EXTRACTION OF CAFFEINE FROM TEA & DOCKING OF ADENOSINE RECEPTORS WITH CAFFEINE

PROJECT REPORT

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CERTIFICATE

Certified that this project report **“Extraction of caffeine from tea and docking of caffeine to adenosine receptors”** is the bonafide work of “Sathyanarayanan.V,Abishek Dinesh,Hridya Divakaran,Shah Jainam” who carried out the project work under my supervision.

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ABSTRACT

Extraction is a method used for the separation of organic compound from a mixture of compound.

Here the organic solvent Dichloromethane is used to extract caffeine from aqueous extract of tea powder because caffeine is more soluble in dichloromethane than it is in water. The dichloromethane - caffeine mixture can then be separated on the basis of the different densities of dichloromethane and water because dichloromethane is much denser than water and insoluble in it.

Residual water is separated from dichloromethane by drain out the dichloromethane through separating funnel, thus dichloromethane passed through the funnel while polar solvents such as water is still remaining in the funnel.

Mainly anhydrous sodium carbonate is used for the removal of water from organic layer. Anhydrous sodium carbonate is an insoluble inorganic solid which will absorb water, thus drying it.

Docking is a method to predict the preferred orientation of a molecule to another when bound to each other to form a stable complex.

We also perform docking studies of caffeine to adenine receptors of the central nervous system. On using Hex 8.0.0 software for molecular docking and UCSF Chimera structural viewer we observe the binding affinity of caffeine to adenosine receptors and we find out which of the four adenosine receptors has higher binding affinity by docking them. The caffeine on binding to these different receptors has different inhibitory or inducing effects to the body.

INTRODUCTION

Extractions of certain solids can be performed by utilizing the different chemical properties of various solvents. The initial solvent used in the extraction of caffeine is water. Caffeine is sparingly soluble in water at ambient temperatures but highly soluble in water at 100^{o}C. The boiling of coffee beans and tea leaves dissolves caffeine and other materials to produce coffee and tea beverages. We will take advantage of the solubility properties of caffeine in water to create an aqueous solution of caffeine at room temperature. First, the caffeine will be dissolved from tea leaves by boiling them in water. The solution will be allowed to cool to room temperature. Although the solubility of caffeine is low at room temperature; the caffeine will remain in solution and must be extracted with another solvent.

Caffeine causes most of its biological effects via antagonizing all types of adenosine receptors (ARs): A1, A2A, A3, and A2B and, as does adenosine, exerts effects on neurons and glial cells of all brain areas. In consequence, caffeine, when acting as an AR antagonist, is doing the opposite of activation of adenosine receptors. Caffeine have other biological actions: they inhibit phosphodiesterases (PDEs) (e.g., PDE1, PDE4, PDE5), promote calcium release from intracellular stores, and interfere with GABA-A receptors. Caffeine, through antagonism of ARs, affects brain functions such as sleep, cognition, learning, and memory, and modifies brain dysfunctions and diseases: Alzheimer's disease, Parkinson's disease, Huntington's disease, Epilepsy, Pain/Migraine, Depression, Schizophrenia. In conclusion, targeting approaches that involve ARs will enhance the possibilities to correct brain dysfunctions, via the universally consumed substance that is caffeine.

MATERIALS USED FOR CAFFEINE EXTRACTION:

PROCEDURE FOR CAFFEINE EXTRACTION:

- Tea bags are used as a source of caffeine for this experiment:
- Take 5 tea bags and record the weight of these tea bags.
- Take 500 ml beaker add 200 ml of distilled water to it. Now place the 5 tea bags in this beaker.
- Boil the contents in the beaker vigorously using a hot plate.
- Allow the mixture to cool for 5 minutes and then decant the mixture into another beaker.
- Gently squeeze the tea bags to liberate the rest of the water.
- Cool the aqueous solution to near room temperature.
- Continue cooling in an ice box, the tea must be cool (20° C) before coming in contact with dichloromethane (boiling point = 40° C).
- Extract the solution three times with 30-mL portions of dichloromethane (CH2Cl2). Do not get dichloromethane on your hands.

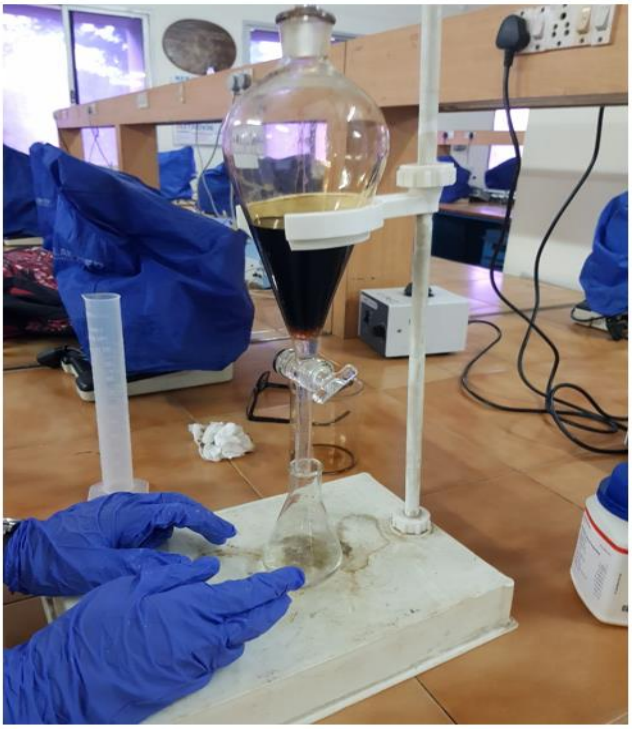
Extraction of tea from tea bags

Filtering extracted tea

Extraction step:

- The tea solution is poured into a separating funnel and 20ml of dichloromethane is added to it. The mixture will separate into two layers - the top layer is the tea layer and bottom layer is the dichloromethane since it is denser than tea.
  B) Remove the funnel from the stand and keep your fingers on the stopper and carefully shake the separating funnel.
  C) Vent the separating funnel periodically (every 30 sec) to relieve vapor pressure created inside the funnel.
  D) When the contents have been sufficiently shaken place the separating funnel back on the ring stand and let the two layers separate.
  E) Drain the bottom layer into a conical flask because now the caffeine is extracted into the dichloromethane layer. Cover the mouth of the conical flask to avoid evaporation of solution.
  F) Repeat steps: **A)** through **E)** twice.
- Dry the combined dichloromethane solutions with anhydrous Sodium carbonate. Add about 1 teaspoon of the drying agent until it no longer clumps together at the bottom of the flask. Mix well and leave it for 10 minutes.

- Decant the dichloromethane into a conical flask (100ml). Evaporate the dichloromethane solvent in a hot water bath.
- When all the solvent is removed you observe a residue of yellowish green - white crystalline caffeine.



Extraction of DCM

The DCM with caffeine is placed inside the water bath until DCM evaporated

After placing in the water bath until DCM evaporates we observe greenish-white residue is in the bottom of the flask

Sublimation step:

- **A)** Take the conical flask containing crystalline caffeine.
  B) Sublime the crude caffeine at atmospheric pressure by placing the flask directly on a pre-heated hot plate. Caffeine melts at 238°C and sublimes at 178°C.
  C) Collect your sublimed caffeine by keeping a test tube on the mouth of the conical flask.
  D) White vapor of caffeine sticking onto the test tube and the walls of the conical flask is observed.
  E) Now cool the conical flask.
- Take a clean watch glass and record its weight in a weigh balance.
- Now strip off the caffeine from the conical flask and the walls of the test tube into the watch glass using a spatula.
- Record the weight of the watch glass + caffeine in a weigh balance and then find out the weight of extracted pure caffeine.

Now we dry heat the caffeine in a hot plate till effervescence comes out and we get a caffeine .

- We were able to extract 0.025 grams of caffeine from 5 tea bags.

RESULT AND CONCLUSION OF CAFFEINE EXTRACTION:

Thus, we have extracted **0.025 grams** of caffeine from 5 tea bags of Brooke Bond-Taj Mahal. This concludes that 1 tea bag of Brooke Bond-Taj Mahal contains **0.005 grams** of caffeine.

DOCKING OF CAFFEINE TO ADENOSINE RECEPTORS:

We performed docking using the software Hex 8.0.0 and we have observed the following results for docking caffeine with A1, A2A, A2B or A3 receptors.

Results from Hex 8.0.0 :

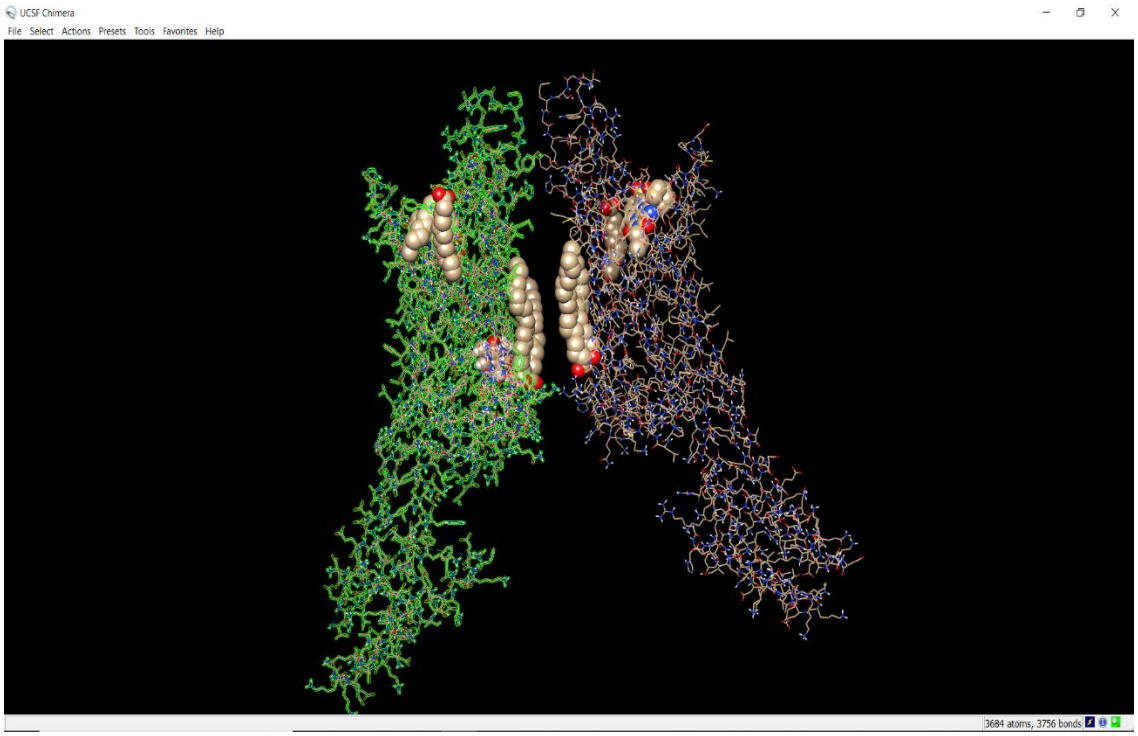
Receptor	Clst	Soln	Models	Etotal	Eshape	Eforce	Eair	Bmp	RMS
A1	1	1	000:001	-154.8	-154.8	0.0	0.0	-1	-1.00
A2A	1	1	000:001	-196.97	-196.97	0.0	0.0	-1	-1.00
A2B or A3	1	1	000:001	-184.6	184.6	0.0	0.0	-1	-1.00

The Etotal value signifies the maximum stability of the ligand binded to the receptor on the docked region.

Using UCSF Chimera we view the docked structures:

For A1 adenosine receptor docked with caffeine:

- The chain A sequence of the receptor, caffeine is binded in between 004-304 of chain A.
- The chain B sequence of the receptor, caffeine is binded in between 006-306 of chain B.



For A2A adenosine receptor docked with caffeine:

- Only has Chain A sequence to which caffeine is binded in between 007-257 which is all of chain A sequence.

For A2B or A3 adenosine receptor docked with caffeine:

- Only has Chain A sequence to which caffeine is binded in between the 251st amino acid sequence of chain A.

From this research journal:

- "https://www.ncbi.nlm.nih.gov/pubmed/20164566" we were able to understand that, different adenosine receptors induced different effects from caffeine binding to them.
- Caffeine on binding to A1 adenosine receptor induced prolonged wakefulness while it on binding to A2A adenosine receptor promoted sleep. So A2A AR antagonists may represent a novel approach as potential treatments for narcolepsy and other sleep-related disorders. But on chronic consumption of caffeine it will alter function.
- A1 AR antagonists with caffeine has been proposed to treat memory disorders and it provides a cognitive effect. Reduced A2A AR activation can improve spatial recognition memory, has cognitive improvements and overexpression can lead to memory deficits.
- Caffeine is known to have stimulatory actions from locomotion due to antagonism of A2A ARs. In animal models on inhibiting caffeine binding to A2A AR decreases the disease symptoms for Parkinson's disease.
- A2A receptors are potent vasodilators, and therefore the influence of caffeine in migraine probably occurs throughA2A receptor antagonism, A2A receptor blockade and consequent attenuation of CGRP-R activation might also contribute to the ability of caffeine to alleviate migraine.
- The influence of A2B or A3 and A2 ARs on GABAA receptor stability is used for seizure control.
- A1 ARs are also probably involved in the antidepressant-like effect of adenosine, which may be the consequence of interactions with the opioid system.

Conclusions:

- We can induce prolonged wakefulness, Antidepressant effect and cognitive boost by making sure caffeine binds to A1 Adenosine receptor. The A1 adenosine receptor docked with caffeine has binding stability of -154.8 and it is binded between aminoacid sequence "004 to 304" of chain A and "006 to 306" of chain B.
- We can potentially treat insomnia and sleep related disorders with the help of A2A AR antagonists as they promote sleep. It has binding stability of -196.97 and it is binded between aminoacid sequence "007 to 257" and has only Chain A.
- Most diseases/conditions can have reduced symptoms or reduced effects by inhibiting A1 and A2A receptors. Hence the caffeine binds to A2B or A3 which only has functions in seizure control etc.

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