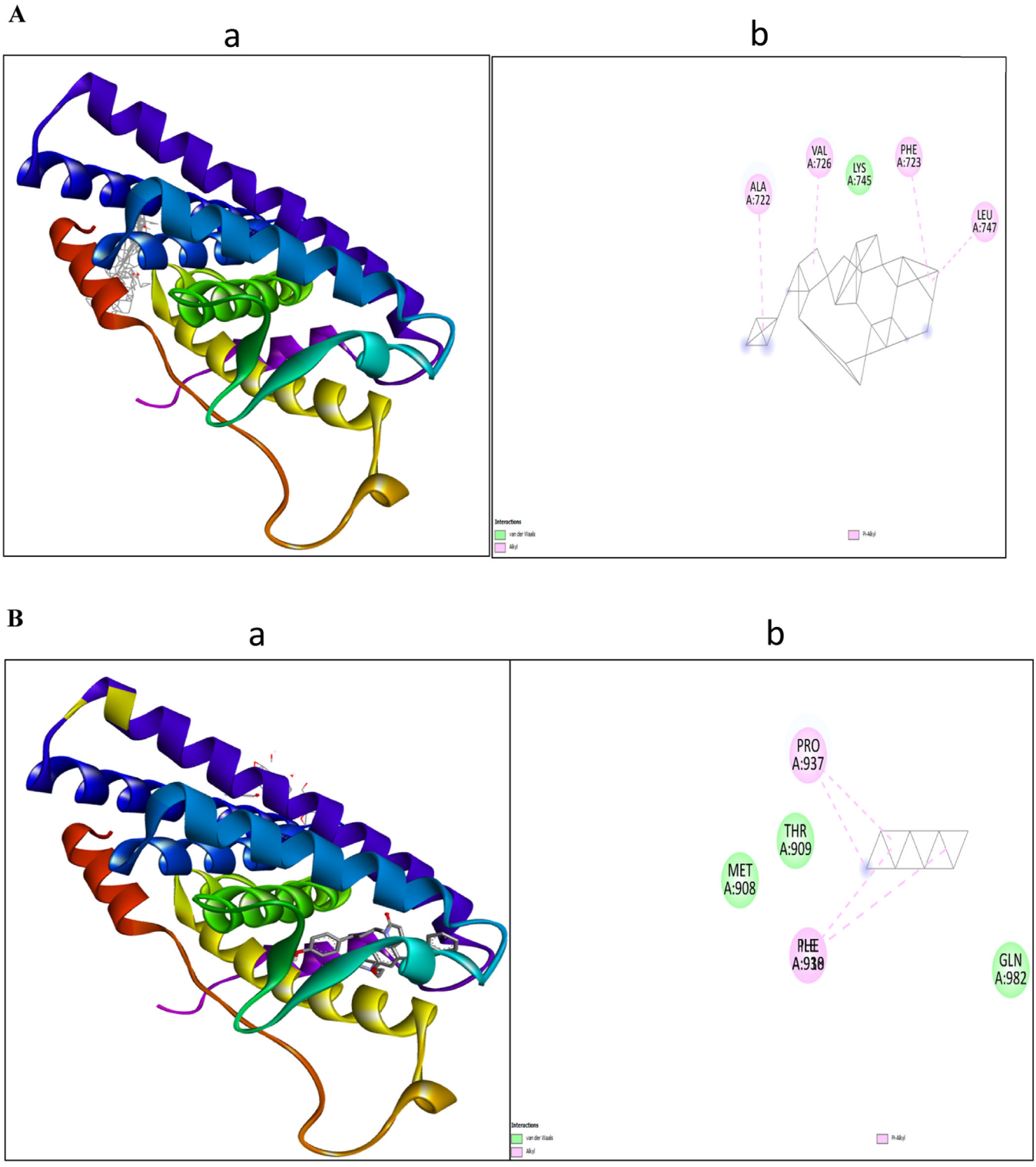


Fig. 8. 3D (a) and 2D (b) interactions of lupeol (A), 8-pentadecanol (B), and 2- butoxyethyl oleate (C) and doxorubicin (D) with Er-α protein.

Table 7
Toxicity and carcinogenicity profiling of selected compounds.

Toxicity	Lupeol		8-Pentadecanol		2-Butoxyethyl oleate		Doxorubicin	
	Profile	Probability	Profile	Probability	Profile	Probability	Profile	Probability
AMES toxicity	NT	0.9420	NT	0.9837	NT	0.8213	T	0.9198
Carcinogen	NC	0.9188	C	0.5066	NC	0.534	NC	0.9534
Acute oral toxicity	III	0.8578	III	0.8149	III	0.5264	III	0.7766
Rate acute toxicity	3.3838	LD_{50} mol/kg	1.7615	LD_{50} mol/kg	1.61	LD50 mol/kg	2.6644	LD_{50} mol/kg

T: Toxic, NT: Non-toxic, C: Carcinogen, NC: Noncarcinogen.

against *E. coli* and *K. pneumoniae*, while the chloroform extract inhibited *Ps. aeruginosa*. Lupeol, a component of the PVSC extract, showed more promising interactions than 8-pentadecanol and 2-butoxyeutyl acetate. Lupeol's prospective therapeutic potential for the treatment of breast cancer was also validated by its nontoxic and non-carcinogenic nature, satisfaction of drug likeliness criteria, and the highest projected anti-breast cancer score. However, the experiments were conducted *in vitro*, and their findings require confirmation *in vivo*, necessitating further research into the mechanism of lupeol's cytotoxic effect, bioavailability, and metabolism.

Consent for publication

All authors have read the manuscript and given consent for publication in this journal.

Ethics approval

This is an observational study. The Research Ethics Committee of the School of Life Sciences, SRTMUN has confirmed that no ethical approval is required.

Funding

The authors did not receive support from any organization for the submitted work.

Availability of data and material

All data related to this manuscript is included during submission.

Declaration of competing interest

"All authors declare no financial or non-financial interests in this paper".

CRediT authorship contribution statement

Pallavi B Jadhav: Resources, Methodology. **Hemlata J Bhosale:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization. **Shailesh V Mamdapure:** Software, Formal analysis. **Sunil B Jadhav:** Software, Methodology, Formal analysis.

Acknowledgments

The authors would like to acknowledge DST-FIST sponsored School of Life Sciences, SRTM University, Nanded, MS, India for providing support and facilities to conduct this research work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2024.11.033.

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