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Phytochemical profiling of peel extracts of *Colocasia esculenta*, *Raphanus sativus*, and *Daucus carota*, and Molecular Dynamics Simulation to explore the Inhibitors for Anti-quorum Sensing Activity.

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Abstract

Membrane based bioreactors are efficient for wastewater treatment but face major challenge of membrane biofouling. This study investigates the potential of compounds present in vegetable peel extracts for anti-quorum sensing (anti-QS) activity using molecular dynamics simulation. Extraction of phytocompounds from Colocasia esculenta, Raphanus sativus, and Daucus carota peels followed by GC/MS analysis was performed. Invitro antioxidant potential of peel extract was estimated using DPPH free radical scavenging assay, Ferric Reducing Antioxidant Power (FRAP) assay. Phenolic content present in the extract of Colocasia esculenta, Raphanus sativus, Dacus carota was around 3.35 mg GAE/ml, 0.43 mg GAE/ml, 3.308 mg GAE/ml respectively whereas total flavonoid content in Colocasia esculenta, Raphanus sativus and Dacus carota was 3.95 mg QE/ml, 1.77 mg QE/ml, 4.87 mg QE/ml respectively. Forty-six, Forty-five and Fifty-three phytocompounds were identified in Colocasia esculenta, Raphanus sativus and Daucus carota respectively. The methanol extract of Colocasia esculenta, Raphanus sativus, Dacus carota exhibited notable antioxidant potential, with percentage inhibition of 78.8%, 29.2% and 60.89% respectively for DPPH assay, and 74% (CE), 26% (R) and 49% (C) for ferrous reducing power assay. Molecular docking studies revealed the strong binding interaction of MTA/SAH nucleosidase with Stigmasterol (-8kcal/mol) and 3-Benzyl-3-hydroxycholestane-2-carbaldehyde (-8.4 kcal/mol). According to MMPBSA Calculations, Free binding energy of carbaldehyde complex was -92.084 ± 2.375 kJ/mol. 3-Benzyl-3-hydroxycholestane-2-carbaldehyde present in the peel of Raphanus sativus could be a potential inhibitor of MTA/SAH nucleosidase. The results provide insights into the chemical diversity of vegetable peels and their potential as a source of novel anti-QS agents

Keyword: Phytocompounds, GC-MS analysis, Antioxidant, Anti-Quorum Sensing Activity, MTA/SHA Inhibitors, Molecular Docking, Molecular dynamic simulation

Introduction

Membrane-based bioreactors (MBR) represent an effective technology for wastewater treatment, integrating biological treatment and membrane filtration(Oh and Lee, 2018). However, MBR encounters challenges due to membrane biofouling, where a layer of bacterial biomass accumulates on the membrane surface, diminishing filtration efficiency. Controlling biofouling is essential as it elevates transmembrane pressure, reduces osmotic flux, and increases energy consumption and operational costs by up to 60%(Jiao et al., 2020). Despite extensive efforts by researchers employing engineering, physiochemical methods, regular cleaning, operational strategies, and chemical additives, satisfactory results remain elusive (Oh and Lee, 2018). Since, it's been reported that there is a direct correlation between the quorum sensing and biofilm formation, Quorum sensing has become a fundamental approach to inhibit biofouling (Oh et al., 2012; Oh and Lee, 2018; Yeon et al., 2009). Additionally, Anti-quorum sensing activity is recently gaining attention, as a promising strategy to target Antimicrobial resistant, Drug discovery, and controlling membrane biofouling in wastewater treatment (Jiao et al., 2020; Peng et al., 2023).

Quorum sensing is a bacterial intercellular communication mechanism which is dependent on bacterial cell population and also responsible for bacterial virulence factors. It is regulated by extracellular signalling molecules known as Autoinducers like autoinducer-2 (AI-2), acylated homoserine lactones (acyl-HSLs), Autoinducing Peptides, hydroxyl-palmitic acid methyl ester (PAME), Pseudomonas quinolone signal molecule (PQS), g-butyrolactone, cyclic diguanylate (c-di-GMP), diffusible signal factor (DSF), and 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS). On the other hand, anti-quorum sensing is inhibition of quorum sensing either by enzymatic method i.e. Quorum Quenching and non-enzymatic methods i.e. Quorum sensing inhibition (QSI) (D. Shah et al., 2023; Liu et al., 2021; Samreen et al., 2022).

Autoinducer-2 are termed as universal signalling molecules present in both gram-positive and gram-negative bacteria. So, targeting AI-2 molecules synthesis could be a good strategy for inhibiting quorum sensing. As illustrated in Figure 1, Methylthioadenosine/S-adenosylhomocysteine nucleosidase (MTA/SAH nucleosidase) and LuxS are the two main enzymes responsible for AI-2 biosynthesis. So, inhibition of either of these enzymes will result in decrease in AI-2 production. In addition, MTA/SAH nucleosidase has shown possible role in the AHLs biosynthesis. Therefore, targeting MTA/SAH nucleosidase is a promising approach for developing new quorum sensing inhibitor (Liu et al., 2021).

The aim of this study is to identify the phytochemical compounds present in the peel extracts of *Colocasia esculenta* (CE), *Raphanus sativus* (R), *and Daucus carota* (C). The fruit and vegetable industry contribute approximately 44% of the global annual waste with food waste in Europe alone estimated at around 89 million tons. This is Anticipated to grow by a factor of 40 in the upcoming years (Manthei et al., 2023). According to Ministry of Food Processing industry, India's fruit and vegetable loss is around 12 to 21 million ton (Bhardwaj et al., 2022). Fruit and vegetable waste (FVW) include the undigestible, nonedible, parts of the fruits and vegetables like peels, seeds. stems that are discarded at any time in the process like production, processing, distribution and consumption. FVW are rich source of polyphenols, dietary fibers and bioactive compounds. It has been reported that the peel of lemon, jackfruit, grapes, avocadoes have 15% more total polyphenolic compound than their pulp (Bhardwaj et al., 2022; Kumar et al., 2020). Inefficient use of FVW results in the loss of valuable nutrition, and its in-proper disposal in landfills contributes to the emission of methane, soil and water pollution. Therefore, Utilization of FVW presents a valuable opportunity to reduce waste, produce value added products, and it also promotes circular economy. Understanding the chemical profile by GC-MS analysis of these peels can provide valuable insights into their potential health benefits and contribute to the development of functional foods, nutraceuticals, and pharmaceuticals. Furthermore, the comparative analysis of phytochemical profiles among these diverse vegetables may reveal unique compounds specific to each species, aiding in the exploration of their distinct bioactivities. Utilization of peel waste for value added products could result in the foundation of sustainable development. By employing an in-silico approach, an inhibitor of the MTA/SAH nucleosidase enzyme was discovered among the compounds found in the peel extract through molecular dynamic simulation studies.

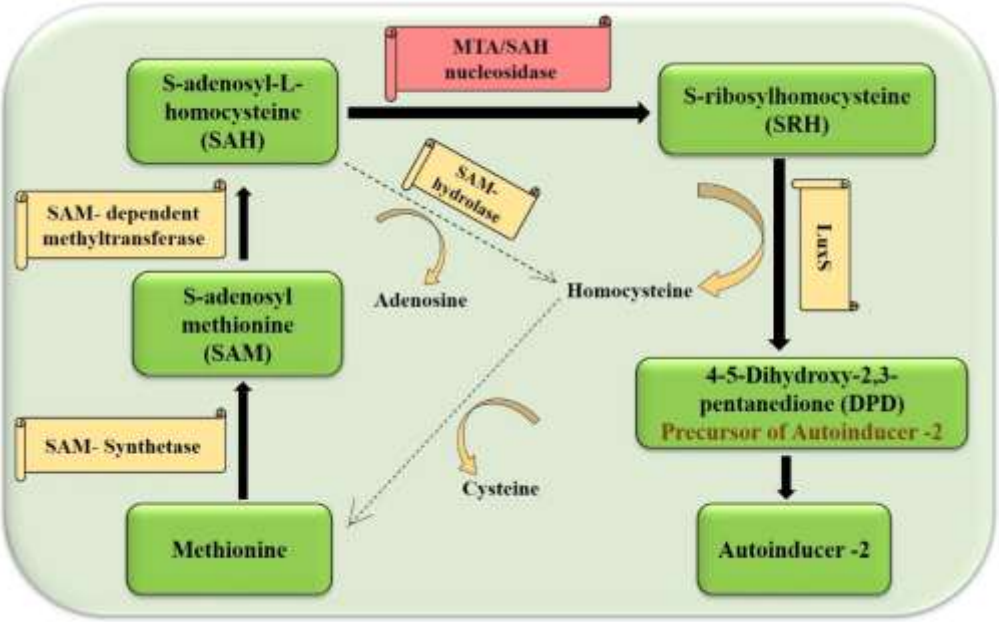

Figure 1: Autoinducer-2 synthesis pathway

Material and Methods

Preparation of peel extract

The peels of *Colocasia esculenta* (CE), *Raphanus sativus* (R), and *Dacus carota* (C) were carefully rinsed using double distilled water and subsequently allowed to air dry for a duration of 24 hours. Following this drying period, the peel specimens were placed in an oven set at 70ºC and kept there until complete dehydration was achieved. The dried sample were then grinded to make a fine powder.

Preparation of solvent extract assisted microwave and sonication

The crushed peels of CE, R, and C were utilized for extraction of secondary metabolite using various organic solvents (Methanol, Chloroform, Ethyl acetate, Hexane), followed by subsequent phytochemical analysis. A total of 5 grams of peel from each sample (CE, R, C) was immersed in 5 ml of corresponding organic solvents (Methanol, Chloroform, Hexane, Ethyl acetate) in a 1:10 ratio. These distinct mixtures were then exposed to microwave radiation for a duration of 10 seconds and left for overnight shaking, followed by a subsequent sonication process at 20MHz for 15 minutes for the extraction of secondary metabolite. The resultant mixture was than centrifuged for 30 mins at 10,000 rpm. The supernatant was transferred to a falcon and concentrated by alternate drying in hot-air oven (50ºC) as well as, air-drying at room temperature (Mohammed and Abdullah, 2022; Tekale et al.,

2017). The dried material was stored at 4ºC until further analysis. The weights of all the obtained extracts were measured, and their percentage yields were calculated based on the following formula (Singh et al., 2023):

% Yield of plant extract =

$$\frac{(weight\ of\ container\ with\ plant\ extract\ (g) - weight\ of\ empty\ container\ (g)}{weight\ of\ plant\ material\ used\ for\ extraction\ (g)} * 100$$

Qualitative phytochemical analysis

Several biochemical tests were performed in extracts for detecting the presence or absence of various secondary metabolites such as Alkaloids, Tannins, Glycosides, Amino acids, and phenolic compounds like Flavonoids, Anthocyanins and Phenolic acids. The qualitative analysis of phytocompounds was done by following test described by (Sunitha Bai et al., 2021; Tekale et al., 2017):

Alkaloids. Dragendroff's test: 400µl of Hydrochloric acid (HCl) was added to 100µl of the extract followed by 0.2 ml of Dragendroff's reagent. Emergence of an orange red precipitate indicates the presence of alkaloid. Tannic acid test: It is done by adding 2-3 drops of tannic acid (10%) to the 0.1ml of plant extract. Its presence is indicated by buff color precipitate.

Glycosides. Legal's test: Few drops of Pyridine was added to 0.1 ml of extract followed by alkaline sodium nitroprusside. Presence of glycosides is confirmed by formation blood red color. Molisch test: 0.1 ml of extracts was mixed with drops of Alcoholic α-naphthol. This was followed by adding concentrated H_2SO_4 (400µl) slowly along the sides of the test tube. The presence of carbohydrates is indicated by the formation of a brown-colored ring at the interface of the two layers.

Tannins. $FeCl_3$ test: Few drops of $FeCl_3$ solution was added in 0.1 ml of extract. Appearance of Green/Blue color indicates the presence of tannins. Alkaline reagent test: Few drops of Sodium hydroxide solution was added to the extract, formation of yellow to red precipitate indicates the presence of Tannins.

Flavonoids. $FeCl_3$ test: Few drops of $FeCl_3$ solution was added in 0.1 ml of extract. Appearance of black precipitate indicates the presence of tannins. Alkaline reagent test: Few drops of Sodium hydroxide solution was added to the extract, formation of yellow to red precipitate that turns colorless on addition of few drops of dilute Hydrochloric acid or Acetic acid.

Sterols and Triterpenoids. Salkowski test: Few drops of concentrated H_2SO_4 was added to the 0.1ml of extract shaken well and allowed to stand for few minutes. Appearance of lower red color indicates the presence of sterols and yellow color indicates triterpenoids.

Terpenoids. Chloroform test: 0.1 ml of the extract was mixed with 1 ml of Chloroform. To it, few drops of concentrated Sulfuric acid was added, shaken well and allowed to stand for few minutes. Formation of red brown color indicates presence of terpenoids.

Saponins. Foam test: Extract was diluted with ddH_2O and shaken vigorously to obtain froth. Several drops of olive oil were added to the froth. Formation of emulsion indicates the presence of saponins.

***Phenolic compounds*.** Ellagic acid test: Few drops of 5% glacial acetic acid and 5% sodium nitrate solution was added to the 0.1 ml of sample. Niger brown precipitate formation indicates phenolics.

Determination of total phenolic content

Total phenolic content of the samples was determined by Folin- Ciocalteu method. Gallic acid standard was prepared with concentration 10 to 100µg/ml (Kakkar and Wadhwa, 2023). For each extract (CE, R, C) 1mg/ml of stock was prepared, from each stock 50 µl, 100 µl, and 200 µl of the sample was taken and mixed with the dd H_2O to make a final volume of 1 ml in a test tube. Followed by the addition of 100 µl of 10% Folin Ciocalteu's reagent and mixed through vortex. After a 5-minute incubation, 1 ml of 7.5% Na_2CO_3 and 400 µl of distilled water were added. Further, the test tubes were incubated in the dark for 30 minutes and final absorbance was taken at 765nm. The phenolic content was reported in mg of Gallic Acid Equivalent (GAE) per gram of sample (mg GAE/g).

Determination of total flavonoids content (TFC)

The estimation of TFC in peel extracts of CE, R, C was done by Aluminium Chloride method with slight modification using Quercetin as a standard (Shekhar et al., 2023). The concentration of peel extract taken was 1mg/ml, from which 0.1ml of sample was added to 900 µl of methanol. To each tube, 75 µl of 5% Sodium Nitrate was added and mix thoroughly. Subsequently, 150 µl of Aluminium Chloride (10%) was added, and the mixture stood for 5 minutes. After that 500 µl of 1M NaOH was added, and the total reaction volume was adjusted to 2.5 ml with distilled water. The absorbance of the extract was taken at

510nm. The total flavonoid content was expressed in terms of quercetin equivalent per gram (QE/gm).

Determination of Antioxidant activity

The antioxidant potential of extracts was determined by diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and Ferrous Reducing Antioxidant power (FRAP) assay as described by (Jabeen et al., 2023). In both the assays, Ascorbic acid was utilized as the reference standard with concentration 100 to 1000 µg/ml. Each extract (CE, R, C) with a concentration ranging from 100 to 1000 µg/ml were prepared. In DPPH assay, 100 µl sample of varied concentration was mixed with 50 µl of prepared 0.1mM DPPH solution and incubated for 30 minutes in dark, followed by measuring the absorbance at 517nm. All the experiments are performed in triplicates and percentage inhibition was determined according to the below mentioned formula:

$$\%\text{Inhibition} = \frac{\text{Absorbance of control without extract (A0)} - \text{Absorbance of sample (A)}}{\text{Absorbance of control without extract (A0)}} * 100$$

In FRAP assay, 0.2 ml of Different extract concentrations was added to 0.5 ml of 0.2M phosphate buffer (6.6 pH). Then, 0.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added to the mixture and incubated for 20 mins at 50ºC. After incubation, once the mixture is cool down 0.5 ml 10% trichloroactetic acid was added and further centrifuged for 10 mins at 3000 rpm. Then, collect 0.5 mL of supernatant and add 0.5ml of deionized water to it. Then, add 0.1 mL of 0.1% ferric chloride solution and proceed to measure the absorbance at 700 nm.

GCMS Analysis

The phytochemical analysis of the peel samples was done by gas chromatography–mass spectrometry instrument Shimadzu QP-2010 Plus with Thermal Desorption System TD20 at the Advanced Instrumentation Research facility (AIRF) at Jawaharlal Nehru University, New Delhi. It is a coupled technique in which chemicals may be separated and identified based on retention time and mass spectra. The dried sample was suspended in 1 ml of methanolic solvent for analysis.

Molecular Docking

Protein preparation: The crystal structure of Methylthioadenosine/S- adenosylhomocysteine nucleosidase (MTA/SAH nucleosidase) was downloaded from the RCSB Protein Data Bank with PDB ID 1JYS and resolution of 1.9Å in the pdb format. 1JYS was selected as a target due to previous studies based on MTA/SAH nucleosidase inhibition (Nandi et al., 2022). Target protein was prepared by a software called Biovia Discovery Studio by removing co-crystallized ligands and addition of Hydrogen atoms. Addition of charges was done in Autodock vina and the structure was saved in PDBQT format. Ligand preparation: All the compounds with highest peak in GC-MS analysis were downloaded from the PubChem database in the SDF format and converted to PDB format through Online Smile converter software. Pre-processing of the ligands like removing Heteroatoms, delete water, add polar hydrogen groups, addition of charges was done in AutoDock Vina and the structure were saved in PDBQT format. The grid setting was determined based on the binding site residues retrieved from the Castp software. The grid center of MTA/SAH protein was positioned at coordinates; x:1.029, y: -37.993, z: 13.172 with 0.1 Å spacing for all docking simulations. Binding affinity was calculated using AutoDock vina, Protein-ligand 2D and 3D interactions were studied and visualised by using Discovery Studio (Dassault Systemes BIOVIA, 2021) and UCSF Chimera software. Further moleculat dynamic simulation was conducted to validate its stability and calculate free binding energy (Kakkar and Wadhwa, 2023).

Molecular Dynamic simulation and MM-PBSA calculations

Molecular dynamics (MD) and simulation was performed to check the stability of the complex in the dynamic system using Groningen Machine for Chemical Simulations (GROMACS) (Adamu et al., 2023). This method is widely accepted to check the conformational stability, motion and trajectory and of the complex in a dynamic environment. It also provides knowledge about the real -time dynamics of protein-ligand interaction, any structural changes on binding of ligand, solvation of the molecule. MD simulation of the compound with highest binding affinity in AutoDock was performed using following conditions: pH 7.4, 0.9% NaCl concentration, 310^{o}K temperature, 1 atm pressure, and 100 ns running time (Hermanto et al., 2022). For determination of structural stability and rigidity of the complex, Root-Mean-Square Deviation (RMSD), Root-Mean-Square Fluctuation (RMSF), Radius of Gyration and Hydrogen bond were analysed. Furthermore, the free binding energy between 1JYS and the compound was calculated using Molecular

Mechanics/Poisson-Boltzmann Surface Area (MMPBSA) in combination with MD, utilizing the 'g_mmpbsa' script of GROMACS (Adamu et al., 2023).

Results and Discussion

Extraction of phytocompounds

Extraction of secondary metabolites was done in various organic solvents with the help of microwave treatment and sonication. Microwave- and Ultrasound-assisted extraction (MAE/ UAE) is a modern and efficient technique which facilitate the extraction process, allowing faster and effective recovery of bioactive compounds (Mohammed and Abdullah, 2022). This technique offers various advantages as compared to the conventional methods (Soxhlet extraction, maceration): MAE decreases the extraction time as it uses high energy which heats the sample rapidly and uniformly resulting in increased interaction with the plant cell and release of compounds in the solvents, The targeted application of microwave energy ensures efficient heat transfer, leading to higher extraction yields in a shorter period of time, MAE often requires smaller amounts of solvents compared to traditional methods, contributing to reduced environmental impact and cost savings (Singh et al., 2023). Ultrasound-assisted extraction (UAE), also known as sonication, is a technique used to extract bioactive compounds from plant materials using high-frequency sound waves. This method enhances the extraction process by applying mechanical agitation and cavitation to the sample-solvent mixture, leading to improved extraction efficiency and shorter extraction times compared to traditional methods. It works by generating ultrasonic waves alternating high-pressure and low-pressure cycles in a liquid medium, causing the formation and collapse of microscopic bubbles. This phenomenon is called cavitation. During bubble collapse, localized high temperatures and pressures are produced, creating intense mechanical forces that disrupt plant cell walls, releasing phytochemicals into the solvent (Ranjha et al., 2021). Microwave-assisted extraction and Ultrasound-assisted extraction (UAE) both offers a more efficient, time-saving, and environmentally friendly alternative to traditional extraction methods for obtaining valuable phytochemicals from plant materials (Kaur et al., 2022; Singh et al., 2023). Extraction yield of phytocompounds present in *Colocasia esculenta* in various organic solvent was in following order: Methanol (2%) > Ethyl Acetate (1.8%) > Chloroform (1.8%) > Hexane (1.4%). For *Raphanus sativus*, the extraction yield was as follows:

Methanol (8.8%) > Hexane (5.8%) > Ethyl Acetate (2%) > Chloroform (2%). Finally, for *Daucus carota*, the extraction yield was in the following order: Methanol (10.4%) > Hexane (4%) > Ethyl Acetate (4%) > Chloroform (1.4%). As shown in Table 1, Methanolic extract has shown good percentage yield in all the samples.

Table 1: Percentage yield of phytocompounds in various organic solvents

Sample	**Methanol (%)**	**Ethyl Acetate (%)**	**Chloroform (%)**	**Hexane (%)**
Colocasia esculenta	2	1.9	1.8	1.4
Raphanus sativus	8.8	2	2	5.8
Daucus carota	10.4	4	1.4	4

Qualitative phytochemical analysis

Phytochemicals, the active compounds found in various plant parts such as stems, bark, leaves, seeds, and flowers, encompass diverse groups such as alkaloids, flavonoids, steroids, terpenoids, tannins, glycosides, and more. These bioactive constituents play a crucial role in numerous biological activities and have been extensively investigated for their potential health benefits and contributions to overall well-being. The assessment of phytochemicals involves both qualitative and quantitative analyses. The examination of methanolic, ethyl acetate, chloroform, and hexane extract from *Colocasia esculenta*, *Raphanus sativus*, and *Daucus carota* revealed the presence of alkaloids, glycosides, tannins, flavonoids, saponins, steroids, terpenoids, and phenols in all samples. While the methanolic extracts of *Raphanus sativus* exhibited a high concentration of phytochemicals, *Colocasia esculenta* demonstrated a moderate concentration, and *Daucus carota* displayed a comparatively lower concentration. Presence of steroid and triterpenoids in the *Daucus carota* extract has been supported by previous study. The phytochemical screening of these extracts identified secondary metabolites, as summarized in Table 2-4, along with their respective test results

Table 2: Qualitative analysis of the phytocompounds present in peel extracts of *Colocasia esculenta*

S.No.	Phytochemical	Test	Methanol	Ethyl Acetate	Chloroform	Hexane
1	Alkaloids	Dragendorff's test	+	+	Not Appeared	+
		Tannic acid test	+	+	+	+
2	Glycosides	Legal's test	+	+	+	+
		Molisch test	++	+	+	+
3	Tannins	$FeCl_3$ test	++	+	+	++
		Alkaline reagent test	++	++	+	Not Appeared
4	Flavonoids	$FeCl_3$ test	-	+	+	-
		Alkaline reagent test	+	+	+	+
5	Saponins	Foam test	++	+	+++	+
6	TriTerpenoids/steroid	Salkowski test	++	+ (steroid)	+	-
7	Terpenoids	Chloroform test	++	+	+	-
8	Phenols	Ellagic acid	+	+	-	+
+++: highly present, ++: moderately present, +: Low, -: absent						

Table 3: Qualitative analysis of the phytocompounds present in peel extracts of *Raphanus sativus*

S.No.	Phytochemical	Test	Methanol	Ethyl Acetate	Chloroform	Hexane
1	Alkaloids	Dragendorff's test	+++	++	+	+
		Tannic acid test	+++	++	+	+
2	Glycosides	Legal's test	+++	+	NA	NA
		Molisch test	+++	+	NA	NA
3	Tannins	$FeCl_3$ test	+++	++	+++	+
		Alkaline reagent test	+	+	+	+
4	Flavonoids	$FeCl_3$ test	+++	++	++	+
		Alkaline reagent test	+	+	+	NA
5	Saponins	Foam test	+	+	+	+

6	TriTerpenoids/ steroid	Salkowski test	Steroids	Triterpenoids	Steroids/ Triterpenoid	-
7	Terpenoids	Chloroform test	+++	++	+	-
8	**Phenols**	Ellagic acid	+	+	-	-
+++: highly present, ++: moderately present, +: Low, -: absent						

Table 4: Qualitative analysis of the phytocompounds present in peel extracts of *Daucus carota*

S.No.	**Phytochemical**	**Test**	**Methanol**	**Ethyl Acetate**	**Chloroform**	**Hexane**
1	Alkaloids	Dragendorff's test	+	+	+	+
		Tannic acid test	NA	+	NA	NA
2	Glycosides	Legal's test	+	NA	+	NA
		Molisch test	+	+	+	+
3	Tannins	$FeCl_3$ test	+	+	+	-
		Alkaline reagent test	+	+	+	NA
4	Flavonoids	$FeCl_3$ test	+	NA	NA	NA
		Alkaline reagent test	+	+	NA	NA
5	Saponins	Foam test	-	-	++	+
6	TriTerpenoids/ steriods	Salkowski test	Steroids	Steroids/ Triterpenoid	Steroids	Steroids/ Triterpenoid
7	Terpenoids	Chloroform test				
8	Phenols	Ellagic acid	+	+	+	-
+++: highly present, ++: moderately present, +: Low, -: absent						

Quantitative analysis

The total phenolic content of *Colocasia esculenta*, *Raphanus sativus*, *Dacus carota* plant extracts were determined spectrophotometrically by Folin- Ciocalteu method. Calibration curves were constructed using Gallic acid as the standard compound. The phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per ml of sample (mg GAE/ml). The methanolic peel extract of *Colocasia esculenta*, *Raphanus sativus*, *Dacus carota* exhibited phenolic content of 3.35 mg GAE/ml, 0.43 mg GAE/ml, 3.308 mg GAE/ml

respectively as shown in Table 5. The total flavonoid content was determined by Aluminium chloride method using Quercetin as a standard. The flavonoid content was expressed in milligrams of Quercetin equivalents (QE) per ml of sample (mg QE/ml). The quantitative analysis of total flavanoid content of peel *Colocasia esculenta, Raphanus sativus, Dacus carota* was found to be 3.95 mg QE/ml, 1.77 mg QE/ml, 4.87 mg QE/ml respectively as shown in Table 5.

Table 5: Quantitative analysis of the phytocompounds present in peel extracts of *Colocasia esculenta, Raphanus sativus, Dacus carota*

	Total Phenolic content (mg GAE/ml)	**Total Flavanoid content (mg QE/ml)**
Concentration of sample	200 µg/ml	100 µg/ml
Colocasia esculenta	3.35	3.95
Raphanus sativus	0.43	1.77
Daucus carota	3.308	4.87

Antioxidant Assay

The antioxidant activity of methanolic extracts of *Colocasia esculenta* (CE), *Raphanus sativus* (R), *Dacus carota* (C) as evaluated using DPPH assay and Ferrous reducing power assay are shown in Figure 2. Ascorbic acid was used as a standard for comparison. The principle behind DPPH assay is based on the ability of the antioxidants to donate electron or hydrogen atom to reduce stable free radical DPPH into its reduced form DPPH-H. This reduction leads to colour change from purple to yellow, which can be measured spectrophotometrically (Baliyan et al., 2022). Ferrous Reducing Power Assay (FRAP): It is based on the ability of the antioxidants to reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to it ferrous form, which is blue coloured and can be measured spectrophotometrically (Bungau et al., 2023). As the concentration of the extract increased, the antioxidant activity also increased. At a concentration of 1000 µg/mL, the methanol extract of CE, R, C showed a significant antioxidant potential with a percentage inhibition of 78.8%, 29.2% and 60.89% respectively for DPPH assay, and 74% (CE), 26% (R) and 49% (C) for ferrous reducing power assay. The results suggest that *Colocasia esculenta* has good antioxidant potential. Figure 3 visually represents the ability of extract to reduce DPPH, *Colocasia esculenta* extract has completely decolourise the purple colour of DPPH followed by *Dacus carota* and *Raphanus sativus* respectively. As indicated by the study, there exists a

correlation between phenolic content and antioxidant activity (Piluzza and Bullitta, 2011). As shown in Table 5, CE exhibits a higher phenolic content compared to C and R, which consequently relates to its superior antioxidant activity.

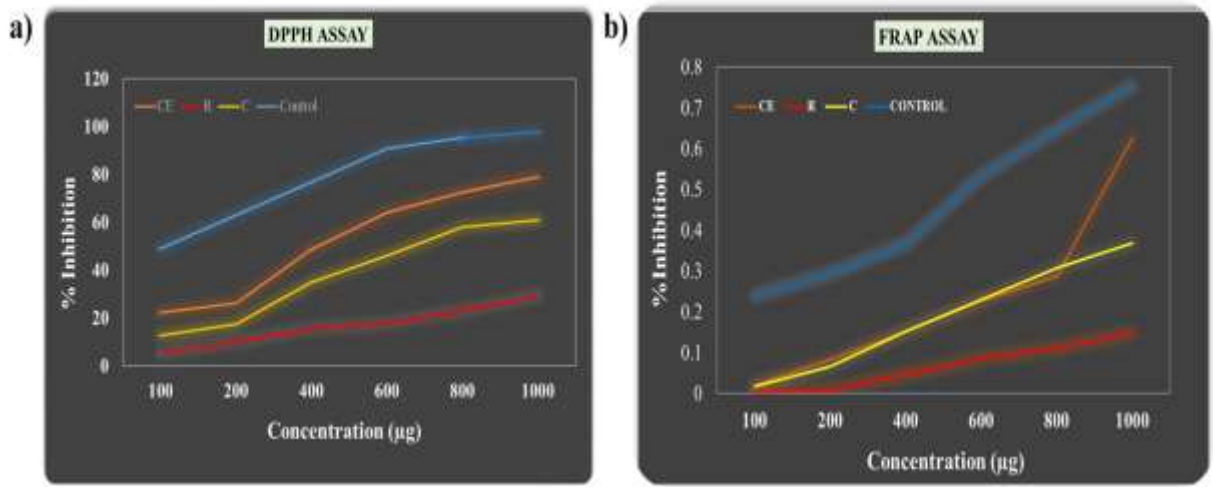

Figure 2: Antioxidant assay: a) DPPH free radical scavenging assay; b) Ferric Reducing Antioxidant Power (FRAP) assay of peel extracts

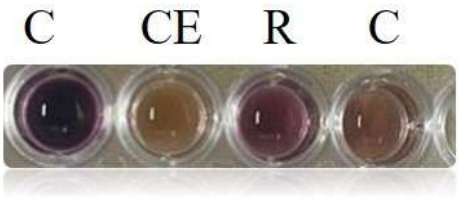

Figure 3: Antioxidant assay using DPPH free radical scavenging assay of peel extracts of *Colocasia esculenta* (CE), *Raphanus sativus* (R), *Dacus carota* (C)

GCMS analysis

Gas chromatography−Mass spectrometry (GC-MS) analysis was performed to determine the phytochemicals present in the peel of *Colocasia esculenta, Raphanus sativus* and *Dacus carota* methanolic extracts. Figure 4 illustrates the GC–MS spectrum of CE, R, and C. A total of forty-six, forty-five, and fifty-three phytocompounds were identified in *Colocasia esculenta, Raphanus sativus, and Daucus carota*, respectively. The highest peak compounds are detailed in Table 6 along with their retention time, percentage area, type and functional properties. Notably, N-Hexadecanoic acid and Oleic acid are present in all three samples. Additionally, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 5-(hydroxymethyl)-2-

furaldehyde, and 1,3,5-Triazine-2,4,6-triamine compounds were found to be common in *Raphanus sativus* and *Daucus carota*. Stigmast-5-en-3-ol was specifically identified in the *Colocasia esculenta* sample, 3-Benzyl-3-hydroxycholestane-2-carbaldehyde was specific to *Raphanus sativus*, and 6-Methoxymellein was unique to *Daucus carota.*

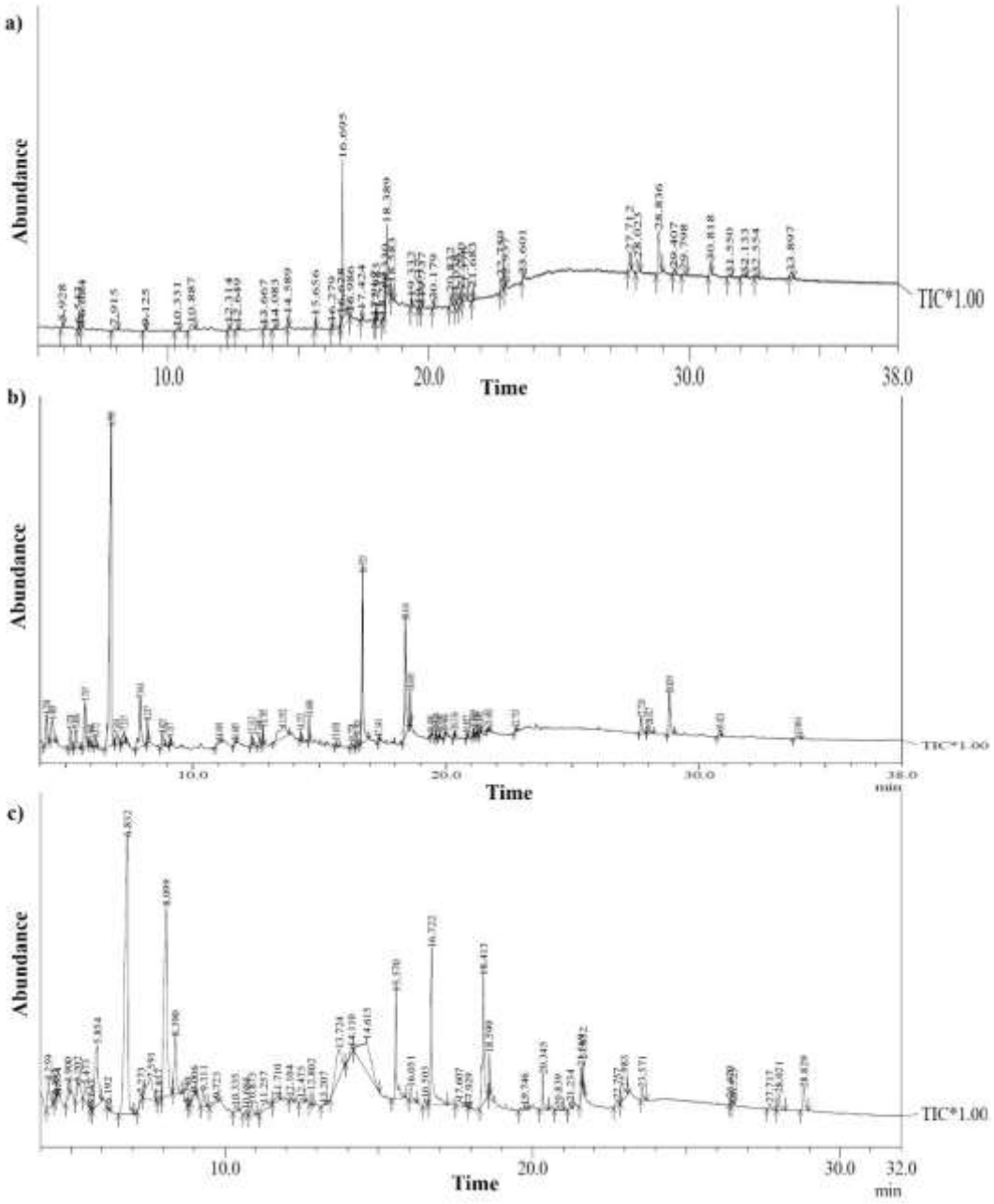

Figure 4: GCMS Chromatogram of a) *Colocasia esculenta* b) *Raphanus sativus* c) *Daucus carota*

Table 6: Compounds with high peaks in GC-MS Chromatogram with their functional properties

Compounds	**Retention Time**	**% Area**	**Compound type**	**Function**
Colocasia esculenta				
N-Hexadecanoic acid (Palmitic acid)	16.695	23.8	Fatty acid	Anti-inflammatory (Aparna et al., 2012), Antioxidant, and anti-atherosclerolic activity (OJATULA, 2023) Pesticide, antiandrogenic, antioxidant, hemolytic, hypocholesterolemic, 5-alpha-reductase inhibitory, nematicides, (Jabeen et al., 2023)
Oleic acid	18.389	11.28	Fatty acid	beneficial effect on cancer, autoimmune, inflammatory diseases and cardiovascular health (Leon-Aparicio et al., 2022; Sales-Campos et al., 2013; Santa-María et al., 2023)
Stigmast-5-en-3-ol	28.836	13.11	Sterols	Reduction of Atherosclerosis, Cardioprotective, Cholesterolytic agent (OJATULA, 2023)
Raphanus sativus				
3,5- diihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	6.795	34.05	Flavonoids	Anti-cancer, antioxidant, anti-inflammatory (Shalini K and Ilango K, 2021)
Hexadecanoic acid	16.725	11.53	Fatty acid	Anti-inflammatory (Aparna et al., 2012), Antioxidant, and anti-atherosclerolic activity (OJATULA, 2023), antiandrogenic, antioxidant, hemolytic, 5-alpha- reductase inhibitory, hypocholesterolemic, nematicides, pesticide (Jabeen et al., 2023). Anti-inflammatory (Aparna et al., 2012), Antioxidant, and anti-atherosclerolic activity (OJATULA, 2023),
Oleic acid	18.414	8.98	Fatty acid	beneficial effect on cancer, autoimmune, inflammatory diseases and cardiovascular health (Leon-Aparicio et al., 2022; Sales-Campos et al., 2013; Santa-María et al., 2023)
Octadecanoic acid	18.6	0.95	Fatty acid	Lipid metabolism regulation, Antiarteriosclerotic, Anticoronary, and antioxidant activities (OJATULA, 2023)

				antiarthritic, antiandrogenic, hepatoprotective, anti-inflammatory, antieczemic, 5-alpha reductase, hypocholesterolemic, antiacne, and antihistaminic effects (Jabeen et al., 2023)
1,3,5-Triazine-2,4,6-triamine	5.767	3.82	Organic compound	laminates, adhesives, molding compounds, textiles, and flame retardants (Khan et al., 2013)
5-(hydroxymethyl)-2-furaldehyde	7.944	5.35	Organic compound	Lipid metabolism regulation, Antiarteriosclerotic, Anticoronary, and antioxidant activities [34]
3-Benzyl-3-hydroxycholestane-2-carbaldehyde	28.839	3.87	Sterol	NA
Daucus carota				
3,5- diihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	6.832	24.56		Anti-cancer, antioxidant, anti-inflamatory (Shalini K and Ilango K, 2021)
5-(hydroxymethyl)-2-furaldehyde	8.099	14.76	Organic compound	Lipid metabolism regulation, Antiarteriosclerotic, Anticoronary, and antioxidant activities [34]
6-Methoxymellein	15.57	3.68	Phenol	inhibits the proliferation and migration of breast cancer cells (Liu et al., 2020)
Hexadecanoic acid	16.722	6.76	Fatty acid	Anti-inflammatory (Aparna et al., 2012), Antioxidant, and anti-atherosclerolic activity (OJATULA, 2023), hemolytic antiandrogenic, 5-alpha- reductase inhibitory, hypocholesterolemic, antioxidant nematicides, pesticide (Jabeen et al., 2023)
Oleic acid	18.413	6.27	Fatty acid	beneficial effect on cancer, autoimmune, inflammatory diseases and cardiovascular health (Leon-Aparicio et al., 2022; Sales-Campos et al., 2013; Santa-María et al., 2023)
1,3,5-Triazine-2,4,6-triamine	5.854	5.24	Organic compound	laminates, adhesives, molding compounds, textiles, and flame retardants (Khan et al., 2013)

Molecular Docking

The molecular docking was performed to investigate the interaction between phytocompounds identified from peel extract of *Colocasia esculenta*, *Raphanus sativus* and *Daucus carota.* These compounds includes n-hexadecanoic acid; Stigmast-5-en-3-ol; 1,3,5-Triazine-2,4,6-triamine; 5-(hydroxymethyl)-2-furaldehyde; Oleic acid; Octadecanoic acid; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; 3-Benzyl-3-hydroxycholestane-2-carbaldehyde; 6-Methoxymellein. The target protein used for docking was the crystal structure of Methylthioadenosine/S-adenosylhomocysteine nucleosidase (MTA/SAH nucleosidase) with PDB ID 1JYS. Among the nine compounds studied, 3-Benzyl-3-hydroxycholestane-2-carbaldehyde showed good binding affinity of -8.4 (kcal/mol) followed by Stigmast-5-en-3-ol with binding affinity -8 kcal/mol as shown in Table 7 along with their interactive residues. The 3D ligand-protein interaction of Carbaldehyde and MTA/SAH nucliosidase is shown in Figure 5 and 2D visualization is shown in Figure 6. Sisir Nandi et al reported that the indazole compounds acts as an inhibitor of MTA/SAH nucleosidase. They studied molecular docking interaction of 40 indazole compounds with MTA/SAH nucleosidase (PDB ID: 1JYS). They reported the binding affinities of 40 compounds between -8 kcal/mol to -14kcal/mol (Nandi et al., 2022). On comparing with this study, it has been concluded that carbaldehyde is an inhibitor of MTA/SAH nucleosidase.

Table 7: Binding affinity of MTA/SAH nucleosidase with phytocompounds isolated from peel of 3 different crop

Phytochemicals	**Molecular weight**	**Molecular Formula**	**PubChem Id**	**Binding Affinity**	**Interacting Residues**
N-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	985	-4.8	ALA A:160, LYS A:170, ILE B:171, HIS B:108, LYS B:170, ALA B:160
Oleic acid	282	$C_{18}H_{34}O_2$	445639	-5.2	HIS A:108, ALA A:160, HIS B:108, ASP B:111, TYR B:119, ALA B:160, ILE B:162, LYS B:170, ILE B:171,
Stigmast-5-en-3-ol	414	$C_{29}H_{50}O$	222284	-8	ALA A:160, ALA B:160, ILE B:162, VAL B:166, LYS B:170

4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	144	$C_6H_8O_4$	119838	-4.6	LYS B:170, ILE B:171, HIS B:108, ASP B:109
1,3,5-Triazine-2,4,6-triamine	126	$C_3H_6N_6$	7955	-4.1	ASP A:109, ASP B:109, TYR B:119, LYS B:170
5-(hydroxymethyl)-2-furaldehyde	126	$C_6H_6O_3$	237332	-4.2	HIS B:108, ASP B:109, TYR B:119, LYS B:170
Octadecanoic acid	284	$C_{18}H_{36}O_2$	445639	-5	HIS A:108, ALA A:160, ILE A:162, LYS A:170, THR B:113
3-Benzyl-3-hydroxycholestane-2-carbaldehyde	506	$C_{35}H_{54}O_2$	634442	-8.4	HIS A:108, ALA A:160, ASP B:111, ALA B:160, VAL B:166, LYS B:170
6-Methoxymellein	208	$C_{11}H_{12}O_4$	83412	-6.2	ALA A:160, PHE A:161, ILE A:162, GLY A:167, HIS A:108

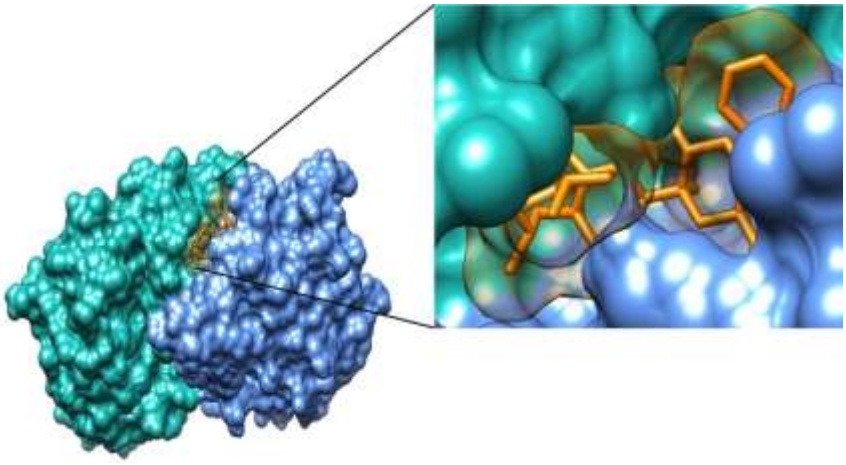

Figure 5: 3D representation of Molecular docking of Carbaldehyde and MTA/SAH nucliosidase

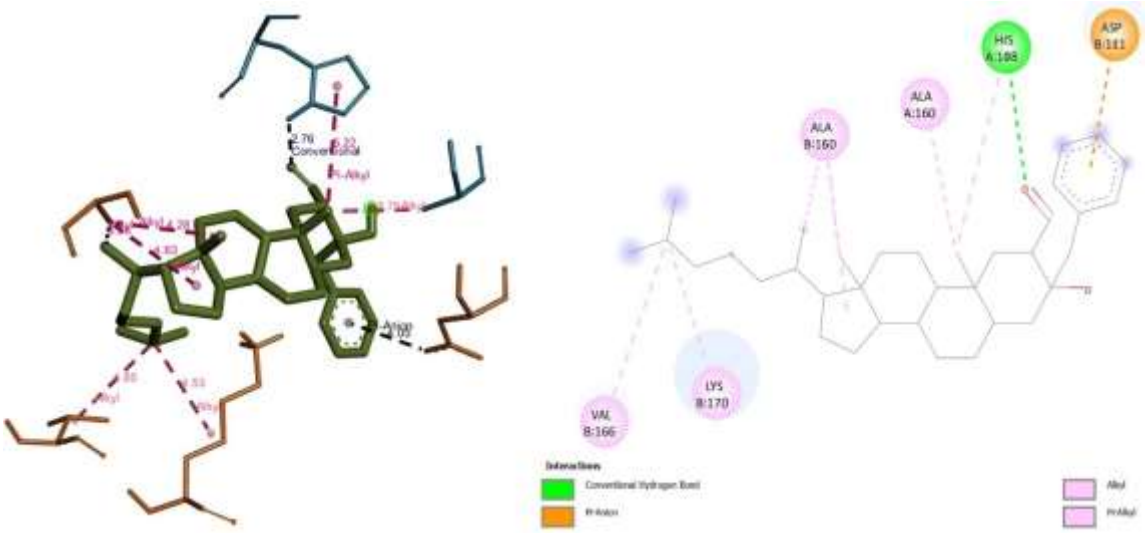

Figure 6: The 3D and 2D binding mode of compound 3-Benzyl-3-hydroxycholestane-2-carbaldehyde using target protein 1JYS.

Molecular Dynamic Simulation

Molecular dynamics (MD) is among the most frequently employed and efficient approaches to studying protein ligand interactions, protein structures, solvation of molecules, or real-time dynamics and structural features of proteins (Adamu et al., 2023). For estimating the overall stability of the system, we measured RMSD from the beginning of the production run to hydrogen bonding and electrostatic contacts that are formed during protein-ligand binding. The RMSD for Cα atoms of the protein indicated that it initially rises from a starting configuration to about 0.15 to 0.2 (1.5 Å – 2 Å) and drops around 1.5 Å in its first 50 ns before stabilizing for about another 100 ns with no noise after that period. The RMSD value of the protein backbone indicated that the presence of Carbaldehyde did not induce significant alterations in the structural backbone of MTA/SAH nucleosidase, as the RMSD of the backbone remained below 3 Å. as shown in figure 7a. The radius of gyration (Rg), which is often utilized in molecular dynamics simulations as well as analysis of structure; it helps to describe conformational dynamics, folding events as well as compactness for proteins during their simulation process. A larger Rg value typically indicates a more extended or less compact structure, while a smaller Rg value suggests a more compact or tightly folded structure (Adamu et al., 2023). Figure 7b depicts that protein folding system was stable throughout the stimulation process and protein did not lose its structure. RMSF stands for Root Mean Square Fluctuation used to quantify the extent of fluctuation or flexibility of individual atoms or groups of atoms within a biomolecular system over the course of a

simulation. The residues that are involved in the binding has shown low fluctuation as shown in figure 7c & d. Overall, MD simulation studies showed that the complex is structurally stable and ligand were tightly bound to the protein. Further estimation of free binding energy using MM-PBSA calculation indicated that complex had a good free binding energy (-92.084 ± 2.375 kJ/mol) as shown in Table 8. SASA, polar solvation energies, Intermolecular van der Waals, electrostatic, constitute free binding energy and they are all combined together to calculate MM-PBSA.

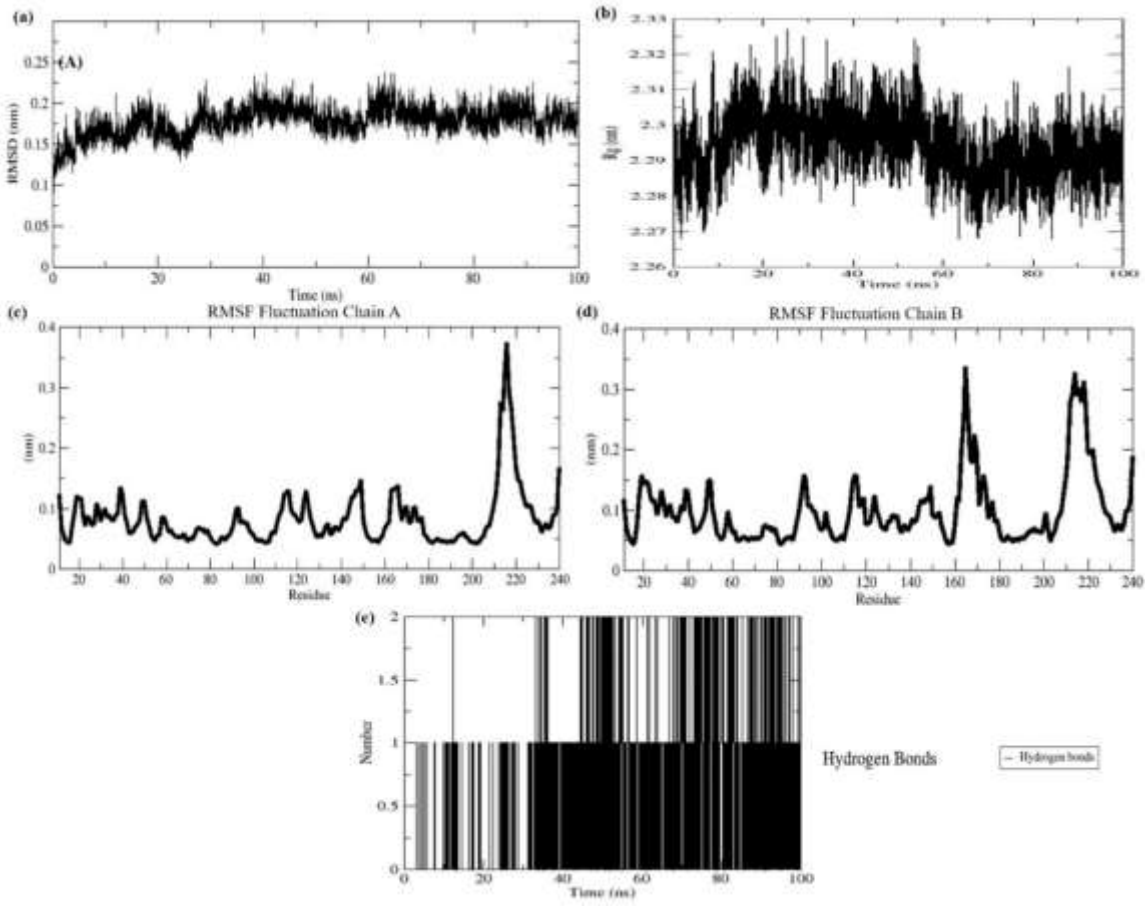

Figure 7: Molecular dynamics trajectory plots a) Root Mean Square Deviation (RMSD), b) Radius of gyration (Rg) , c) Root Mean Square Fluctuation Chain A (RMSF), d) Root Mean Square Fluctuation Chain B (RMSF); e) Analysis of Hydrogen bonds in a 100ns simulation

Table 8: Computed MM-PBSA binding energy of MTA/SAH nucleosidase and carbaldehyde complex

	Van der Waal energy (kJ/mol)	**Electrostatic energy (kJ/mol)**	**Polar solvation energy (kJ/mol)**	**SASA energy (kJ/mol)**	**Binding energy (kJ/mol)**
MTA/SAH nucleosidase and carbaldehyde complex	-138.972 +/- 2.698 kJ/mol	-43.097 +/- 2.482 kJ/mol	105.083 +/- 4.485 kJ/mol	-15.140 +/- 0.310 kJ/mol	-92.084 +/- 2.375 kJ/mol

Conclusion and Future Perspective

Management of Fruit and vegetable waste (FVW) is important for sustainable development. There is an urgent requirement for alternative solutions that can enable us optimally utilize FVW to achieve economic, environmental and social benefits of these waste materials. It is promising and aligned with ongoing trends in natural product research, sustainable agriculture, and functional food development(Bhardwaj et al., 2022). Here are some potential future directions and implications of FVW: Health benefits and Functional food: Peels contain various bioactive compounds such as polyphenols, flavonoids, vitamins, and dietary fibers, which have demonstrated antioxidant and anti-inflammatory properties in numerous studies. Utilizing vegetable peels as a source of these compounds can lead to the development of functional foods and dietary supplements targeting specific health conditions associated with oxidative stress and inflammation, such as cardiovascular diseases, cancer, and neurodegenerative disorders. Kanchan et al. reported the presence of diverse polyphenolic compounds in the peels of fruits and vegetables such as onion, banana, and garlic. They highlighted the antioxidant properties of these compounds and discussed their potential utilization in the development of functional foods (Bhardwaj et al., 2022). Harsh et al. reported the use of Fruit and vegetable peel for industrial application like fortified probiotic, green nanoparticles, biochar, edible coating, peel based microbiological media, biosorbents and carbon quantum dots (Kumar et al., 2020).

Sustainability and Waste Reduction: Repurposing vegetable peels for their health-promoting compounds contributes to reducing food waste and promoting sustainable agricultural practices. By valorizing vegetable peels, food processing industries can minimize

environmental pollution associated with disposal while simultaneously creating value-added products (Isah and Ozbay, 2020; Salem et al., 2023; Sharma et al., 2021).

Quorum Sensing Inhibition and Antibacterial Activity: Some compounds found in vegetable peels have been shown to possess quorum sensing inhibition (QSI) properties, interfering with bacterial communication mechanisms and potentially mitigating bacterial virulence and biofilm formation. Exploration of vegetable peel extracts for QSI activity could lead to the development of novel antibacterial agents and strategies to combat bacterial infections, including antibiotic-resistant strains. One study reported the antibacterial and anti-quorum sensing activity of *Punica granatum L.* peel extracts against *P. aeruginosa* (Hamrita et al., 2022). Quorum sensing (QS) inhibition is used for controlling membrane biofouling in membrane-based reactors for biological wastewater treatment (Kim et al., 2021). Various compounds are reportedly used as QS inhibitors like Vanillin (4- hydroxy-3-methoxy benzaldehyde), Cladodionen, furanone, ϵ-polysine, gingerol, alkaloids, homoserine lactone-like TGK series etc. It's been reported that application of Vanillin on the reverse osmosis membrane decrease the biofilm formation by 45%. One study showed the role of 3,3′,4′,5-tetrachlorosalicylanilide (100 ug/L) as QS inhibitor, it decreases the biofilm formation by 50% and Autoinducer-2 production by 30% (Sahreen et al., 2022). Role of AI-2 in the treatment of biological wastewater treatment is confirmed by various researches. Quorum sensing regulation method has been applied to various water treatment systems like activated sludge, biofilm, granular sludge, biological denitrification etc (Liu et al., 2021). So, vegetable peels can be explored for the novel quorum sensing inhibitors.

In this study, phytochemical analysis of *Colocasia esculenta, Raphanus sativus and Dacus carota* peel extract was determined. Total phenolic content present in the extract of *Colocasia esculenta, Raphanus sativus and Dacus carota* was around 3.35 mg GAE/ml, 0.43 mg GAE/ml, 3.308 mg GAE/ml respectively. Likewise, the total flavonoid content was around 3.95 mg QE/ml, 1.77 mg QE/ml, and 4.87 mg QE/ml for *Colocasia esculenta, Raphanus sativus*, and *Daucus carota*, respectively. At a concentration of 1000 µg/mL, the methanol extract of CE, R, C showed a significant antioxidant potential with a percentage inhibition of 78.8%, 29.2% and 60.89% respectively for DPPH assay, and 74% (CE), 26% (R) and 49% (C) for ferrous reducing power assay. Moreover, all samples demonstrated significant anti-inflammatory activity, with Colocasia esculenta showing particularly promising results. Through GC-MS analysis, presence of Forty-six, Forty-five and Fifty-three phytocompounds was confirmed in *Colocasia esculenta*, *Raphanus sativus* and *Daucus carota* respectively.

Molecular docking studies revealed the strong binding interaction of MTA/SAH nucleosidase with Stigmasterol (-8kcal/mol) and 3-Benzyl-3-hydroxycholestane-2-carbaldehyde (-8.4 kcal/mol), biomolecules found in peel of *Colocasia* and *Raphanus*. Molecular dynamic simulation studies based on RMSD, RMSF, Radius of gyration confirmed that the protein ligand complex (1YJS and carbaldehyde) was stable. Free binding energy calculated using MM-PBSA was --92.084 ± 2.375 kJ/mol. It is concluded that peel of *Colocasia* and *Raphanus* is a good source of anti-Quorum agents and also have antioxidant and anti-inflammatory potential.

Advances in biotechnology can facilitate the development of innovative extraction techniques, formulations, and delivery systems to enhance the bioavailability and efficacy of FVW derived compounds. Therefore, future outlook for utilizing vegetable peels as a source of value-added products is promising, with potential applications spanning functional foods, pharmaceuticals, and sustainable agriculture, wastewater treatment contributing to both human health and environmental sustainability. Control of membrane biofouling in membrane-based bioreactors can be achieved through the inhibition of quorum sensing. The results provide insights into the chemical diversity of vegetable peels and their potential as a source of novel anti-QS agents.

Disclosure Statement

The authors declare that they have no conflict of interest and no funding was received to assist with the preparation of this manuscript.

Data availability

All data generated or analysed during this study are included in this article

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