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Research Article

Exploring the anti-inflammatory effects of bioactive compounds from assam tea clones: *in silico* and i*n vitro* approaches

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Abstract: The consumption of tea, derived from *Camellia sinensis*, ranks second globally after water. This research explores the anti-inflammatory properties of tea plant varieties from Assam, including TEEN ALI, TV-17, and TV-22, through a comprehensive approach that combines experimental and computational techniques. The Albumin Denaturation Method revealed significant anti-inflammatory effects in all three varieties, with TV-22 demonstrating the highest inhibition rate at 82.11%. This underscores its potential as a powerful anti-inflammatory agent, warranting further investigation. In this study, comparative analysis indicates a link between the composition of tea plant samples and their anti-inflammatory efficacy. *In silico* modeling, particularly molecular docking, was utilized to evaluate the interaction of selected bioactive compounds with key inflammatory receptors—COX-2, IL-1, and IL-18. Compounds like 2,4-di-tert-butylphenol and caffeine displayed interactions and have energy values comparable to or superior to standard drugs (Diclofenac and Aspirin), suggesting their potential as promising drug candidates. However, the valuable insights the results provided underscore the importance of conducting thorough experimental validations, such as *in vitro* and *in vivo* studies, to confirm the efficacy and safety of identified compounds. It opens up avenues for future research by stressing the need to extensively explore specific bioactive compounds, especially in TV-22, which could lead to the development of new anti-inflammatory therapeutics. This interdisciplinary study establishes a groundwork for understanding the therapeutic potential of tea plants and provides a roadmap for creating anti-inflammatory drugs from in natural sources.

1. INTRODUCTION

Teas made from *Camellia sinensis* L., mostly black and green varieties, are said to be the most popular beverage in the world, drank after water. The primary distinction between the teas lies in the auto-oxidation process, which is facilitated by the enzymes peroxidase and polyphenol oxidase (PPO). In essence, oxidation is the darkening process that occurs when *C. sinensis* leaves contact with oxygen during processing (such as chopping, crushing, or drying). Accordingly, teas derived from *C. sinensis* are categorized as fermented, semi-fermented, unfermented, green, oolong, black, and Pu'erh (Engelhardt, 2010; Ho *et al.*, 2008). With a long history of nutritional and medical uses, tea is a significant cash crop that is particularly valued

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in Asian nations like China, Thailand, Japan, and India. Antibacterial, antioxidant, anti-cancer, hypoglycemic, and hypolipidemic are only a few of its biological properties (Cipriani *et al.*, 2006; Scoparo *et al.*, 2012; Sharangi, 2009). Tea's unparalleled appeal stems from both its distinct flavor and scent as well as the health advantages of consuming it. Studies and investigations have been conducted on the main bioactivities of tea, which include antioxidation, hypoglycemic, antibacterial, hypolipidemic, and anti-cancer properties.

The culinary, pharmaceutical, and healthcare sectors have also made extensive use of tea (Cipriani *et al.*, 2006; Wang *et al.*,2001). The variety of chemicals in tea is primarily responsible for its biological and pharmacological properties. Tea's chemical composition mostly consists of inorganic elements, catechins, theanine, tea proteins, tea polyphenols (TPPs), and tea polysaccharides (TPSs) (Sharangi, 2009). A growing body of research has demonstrated the superior antioxidant capabilities of tea polyphenols, which have long attracted interest (Cipriani *et al.*, 2006). Modern pharmacological research has demonstrated that TPS, a significant bioactive component together with TPP, is also the major tea chemical that helps decrease blood glucose and triglycerides, resist oxidation, and increase the body's immunological function (Wang *et al.*, 2001; Wang *et al.*, 2009; Xie & Nie, 2006). Furthermore, there is a great deal of potential for its improvement and application in the cosmetics industry (Zhou *et al.*, 2001). When the grade or quality of tea rises, the content of TPS often falls (Xiao & Jiang, 2015). The TPS concentration in low-grade tea was observed to be double that of high-grade tea (Zheng *et al.*, 2016). As a result, employing low-quality tea as a starting point for TPS extraction promotes the full use of tea resources and offers significant health benefits for both illness prevention and enhancement.

1.1. Cyclooxygenases-2 (COX-2)

Tumour necrosis factor-α (TNF-α), prostaglandin (PG), interleukin 6 (IL-6), and nitric oxide (NO) are generated by immune cells and cytokines in response to wounds and infections that trigger inflammatory processes in the tissue. On the other hand, inflammation frequently results in the extrication of NO and PG (Liu *et al.*, 2020; Sanlier *et al.*, 2018). The human body's inflammatory symptoms can lead to chronic inflammation, which in turn promotes cancer. It's important to stop the inflammation from getting worse (Yang *et al.*, 2018). The inflammatory region can react with free radicals like hydroxyl (·OH), superoxide ($O2^{-}$), and peroxyl (·OOH, ·OOR), leading to more severe diseases. Inhibiting the activity of cyclooxygenase-2 (COX-2) is a potential target for inflammatory therapy, as it is a stimulus enzyme (Ding *et al.*, 2017; Zhu *et al.*, 2021). Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to be effective in treating inflammatory illnesses. Although this medicine is effective as an anti-inflammatory, it does not suppress COX-2 (Khan & Mukhtar, 2018; Orem *et al.*, 2017). Long-term usage of NSAIDs may induce stomach irritation, haemorrhage, renal, bronchus, cardiovascular system, and perforation (Wang *et al.*, 2001; Wang *et al.*, 2009; Xie & Nie, 2006).

1.2. Interleukin 1

Interleukin 1 (IL-1) is a key regulator of the acute-phase inflammatory response and is implicated in tissue damage and dysfunction in chronic inflammatory diseases like diabetes, rheumatoid arthritis, and amyotrophic lateral sclerosis. In neurodegenerative diseases such as Alzheimer's disease (AD), IL-1 is believed to drive neuroinflammation (Chen *et al.*, 2018). IL-1 signals through the binding of its receptor to the IL-1α and IL-1β isoforms (Yuan *et al.*, 2015). This interaction, along with the recruitment of the IL-1 receptor accessory protein (IL-1RAcP), triggers the release of inflammatory mediators via NF-κB and MAPK signaling pathways. IL-1 antagonists, such as IL-1 receptor antagonist (IL-1Ra), IL-1-neutralizing antibodies, and the soluble IL-1 receptor (sIL-RI) and IL-1RAcP fusion protein (IL-1 trap), have shown promise in clinical trials for various inflammatory conditions. IL-1Ra binds to IL-1RI without initiating signaling, as it does not engage IL-1RAcP (Zhu *et al.*, 2021). Anakinra (Ana), a recombinant protein of human IL-1Ra, effectively blocks IL-1's effects without any agonist activity and has been used to treat conditions like rheumatoid arthritis, osteoarthritis, juvenile idiopathic

arthritis, adult-onset Still's disease, and type 2 diabetes (Wang *et al.*,2009; Zhang *et al.*, 2019). However, the therapeutic use of current IL-1 antagonist proteins in treating neuroinflammation is limited due to their relatively poor penetration of the blood-brain barrier (BBB) (Yatam *et al.*, 2018; Zampelas & Micha 2015).

1.3. Interleukin-18

Interleukin-18 (IL-18), a member of the IL-1 cytokine family, plays a key role in regulating inflammatory responses. Initially identified as an interferon-gamma-inducing factor, IL-18 is primarily produced by activated macrophages and dendritic cells. It interacts with the IL-18 receptor complex, comprising IL-18Rα and IL-18Rβ subunits, to trigger intracellular signalling pathways that activate transcription factors such as AP-1 and NF-κB. This activation leads to the release of pro-inflammatory mediators, including chemokines, adhesion molecules, and cytokines. IL-18 is also linked to various inflammatory diseases, such as atherosclerosis, inflammatory bowel disease, and rheumatoid arthritis, with genetic variations in the IL-18 gene potentially increasing the risk of these conditions. Despite the established role of IL-18 in inflammation, further research is needed to fully understand its production, processing, and interactions with other immune mediators.

1.4. Albumin Denaturation Method

The albumin denaturation method evaluates anti-inflammatory activity based on the interaction between the test substance and protein molecules, typically bovine serum albumin (BSA). Heating BSA causes its tertiary structure to unfold, leading to protein denaturation. Anti-inflammatory substances can inhibit or reduce the denaturation of BSA, indicating their ability to stabilize protein structures under stress conditions (Oliveira *et al.*, 2017). The degree of protein denaturation is measured by monitoring changes in absorbance or turbidity of the BSA solution at a specific wavelength using a spectrophotometer. Increased absorbance or turbidity indicates greater protein denaturation. Test samples that inhibit BSA denaturation to a significant degree are considered to possess potential anti-inflammatory properties. This method provides a relative measure of the effectiveness of test substances in stabilizing protein structures, correlating with their potential to modulate inflammatory processes (Oliveira *et al.*, 2017).

1.5. Human Red Blood Cell Membrane Stabilization Method

HRBC Membrane Stabilization Method evaluates the anti-inflammatory properties of natural substances or medicines using HRBCs as a model. Inflammatory substances can cause damage to HRBCs. Inflammation often involves the release of inflammatory mediators that can damage cell membranes. The test compound is incubated with HRBCs and then challenged with an inflammatory agent like hypotonic saline or heat. The degree of protection offered by the test compound is assessed by measuring hemolysis or hemoglobin release from damaged cells. A lower level of hemolysis or hemoglobin release indicates higher membrane stabilization and anti-inflammatory activity (Hossain *et al.*, 2014). This method is valuable for preliminary screening of natural products or synthesized compounds for potential anti-inflammatory activity before further *in vivo* studies (Hossain *et al.*, 2014).

Predicting bioactive components from Assamese tea plants is the current scientific endeavor to mitigate the unwanted side effects of chronic synthetic medication usage. An in-silico method called molecular docking forecasts a drug candidate's (ligand's) propensity to connect to a protein and create a stable complex. This approach simplifies *in vivo, in vitro*, and pharmacy research in drug modeling (Levita *et al.*, 2017; Shah *et al.*, 2019) and reduces costs and time associated with drug design. Protein denaturation occurs when a protein is subjected to external stimuli such as heat, acids, or organic solvents, causing structural disturbances to its secondary and tertiary structures. Enzymes become inactive if substrates cannot bind to the active site (Hossain *et al.*, 2014; Oliveira *et al.*, 2017). Because of their shown ability to lessen pain and inflammation, NSAIDs are frequently given. This is because they prevent protein denaturation,

which acts as an antigen and can cause autoimmune disorders.

2. MATERIALS AND METHODS

Tea plant varieties TV-17, TV-22, TEEN ALI were collected from Jorhat, Assam at 6 to 7 A.M. It was identified and authenticated as *Camellia sinensis* by Dr. N. Senthilkumar Associate Professor & Head, Center for Research and PG Studies in Botany, Ayya Nadar Janaki Ammal College, Sivakasi. The collected samples were washed with water and then they were dried at room temperature and the dried samples were powdered using a Mortar and Pestle.

2.1. GC-MS Analysis

The Tea Plants from Assam were collected and they were grinded. They were added in Ethyl acetate solvent for extraction of Bioactive molecules. After extraction the samples were submitted for GC-MS analysis.

2.2. Molecular Docking

The CoX-2, IL-1 and IL-18 receptors were retrieved from PDB database (PDB ID: 5IKR, 1ILR, 4R6U) (Berman *et al.*, 2000) and was prepared in chimera (Version 1.15) (Pettersen *et al.*, 2004) tools. The chemicals compounds were also retrieved from PubChem database (Benzyl alcohol PubChem ID: 244, Eicosane (C20H42): PubChem ID: 8222, Carbonic acid, eicosyl vinyl ester PubChem ID: 91693137, 2,4-Di-tert-butylphenol PubChem ID: 7311, Tetradecanoic acid PubChem ID: 11005, Caffeine PubChem ID: 2519, n-Hexadecenoic acid PubChem ID: 985, though exact confirmation is recommended, Phytol PubChem ID: 5280435, Oleic acid PubChem ID: 445639, Vitamin E PubChem ID: 14985) (Kim *et al.*, 2019). Docking is done using 100 iterations of the Lamarckian Genetic Algorithm with a grid box size of 40 × 40 × 40 and spacing of 0.375 Å on Autodock (Version 4.2.6) (Morris *et al.*, 2009) software. The docking findings are then analysed using Autodock Tools software, and the interaction is visualized with Biovia Discovery Studio Visualizer (Version 2020) (Dassault Systèmes, 2020).

2.3. ADME Parameters

The analysed chemicals compounds were tested for its ADME properties in SwissADME Website. The analysis focuses on the following parameters:

- Absorption: GI absorption, BBB permeability, and Pgp substrate status.
- Solubility: ESOL, Ali, and Silicos-IT solubility data.
- Metabolic Interactions: Inhibition of CYP enzymes.
- Physicochemical Properties: Molecular weight, log P values, hydrogen bond donors/acceptors.
- Drug-likeness: Lipinski's rule of five violations, bioavailability score, PAINS alerts.

2.4. *In Vitro* Anti-Inflammatory Activity

2.4.1. *Albumin denaturation method*

20 mL of water was used to dilute 0.2g of sample powder. It was held for fifteen minutes at room temperature and then for twenty minutes at eighty degrees Celsius. A muslin cloth filter was used to remove the extract. After that, it was kept at -4°C in a refrigerator until it was needed. For this investigation, aspirin was used as a control, finely powdered. We diluted 0.2g of aspirin with 20mL of water.

For the reference medication, aspirin, a serial dilution from 1000 µg/mL to 0.01 µg/mL was carried out. The total volume of each sample was 5.0 millilitre. To make the reaction solutions, 2.8 mL of phosphate buffered saline (pH 6.4) and 0.2 mL of egg albumin were combined. Next, reaction mixtures were gently combined with 2 millilitres of extract. Aspirin, the reference medication, underwent a similar process and served as the study's positive control. Distilled water was also employed as a negative control. After being incubated for 15–20 minutes at 37°C ± 2°C in a water bath, reaction mixtures were heated to 70°C and kept there for 5 minutes. After that, the reaction mixture was given fifteen minutes to cool at room temperature. Using a

colorimeter, the absorbance of the reaction mixture was measured at 680 nm for each concentration (1000 µg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL, 0.1 µg/mL, and 0.01 µg/mL) before and after denaturation. The mean absorbance was measured after each test was conducted three times. The following formula was used to calculate the percentage of protein inhibition on a percentage basis relative to the control.

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \text{ x100}$$

2.4.2. *The human red blood cell membrane stabilization method*

By stabilizing the membrane of human red blood cells (HRBCs), the anti-inflammatory activity has been investigated *in vitro*. A healthy volunteer's blood was extracted two weeks before to the experiment and mixed with an equivalent volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42 percent NaCl). Every blood sample was stored for a full day at 4 °C before being used. The supernatant was removed following five minutes of centrifuging at 2500 rpm. The cell suspension was centrifuged at 2500 rpm for five minutes following rinsing with sterile saline solution (0.9 % w/v NaCl). This was done three times, and the resultant supernatant was clear and colourless when the packed cell volume was assessed. Following its reconstitution in phosphate buffered saline (10 mM, pH 7.4), the cellular component was used in the assays at a 40% suspension (v/v).

Ten millilitres of water were used to dilute one gram of sample powder, which was then held for fifteen minutes at room temperature and then for twenty minutes at eighty degrees Celsius. Once it was used, it was refrigerated at four degrees Celsius for 30 minutes. After incubation one millilitre of phosphate buffer, two millilitres of hyposaline, and half a millilitre of HRBC suspension. It was then incubated for thirty minutes at 37 degrees Celsius and centrifuged for twenty minutes at three thousand rpm. The supernatant solution's haemoglobin concentration was determined by spectrophotometry, with aspirin (100 µg/mL) serving as the reference standard. A control was created by omitting the extracts. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde *et al.*, where: OD1 = Optical density of hypotonic-buffered saline solution alone.OD2 = Optical density of test sample in hypotonic solution.

3. RESULT

The GC-MS Results of tea samples revealed many chemical compounds (Figure 1). Among them compounds like caffein, Tetradeconoic acid, vitamin E and few more compounds were found to have biological activities. So, the ten Compounds with anti-inflammatory, antibiotic and other biological activity were selected for the Insilico analysis in this study. They were mentioned in Table 1.

Table 1. Selected bioactive compounds of tea plant from GC-MS analysis.

S. No	Name of the Bioactive compounds	Molecular formula	Molecular weight	Abundance/ Quality
1	Benzyl alcohol	C_7H_8O	108.14	0.25% / 95%
2	Eicosane	$C_{20}H_{42}$	282.5	0.73% / 64%
3	Carbonic acid, eicosyl vinyl ester	$C_{23}H_{44}O_3$	368.59	0.27% / 91%
4	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	0.68% / 97%
5	Tetradeconoic acid	$C_{14}H_{28}O_2$	228.37	0.73% / 99%
6	Caffeine	$C_8H_{10}N_4O_2$	194.19	80.61% / 96%
7	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256.42	0.84% / 99%
8	Phytol	$C_{20}H_{40}O$	296.5	4.85% / 91%
9	Oleic acid	$C_{18}H_{34}O_2$	282.5	0.71% / 99%
10	Vitamin E	$C_{29}H_{50}O_2$	430.7	0.44% / 99%

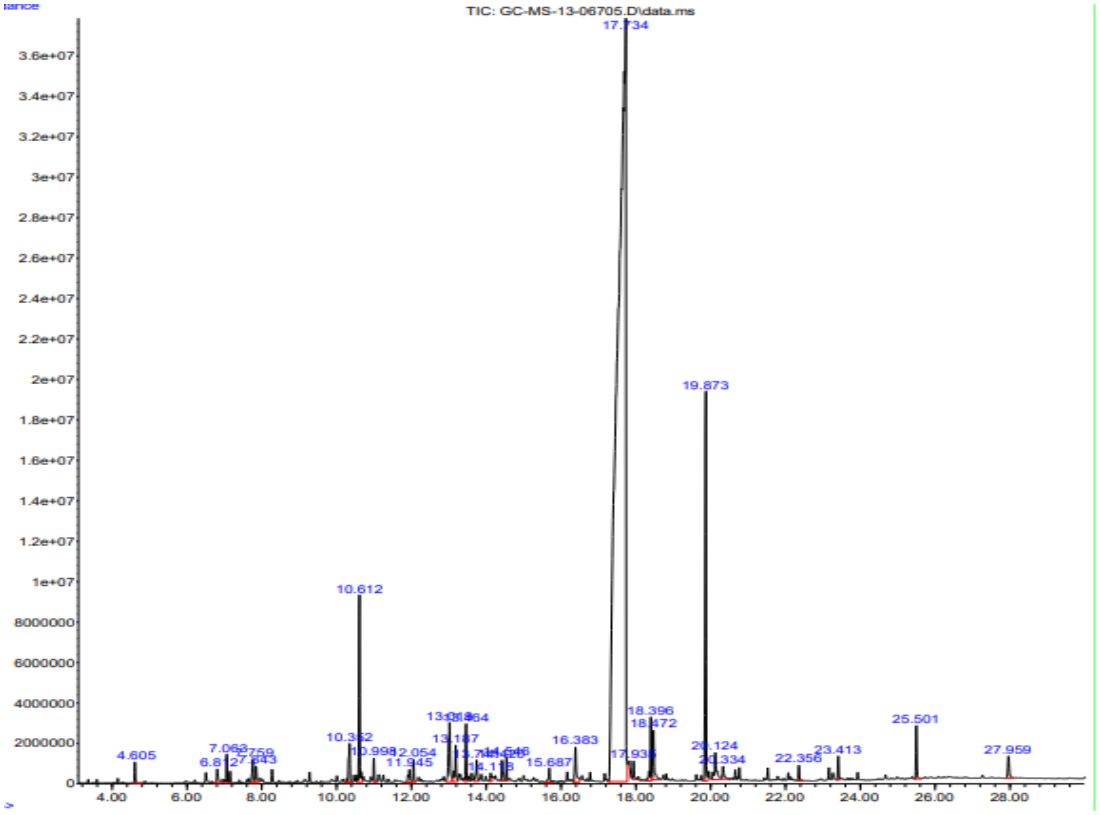

Figure 1. GC-MS results of assam tea samples.

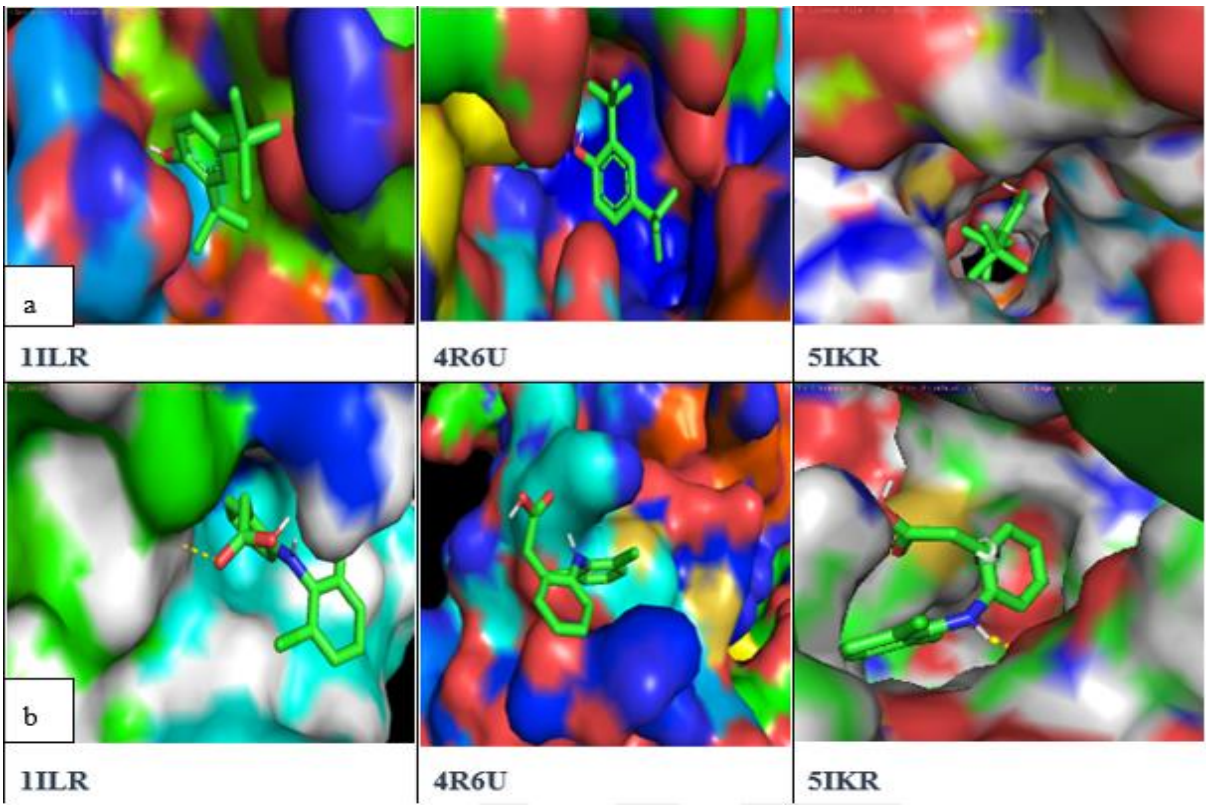

Figure 2. Aspirin(a) and Diclofenac(b) Interaction with three different receptors.

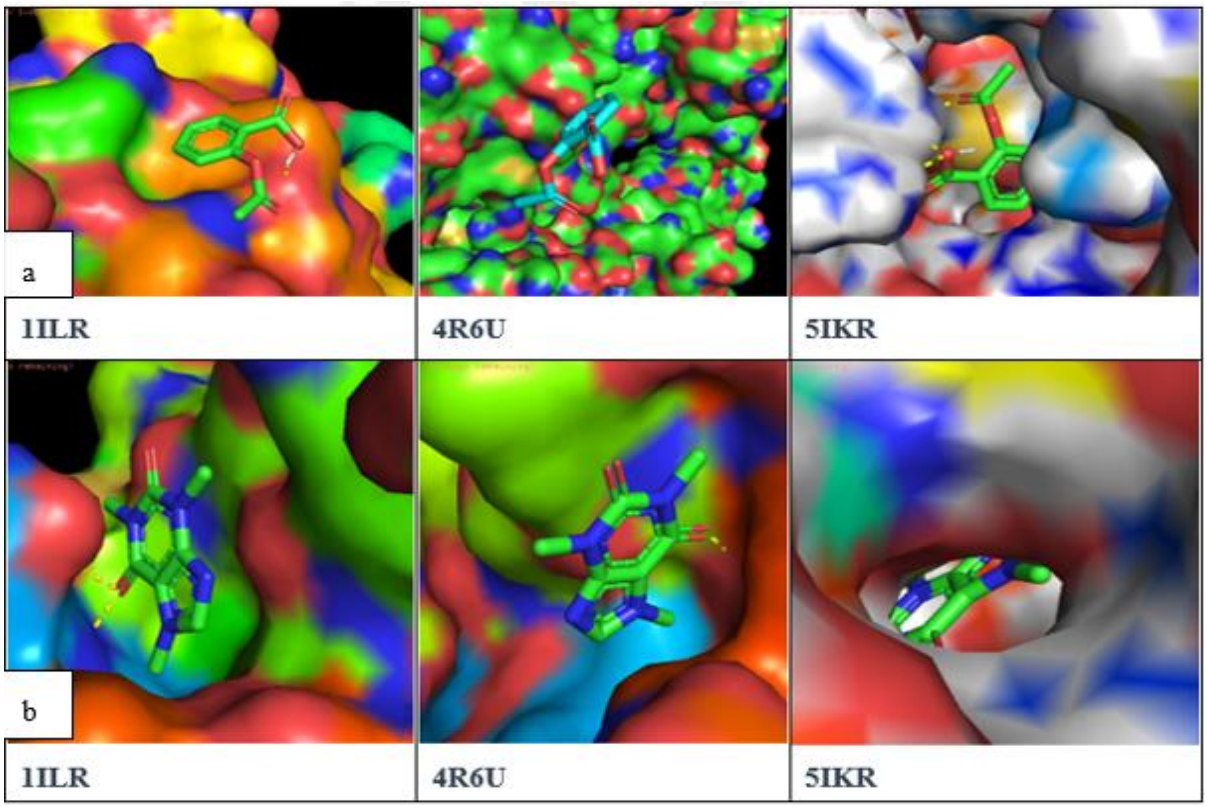

Figure 3. 2,4-di-tert-butylphenol(a) and Caffein(b) Interaction with three different receptors.

3.1. In-Silico Modelling

The in-silico approach is widely employed for predicting and validating drug designs. In this study, molecular docking simulations were performed using the Autodock program (MGL Tools) to investigate the interactions between selected phytochemicals from tea plants and three target receptors: CoX-2 (PDB ID: 5IKR), IL-1 (PDB ID: 1ILR), and IL-18 (PDB ID: 4R6U). The docking results are summarized in Table 2.

3.1.1. *Diclofenac and aspirin (Reference drugs)*

Diclofenac and Aspirin exhibited strong interactions with the CoX-2 receptor (5IKR), as indicated by highly negative binding energy values and low inhibition constants. For IL-1 (1ILR) and IL-18 (4R6U), Diclofenac displayed moderate interactions, whereas Aspirin showed weaker binding characterized by higher positive energy values and an absence of inhibition constants. The interactions of both reference drugs with all three receptors are illustrated in Figure 2 (a and b).

b

3.1.2. *2,4-Di-tert-butylphenol*

2,4-Di-tert-butylphenol demonstrated consistently favorable interactions with all three receptors, as evidenced by negative binding energy values and moderate inhibition constants (Figure 3a). For 1ILR, the interaction showed highly negative energy values and a low inhibition constant, indicating strong binding affinity. With 4R6U, the interaction was characterized by moderately negative energy values and a moderate inhibition constant, reflecting favorable binding. A reasonably favorable interaction was observed with 5IKR, supported by negative energy and a moderate inhibition constant. The compound's particularly strong interaction with 1ILR suggests its potential as a promising therapeutic agent.

Table 2. Docking values of selected ligands against inflammatory receptors.

	1ILR			4R6U			5IKR		
Ligands	Energy Values (kcal/mol)	Inhibition Constant (Ki)	Ligand Efficiency	Energy Values (kcal/mol)	Inhibition Constant (Ki)	Ligand Efficiency	Energy Values (kcal/mol)	Inhibition Constant (Ki)	Ligand Efficiency
Diclofenac	+0.23	0	0.01	+3.79	0	0.2	-5.37	115µM	0.28
Aspirin	+1.80	0	0.14	+4.26	0	0.33	-4.90	255µM	0.32
2,4-di-tert-butylphenol	-4.91	249µM	0.33	-3.97	1.22mM	0.26	-4.64	395µM	0.31
Benzyl Alcohol	-3.51	2.67mM	0.44	-2.80	8.83mM	0.35	-3.61	2.27mM	0.45
Caffein	-3.52	2.65mM	0.24	-3.07	5.58mM	0.22	-3.98	1.21mM	0.28
Carbonic Acid	-0.65	335.mM	0.02	-0.80	260mM	0.03	-0.98	191mM	0.04
Eicosane	-2.41	17mM	0.12	-1.92	38.9mM	0.1	-1.27	117mM	0.06
n-Hexadecenoic acid	+2.63	0	0.15	+7.61	0	0.2	-2.13	27.34mM	0.12
Oleic Acid	+2.93	0	0.15	+8.9	0	0.01	-2.14	26.83mM	0.11
Phytol	-3.02	6.13mM	0.14	-1.32	10.7mM	0.06	-2.44	16.3mM	0.12
Tetradeconoic acid	+2.26	0	0.14	+7.19	0	0.02	-3.22	4.53mM	0.2
Vitamin E	-2.44	16.2mM	0.08	-2.96	6.79mM	0.10	-4.16	889µM	0.13

Table 3. ADME Analysis of selected compounds from assam tea sample.

Molecule	SA	GI absorption	BBB permeant	Pgp substrate	Bioavailability Score	MW(g/mol)	nHBA	TPSA (Å²)	iLOGP	WLOGP
2_4_Di_tert_butylphenol	1.83	High	Yes	No	0.55	206.32	2	26.3	2.91	3.82
Benzyl Alcohol	1.0	High	Yes	No	0.55	108.14	1	20.23	1.66	1.03
Caffeine	2.03	High	No	No	0.55	194.19	3	61.82	1.79	-1.03
Carbonic Acid	1.36	High	No	No	0.85	62.02	3	57.53	-0.22	0.22
Eicosane	2.72	Low	No	Yes	0.55	282.55	0	0.0	5.64	8.05
n-Hexadecenoic Acid	4.57	Low	No	Yes	0.55	326.59	2	26.3	5.45	6.62
Oleic Acid	3.07	High	No	Yes	0.85	282.46	2	37.3	4.27	6.11
Phytol	4.3	Low	No	Yes	0.55	296.53	1	20.23	4.71	6.36
Tetradecanoic Acid	2.68	High	Yes	Yes	0.55	270.45	2	26.3	4.68	5.64
Vitamin E	3.47	High	No	No	0.56	176.12	6	107.22	0.39	-1.41
Aspirin	1.52	High	Yes	No	0.85	180.16	4	63.6	1.3	1.31

3.1.3. *Caffeine*

Caffeine exhibited moderate interactions with all three receptors, supported by negative binding energy values and high inhibition constants (Figure 3b). With 1ILR, moderately negative energy values combined with a high inhibition constant indicated a relatively weak interaction. For 4R6U, negative energy values and a high inhibition constant suggested moderate binding. For 5IKR, negative energy values and a moderate inhibition constant reflected a reasonable interaction. Despite its moderate activity, caffeine's consistent binding across receptors and its high abundance in the tested sample position it as a viable candidate with activity comparable to the reference drugs

3.2. ADME Analysis

3.2.1. *Absorption*

Among the compounds studied, 2,4_Di_tert_butylphenol, Benzyl Alcohol, Caffeine, Carbonic Acid, and Aspirin demonstrate significant gastrointestinal (GI) absorption (Table 3). This high level of GI absorption is advantageous for oral medications, indicating that these compounds may be efficiently absorbed when taken orally. On the other hand, Eicosane, n-Hexadecenoic Acid, Oleic Acid, Phytol, and Tetradecanoic Acid exhibit poor GI absorption, making them less suitable for oral administration. The limited absorption of these compounds can have a notable impact on their efficacy and bioavailability.

3.2.2. *Solubility*

Benzyl Alcohol, Caffeine, Carbonic Acid, Vitamin E, and Aspirin exhibit a very high level of solubility, which is typically associated with increased bioavailability. This enhanced solubility allows for easy dissolution of the drug in bodily fluids, aiding in absorption. 2,4_Di_tert_butylphenol, Oleic Acid, and Phytol, on the other hand, have a moderate level of solubility. While they show some promise, their bioavailability may not be as high as that of highly soluble compounds. Eicosane, n-Hexadecenoic Acid, Oleic Acid, Phytol, and Tetradecanoic Acid, however, are either poorly soluble or insoluble, presenting significant obstacles in terms of bioavailability and efficacy as pharmaceuticals.

3.2.3. *Metabolism and CYP enzyme inhibition*

2,4_Di_tert_butylphenol, Caffeine, Carbonic Acid, Vitamin E, and Aspirin do not hinder any major CYP enzymes. The absence of CYP enzyme hindrance is essential as it reduces the likelihood of drug-drug interactions, thus enhancing the safety of these substances for simultaneous use with other medications. Benzyl Alcohol hampers CYP1A2, while n-Hexadecenoic Acid, Oleic Acid, Phytol, and Tetradecanoic Acid impede various CYP enzymes. This hindrance can result in notable drug-drug interactions, rendering these substances less desirable.

3.2.4. *Lipophilicity and drug-likeness*

Lipophilicity, as determined by log P values, reflects the equilibrium between hydrophilicity and lipophilicity. Ideal log P values (typically ranging from 1 to 3) are indicative of enhanced absorption and distribution. 2,4_Di_tert_butylphenol, Benzyl Alcohol, Caffeine, Carbonic Acid, Vitamin E, and Aspirin exhibit advantageous log P values, rendering them more drug-like. Conversely, Eicosane, n-Hexadecenoic Acid, Oleic Acid, Phytol, and Tetradecanoic Acid display elevated log P values, signifying increased lipophilicity. Consequently, this may result in diminished aqueous solubility and complexities in drug formulation.

3.2.5. *Bioavailability and PAINS alerts*

Bioavailability scores serve as a measure of a drug's absorption and utilization within the body. Many compounds, such as 2,4_Di_tert_butylphenol, Benzyl Alcohol, Caffeine, Carbonic Acid, Vitamin E, and Aspirin, exhibit favorable bioavailability scores of 0.55 or above. PAINS (Pan-Assay Interference Compounds) alerts warn of potential binding promiscuity to various targets, which can result in false positives during bioassays. The absence of PAINS alerts in the

analysed compounds suggests that they are likely to interact specifically with their intended targets.

3.3. Invitro Analysis

3.3.1. *Albumin denaturation method*

This approach, which evaluates the capacity of samples to suppress protein denaturation, is an important step towards understanding the chosen tea plants' possible anti-inflammatory benefits. The results revealed a significant inhibition of protein denaturation by all three tea plant varieties. TV-22 exhibited the highest rate of inhibition, suggesting a pronounced anti-inflammatory effect (Table 4). This outcome positions TV-22 as a promising candidate for further investigation and potential therapeutic applications. A comparative analysis of the rate of inhibition demonstrated an ascending trend from TEEN ALI to TV-17 and reaching its peak in TV-22. TV-22, with the highest inhibition rate, showcased its potential as a potent anti-inflammatory agent Figure 4.

Table 4. Anti-inflammatory activity using albumin denaturation method**.**

Sample	Rate of inhibition (%)
Teen Ali	72.31
Tv-17	79.00
Tv-22	82.11

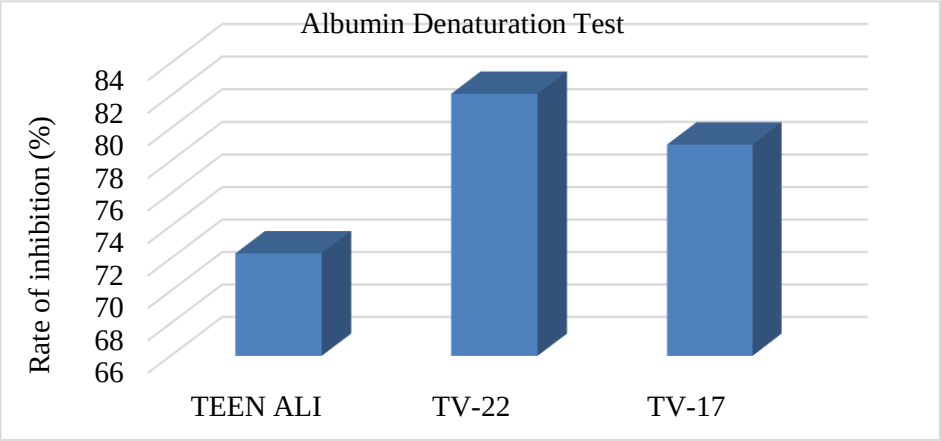

Figure 4. Anti-inflammatory activity using albumin denaturation method.

3.3.2. *Human red blood cell membrane stabilization method*

The HRBC membrane stabilization assay results indicate that the studied substances may have anti-inflammatory action. This test works on the concept that drugs having anti-inflammatory characteristics can prevent red blood cell lysis caused by a hypotonic solution, therefore stabilizing the cell membrane. In this study, three samples (TV-17, TV-22, and TEEN ALI) were evaluated for their ability to inhibit membrane lysis. The samples were prepared and tested according to a standardized protocol, with aspirin used as a reference standard (Figure 5). The percentage inhibition of haemolysis was calculated for each sample (Table 5).

Table 5. Human red blood cell membrane stabilization method**.**

Sample	Rate of inhibition (%)
Tv-17	234.8
Tv-22	205.6
Teen Ali	140.4

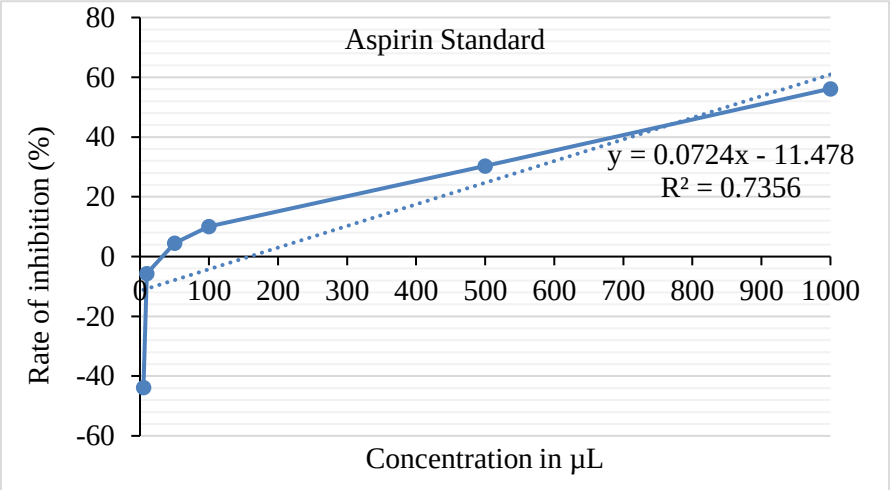

Figure 5. Aspirin standard curve for albumin denaturation method.

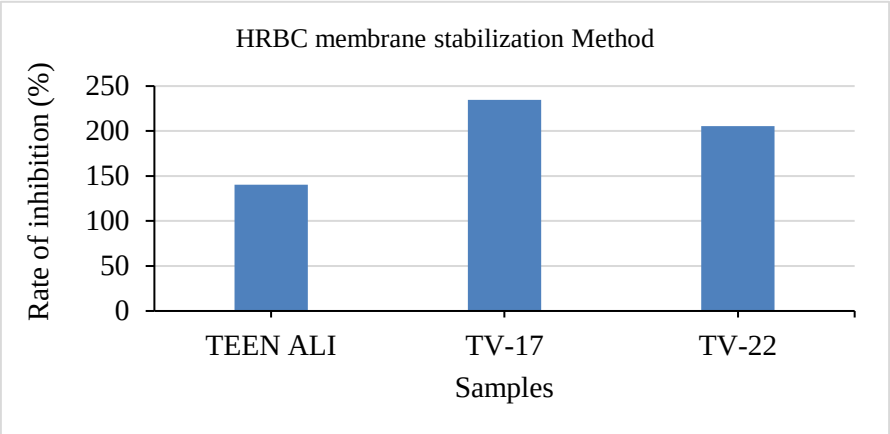

Figure 6. Human red blood cell membrane stabilization method.

Among the samples tested, TV-17, and TV-22 exhibited significant inhibition of haemolysis. These results suggest that these samples contain compounds that could potentially be used as anti-inflammatory agents (Figure 6). TEEN ALI also showed some level of inhibition, although less pronounced compared to the other samples. This indicates that these samples may contain weaker or fewer anti-inflammatory compounds. This value indicates the potency of the samples in inhibiting membrane lysis and further supports their potential as anti-inflammatory agents.

4. DISCUSSION

The research findings align with prior studies on tea's anti-inflammatory properties, particularly the role of bioactive compounds like caffeine and vitamin E in modulating inflammatory pathways. Notably, the inhibition rate of TV-22 (82.11%) in the Albumin Denaturation Method surpasses values reported in studies on other *Camellia sinensis* varieties, suggesting superior anti-inflammatory potential. For instance, Sharangi *et al.*, (2009) reported anti-inflammatory effects of black tea polyphenols with inhibition rates ranging from 60% to 75%, highlighting the distinct efficacy of Assam tea clones. Molecular docking studies further emphasize significant interactions between TV-22 compounds and COX-2, IL-1, and IL-18 receptors, comparable to the performance of standard NSAIDs like diclofenac. This aligns with findings by Yatam *et al.*, (2019), who identified COX-2 inhibition as a critical target for anti-inflammatory agents derived from natural sources, including tea flavonoids. Specifically, compounds like 2,4-di-tert-butylphenol demonstrated consistently negative binding energy, suggesting stable receptor-ligand complexes, a finding supported by Levita *et al.*, (2017), who also reported strong receptor binding by phenolic compounds.

Unlike prior studies focusing predominantly on polyphenols, this research underscores the significance of non-polyphenolic compounds, such as 2,4-di-tert-butylphenol, which exhibited robust interactions across all receptors. This observation is consistent with the work of Hossain *et al.,* (2014), who highlighted the role of phenolic derivatives in inflammation inhibition. Additionally, the high abundance of caffeine in TV-22 aligns with reports by Yang *et al.,* (2018), identifying caffeine as a significant contributor to the anti-inflammatory effects of tea through modulation of inflammatory cytokines.

Furthermore, the combination of in vitro and in silico methods provides a holistic view of the anti-inflammatory potential of Assam tea clones. Studies by Scoparo *et al.,* (2012) and Xiao *et al.,* (2011) similarly highlighted the importance of combining computational and experimental approaches to validate the bioactivities of tea-derived compounds. Future studies could explore the synergistic effects between these bioactive compounds and other anti-inflammatory agents. Additionally, scaling these findings to in vivo models and assessing long-term safety will be crucial for therapeutic applications. Expanding the scope to include other tea varieties and bioactive compounds could further strengthen the understanding of tea's therapeutic potential.

5. CONCLUSION

The study emphasizes the need for further investigation into the specific components responsible for the observed anti-inflammatory effects. It acknowledges the limitations of *in silico* modeling and underscores the importance of experimental validation for potential drug candidates. The combined results from the Albumin Denaturation Method, HRBC membrane Stabilization method, and *in silico* modeling offer a strong foundation for future research. Key compounds identified, such as 2,4-di-tert-butylphenol, caffeine, and Vitamin E, show promise, but their docking results indicate only moderate binding affinity, suggesting that their therapeutic potential should be further validated through in vitro and in vivo studies before consideration as viable drug candidates. Caffeine and carbonic acid stand out due to their favorable properties, including high GI absorption and solubility, making them particularly promising. Assam tea varieties, especially TV-22 and TV-17, demonstrated the highest anti-inflammatory activity, supporting their potential as natural anti-inflammatory agents with therapeutic benefits for chronic inflammatory diseases.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

The first author **Nishanth Pitcham** has contributed in Complete work done, Data curation, Formal analysis, Visualization, Writing – original draft. The Corresponding author **Hariram Natarajan** has contributed in Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review and editing.

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