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***Research Article** (Open Access)*

MOLECULAR DOCKING AND ADMET STUDIES FOR COSTUS IGNEUS LEAF EXTRACT COMPOUNDS AGAINST AROMATASE PROTEINS TOWARDS THE TREATMENT OF BREAST CANCER

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ABSTRACT
Breast cancer is the most common cancer among women in India, with rising incidence, especially in younger age groups (30-50 years). While survival rates are lower than in Western countries, early detection through awareness and screening as well as bound therapeutic strategies are crucial. Although various chemotherapeutic strategies have been developed over the last two decades but most of them either suffer from toxicities or proven to be highly expensive. So, to combat this situation, a robust herbal anti-cancer product is the need of the hour. A well-known plant *Costus igneus*, may serve the purpose with two compounds Stigmasterol (-10.4 kcal/mol) and Alpha Tocopherol (-8.5 kcal/mol) showing better binding energy to the aromatase receptor as compared to standard Tamoxifene (-8.4 kcal/mol). With high druggability in ADMET prediction studies and very high LD50 report indicate their safe usage in human body. Molecular dynamics (MD) study using iMODS, for the top scoring molecules showed stable interaction. With further in vitro and in vivo studies, more assurance regarding the compounds can useful against breast cancer.

***Keywords**: Costus igneus, Molecular Docking, Ligand, ADMET Studies, Molecular Dynamics*

INTRODUCTION
Breast cancer remains the top cause of cancer-related death among women globally. The oestrogen receptor, namely the aromatase enzyme, is critical to the genesis and progression of hormone-dependent breast cancers. Aromatase inhibitors, therefore, represent an important treatment option. However, issues such as drug resistance and undesirable side effects need the development of new, perhaps safer, and more effective treatment alternatives (Arnold *et al*., 2022).
Natural products, particularly those derived from medicinal plants, have historically served as a rich source of bioactive compounds with diverse pharmacological activities. *Costus igneus*, traditionally used in folk medicine for its purported antidiabetic and anti-inflammatory properties, contains a variety of phytochemicals that may possess unexplored anticancer potential (Chumsri *et al*., 2011; Miziak *et al*., 2023). This study aims to investigate the potential of compounds identified in *Costus igneus* leaf extract to inhibit aromatase proteins, utilizing molecular docking and ADMET (absorption, distribution, metabolism, excretion, and toxicity) studies. By employing computational approaches, we seek to identify promising lead compounds that could contribute to the development of novel therapeutic strategies for breast cancer, offering a foundation for future *in vitro* and *in vivo* validation (Dias *et al.*, 2012). This research bridges the gap between traditional medicinal knowledge and modern drug discovery, potentially paving the way for the development of safer and more effective breast cancer treatments.

MATERIALS AND METHODS
Details on plants
Costus igneus, sometimes known as the Insulin Plant or Fiery Costus, is a member of the Costaceae family, which includes spiral gingers (Fig. 1). It is a herbaceous perennial plant native to South America,

specifically Brazil. It develops from underground rhizomes and can reach heights of 2 to 5 feet.Large, smooth, fleshy green leaves grouped spirally around tall stems are a distinguishing trait of the Costaceae family. The plant produces brilliant, stunning orange tubular flowers that emerge from cone-like bracts and are commonly found around the stem base. Its colloquial name refers to its traditional usage, although botanically, it is classified as spiral ginger (Hegde *et al*., 2014).

Figure 1: *Costus igneus* plant

Ligands preparation and optimization

As reported by Vijaya et al and Sivakumar et al., 11 ligands from *Costus igneus* leaf reported in various literatures, were drawn in Chem Draw Professional 8.0 (Fig. 2). Three-dimensional structures of the ligands were created in Open Babel and saved as SDF format for further preparation and molecular docking analysis (Vijaya *et al*., 2022).

Drug like properties of the ligands

The cutoff values for the physicochemical parameters of all ligands were determined using LogS, LogP, Lipinski's rule of five, and the bioavailability score. Drug probability was evaluated using the following molecular parameters: MW (molecular weight), HBD (hydrogen bond donor), HBA (hydrogen bond acceptor), log P (lipophilicity log), and log S (aqueous solubility). The parameters were created with the SWISSADME server (www.swissadme.ch/index.php). Protein fabrication and optimisation for an aromatase inhibitor that targets the breast cancer receptor.

The crystallographic structures of the aromatase inhibitor protein (PDB ID: 3EQM), which targets breast cancer, were obtained from the protein data bank. The water molecules were removed from the protein to prepare it for molecular docking, and hydrogen atoms were supplied using the BIOVIA Discovery Studio 2021 Client application to correct the ionisation of the amino acid residues (Nonglang *et al.*, 2024).

Molecular docking analyses and visualization

The proteins in pdb format were saved and loading into the PyRx application, molecular docking was carried out using the Auto dock Vina tool. To find the most dependable conformer, the PyPx program was

utilised. The grid volume was 54.68 Å x 65.23 Å x 56.91 Å. The Discovery Studio 2021 Client software was used to identify and visualise the intermolecular interactions between the aromatase inhibitor protein (3EQM) and the *Costus igneus* leaf ligands (Palei *et al*., 2025).

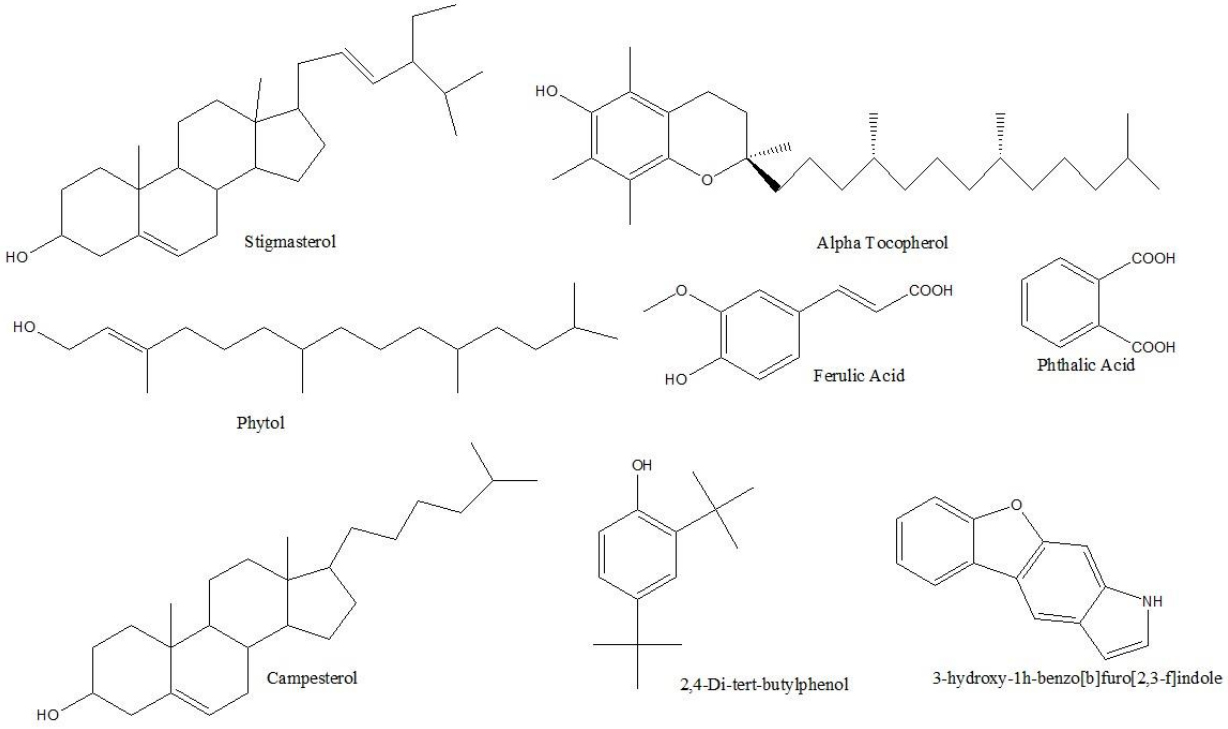

Fig. 2. Ligands of *Costus igneus*

Toxicity prediction

ProTox III software was used to predict the toxicity of the top-scoring ligands in human cells (https://tox-new.charite.de/protox_III/). The online server offers the potential toxicity profile of the chemical for 14 models with confidence scores based on a two-dimensional chemical structure as input (Palei *et al*., 2025).

Molecular Dynamics Simulation

The iMod server (iMODS) enhanced simulations by providing increased normal mode analysis (NMA) and an intuitive interface for exploring different pathways and interacting with 3D objects. Docking simulations used RMSD to evaluate structural stability, ligand-protein interactions, and binding energies. MD simulations using iMod helped analyse dynamic behaviour and binding interactions in ligand-protein complexes, yielding crucial insights into structural dynamics and functional implications. This study used molecular dynamics (MD) simulations, a computer method for analysing atomic and molecule movement over time, to understand the dynamic features of biological systems (Mandal and Mandal, 2024).

Statistical analysis

The values are expressed as the mean ± standard error of the mean.

RESULTS

Molecular docking studies

The binding affinities and important interactions between the phytochemical ligands derived from *Costus igneus* and the breast cancer receptor-targeting aromatase inhibitor protein (3EQM) were ascertained

using PyRx docking software. The discovered ligands' binding affinities were contrasted with those of the common anti-breast cancer medication, tamoxifen. The binding affinities of the protein-bound ligands and the common medication Tamoxifen are displayed in Table 1. The ligands based on *Costus igneus* had binding affinities ranging from -10.4 to -4.9 kcal/mol. The molecular interactions between the most active ligands and the active site of the aromatase inhibitor protein that targets the breast cancer receptor were visualised using the Discovery Studio 2021 Client application (Fig. 3). Strong antagonistic qualities against the aromatase inhibitor protein, which targets the breast cancer receptor, were suggested by these samples' predictable interactions with the amino acids in the protein's active region. The maximum binding affinity for 3EQM was -10.4 kcal/mol for stigmasterol and -8.5 kcal for alpha-tocopherol. Isopropyl myristate had the ligand with the lowest binding affinity (-4.9 kcal/mol). The binding affinities of two natural compounds (Stigmasterol, Alpha-Tocopherol) obtained from Costus igneus were found to be higher than those of the widely used aromatase inhibitor medication Tamoxifen (-8.4 kcal/mol), suggesting their potential utility in the suppression of breast cancer. Studies on the toxicity and molecular dynamics of these ligands were conducted (Samajdar and Mondal, 2023).

Table 1: Docking score of *Costus igneus* ligands

Ligands	Binding Affinity (cΔG in kcal/mol)
	3EQM
Stigmasterol	-10.4
Alpha Tocopherol	-8.5
Tamoxifene	-8.4
Acridine	-7.6
Phytol	-6.9
3-hydroxy-1h-benzo[b]furo[2,3-f]indole	-8.3
Phthalic Acid	-6.5
Ferulic Acid	-6.8
Campesterol	-8.0
2,4-Di-tert-butylphenol	-6.5
Isopropyl Myristate	-4.9
Gamma Sitosterol	-8.2

ADMET studies

According to SwissADME, the ligands' lipophilic character was demonstrated by their molecular weights, which varied from 166.13 to 430.71 g, and their LogP values, which ranged from 0.60 to 6.04. With no more than one infraction, the prediction values for each ligand set were all within the range of Lipinski's rule of five cutoffs. This is due to the fact that all ligands have drug-like characteristics that satisfy the Ghosh's rule criterion, and compounds with log P values in this range are soluble in fats, oils, lipids, and nonpolar solvents. Using ProTox III software, the toxicity of the same set of ligands was examined. The results showed that the top-scoring ligands, stigmasterol and alpha tocopherol, had projected class 4 to class 5 toxicity with extremely high LD50 values (890-5000 mg/ml), suggesting that they are safe for human use (Ganesan *et al*., 2024) (Table:3).

Molecular Dynamics studies

The iMod server evaluated protein and ligand movements in docked complexes of Stigmasterol_3EQM using normal mode analysis and molecular dynamics simulations. Both complexes showed remarkably flexible locations on the main-chain deformability graph, with atomic index 1000 showing the greatest peak with a deformability value of about 1. The B-factor values, which reflect temperature variations and protein structural flexibility, were computed by iMod. Greater atomic mobility and structural changes in stigmasterol with 3EQM protein are shown by increased Bfactor values. The vibrational frequencies linked to collective atom motions are represented by the eigenvalues produced by iMod, which offer insights into system dynamics, structural alterations, and flexibility. Slower global movements of the chemical in attachment to 3EQM proteins are indicated by lower Eigen values in both situations. iMod covariance map displays a value matrix, with each member indicating the covariance between atom pairs. A covariance matrix, which shows whether two residues are correlated (red), uncorrelated (white), or anticorrelated (blue), is frequently used to describe the values (Fig. 4). Stigmasterol docking with the protein in iMod simulations shows that it is flexible, especially at the hinge. Atomic pair interactions in both proteins are represented by the covariance map in iMod. It pinpoints areas where movements are either correlated or anti-correlated and are most likely related to protein activity. Our knowledge of protein dynamics and structure-function relationships is expanded by this map (Sarkar *et al*., 2023)

Table 2: ADME parameters of each ligands in SwissADME

Ligands	Mol Wt. (g)	Log P	HBD	HBA	Violation	BB barrier Yes/No	GI Absorption	Log S
Isopropyl Myristate	270.45	4.68	0	2	1	Yes	High	-5.14
Gamma Sitosterol	414.71	5.07	1	1	1	No	Low	-7.9
2,4-Di-tert-butylphenol	206.32	3.08	1	1	0	Yes	High	-4.55
Campesterol	400.68	4.97	1	1	1	No	Low	-7.54
Ferulic Acid	194.18	1.62	2	4	0	Yes	High	-2.11
Phthalic Acid	166.13	0.6	2	4	0	No	High	-1.57
3-hydroxy-1h-benzo[b]furo[2,3-f]indole	223.23	1.79	2	2	0	Yes	High	-4.08
Phytol	296.53	4.85	1	1	1	No	Low	-5.98
Acridine	179.22	2.24	0	1	0	Yes	High	-3.83
Alpha Tocopherol	430.71	6.04	1	2	1	No	Low	-8.6
Stigmasterol	412.69	5.08	1	1	1	No	Low	-7.46

Table 3: Toxicity prediction of ligands

Ligands	Level of Toxicity (1=highly toxic; 6= safe)	Predicted LD50 (µg/ml)
Isopropyl Myristate	5	5000
Gamma Sitosterol	4	890
2,4-Di-tert-butylphenol	4	700
Campesterol	4	890
Ferulic Acid	4	1772
Phthalic Acid	5	2530
3-hydroxy-1h-benzo[b]furo[2,3-f]indole	4	1200
Phytol	5	5000
Acridine	4	331
Alpha Tocopherol	5	5000
Stigmasterol	4	890

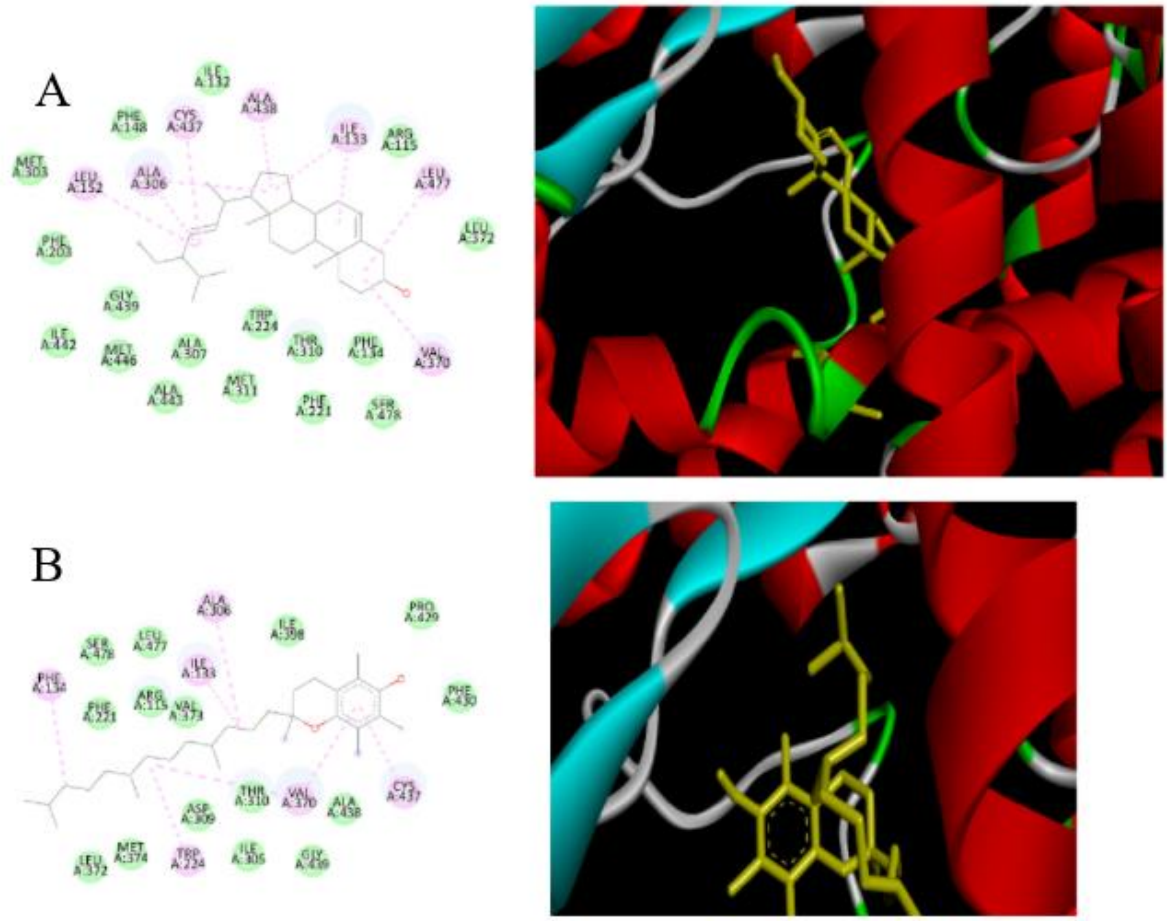

Figure 3: Interaction diagram of A. Stigmasterol B. Alpha Tocopherol

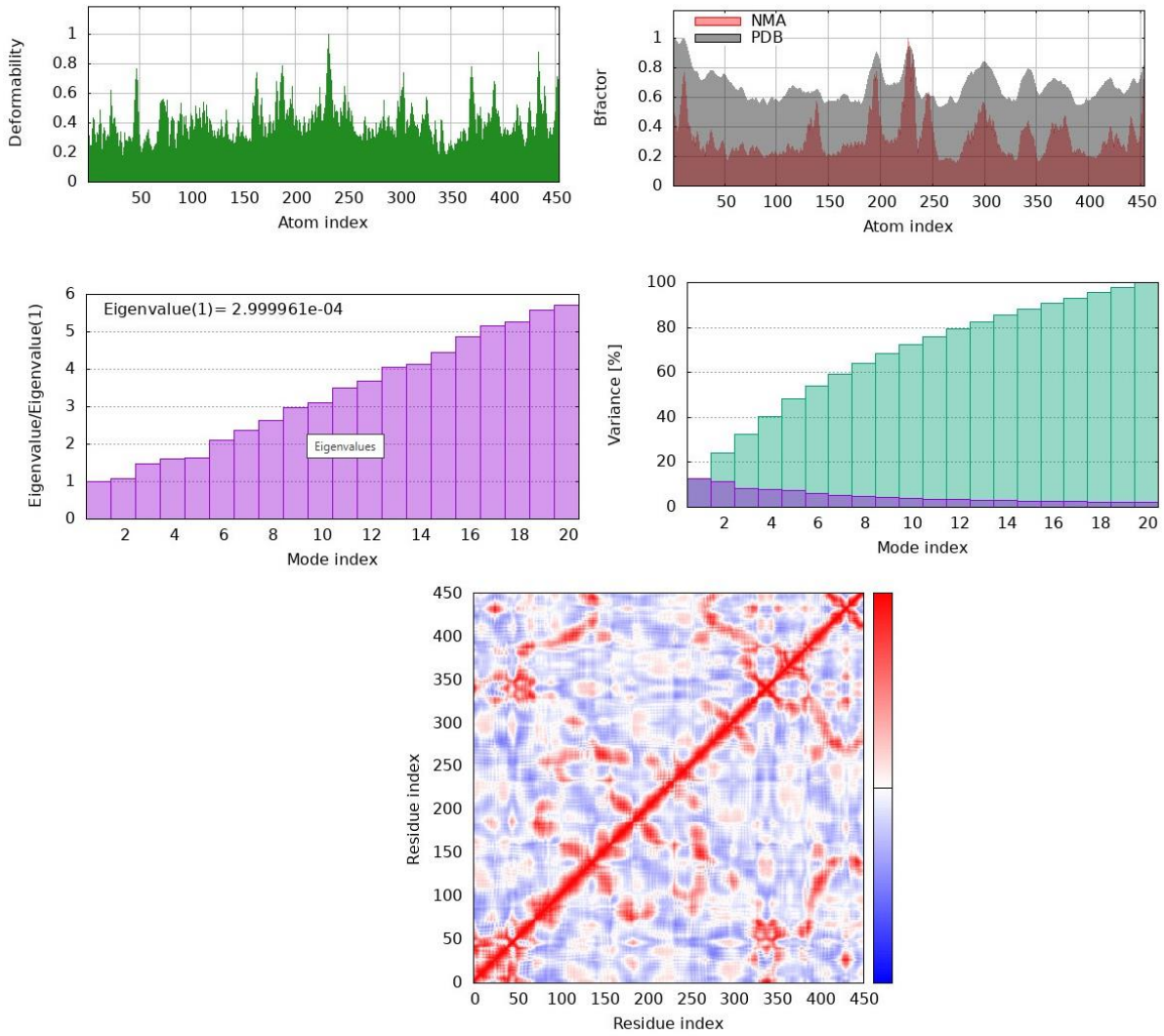

Figure 4: Outputs of Molecular dynamic simulation in iMODS for Stigmasterol with protein 3EQM showing deformability factor plot, Bfactor plot, Eigen value, Variance plot and Covariance plot

DISCUSSION

Molecular docking, molecular dynamics and some of the other in silico prediction techniques are widely used to understand the ligand receptor interactions during drug discovery process. These processes of virtual screening and use of herbal medicine as alternative therapeutics are the need of the hour for prevention and cure of the ever-increasing disease of breast cancer. Additionally, exploration of herbal drug pool is a particularly efficient drug discovery method because it requires less capital investment and time than de novo drug discovery. In this study eleven ligands derived from the *Costus igneus* reported by Vijaya et al and Sivakumar et al. has been obtained for their usage in breast cancer using multiple in silico approaches. According to Sahu *et al*. (2023), molecular docking studies using the aromatase receptor protein with all eleven ligands were compared against the standard aromatase inhibitor Tamoxifene. The results showed that the ligands with Stigmasterol and Alpha Tocopherol had exceptional binding energies of – 10.4 kcal/mol and – 8.5 kcal/mol, respectively. This suggests that these molecules

may be used extensively in breast cancer (Sahoo *et al.*, 2024). All eleven ligands were in toxicity categories 4 and 5 with high IC50 values, meaning they were safe for human use, according to oral toxicity studies on the ligand conducted using Protox III database program. ADME studies indicated that all compounds had no or less number of Lipinski violation with Log P values on higher side indicating within the range of highly soluble in fats, oils, and lipids (Srivastava *et al.*, 2022) [14]. Molecular dynamics (MD) study of the top scoring molecules showed stable interaction.

CONCLUSION

Molecular docking, ADME profiling, toxicity prediction, and MD modelling were used to identify a novel drug against breast cancer derived from previously reported Costus igneus ligands. Compound Stigmasterol and Alpha Tocopherol have a higher negative binding energy than conventional Tamoxifene in 3EQM aromatase protein. MD simulation also confirms that the top-scoring molecule maintains the same interaction with particular stability, low Eigen value, and high covariance. In ADME experiments, all ligands, including sigmasterol and alpha tocopherol, demonstrated drug-like characteristics with minimal structural violations. The oral toxicity testing revealed that the top-scoring Stigmasterol and Alpha Tocopherol are safe for human use. In the future, reducing the toxicity potency and improving the ADME profile might necessitate structural modification or a different drug delivery strategy. Subsequently, the in vivo toxicity assessment remains necessary to guarantee the precision of the human mutagenicity, carcinogenicity, and hepatotoxicity assessments.

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DECLARATION OF INTEREST

None

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