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Research paper

Isolation of natural caffeine from lipton™ black tea through acid-base liquid-liquid extraction approach, its medical significance and its characterization by thin layer chromatography and IR analysis

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Abstract

To isolate the caffeine from Lipton™ Black Tea Brand, a sequence of practices was applied. An Acid-Base Liquid-Liquid Extraction approach carried out to force the caffeine to be isolated in upper organic layer as extractant. By this method, first caffeine was isolated from tea bags through passing different steps of extraction, then caffeine was isolated from tea with the use of both Solid-Liquid approach as well as liquid-liquid extraction approach. A product of 0.05 g of pure caffeine was obtained giving the percentage yield or percent recovery of 1.22%. Calculated percent recovery was 1.2 %, this percentage yield clarified that in this tea brand, very small caffeine is investigated, this deduces a significant loss of product throughout the procedure which are due to formation of emulsions and not due to washing thoroughly with DCM to extract maximum yield. It is also significant to be considered that reactions of precursor with solvent pair may not be completed, so 100% yield is not conceivable. Due to much transfers in all processes, this loss might be occurred. Due to much transfers in all processes, this loss might be occurred that's why repeated the process three times again. It is also revealed that as much water was added which decreased the concentration of Caffeine. On analysis with IR, Peak at f=3000Hz indicates the presence of -NH2 and -CONH2 groups while the peaks at f=1600 Hz and f=1750 Hz indicates the presence of alkene portion of the caffeine molecule which concluded that Caffeine is a purine base.

Keywords: *Liquid-Liquid Extraction; Sublimation; Alkaloids; Structure Analogous; Gallic Acid.*

1. Introduction

Tea, one of universal caffeinated brews used in whole world, contains an alkaloid named caffeine (Bunker & McWilliams, 1979). Alkaloids, basic nitrogen comprising compounds exist in plants and herbs. The Caffeine ($C_8H_{10}N_4O_2$) (Figure.1) present in tea is of bitter taste (Clementz & Dailey, 1988), white colored, apparently crystalline methyl xanthine. Caffeine is a usually used chemical compound found in tea, coffee, soft and energy drinks, as well as in chocolates (Barone & Roberts, 1984). It can be extracted from cola nuts, black and green tea leaves, as well as from cacao beans. In 1821, first time great French chemist "Pierre Jean Robiquet" isolated the Caffeine from black coffee. In pure form, Caffeine is an strongly bitter in taste and white colored solid powder. Its configuration has impacts on its functions and applications. Caffeine, is one of most applied alkaloids physiologically active in humans and are known CNS stimulants and diuretics (Grosso et al., 2017). Caffeine also enhances the breathing and heart beat rates, as well as nervousness and insomnia (Nawrot et al., 2003). It is extremely addictive and one of most extensively abused drugs. Symptoms of Caffeine drawing may embrace the lethargy, headache, vomiting, and sickness. Though caffeine has verified to have physical dependence, it is also accomplished to improve the alertness, knowledge capacity, and bodybuilding concert (Costill et al., 1978). Tea shrubberies containing the Caffeine, also have acidic compound namely tannins, cellulosic materials, pigments and chlorophyll. To isolate the Caffeine from these tea shrubberies and leaves, it must be available in free basic forms.

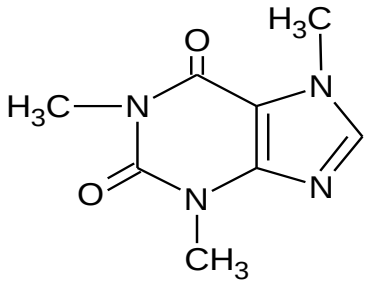

Fig. 1: Chemical Structural Formula of Caffeine.

Caffeine is invigorating due to cyclic backbone arrangement analogous to structure of a base named purine of DNA (Figure.2), enabling it to modify biological natural pathways in body.

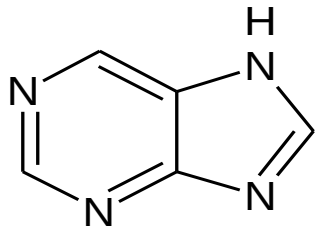

Fig. 2: Chemical Structural Formula of Purine.

Pure Caffeine is being found as odorless (Ruan et al., 2017), crystalline, white colored, fluffy, sparkly powder needles like. Caffeine has weight = 194.19g, m.p = 236ºC, Sublimation temperature=178ºC at 1atm, vapor pressure = 760 mm Hg at 178ºC, pH = 6.9 (1% solution), specific gravity = 1.2, vapor density = 6.7, volatility = 0.5%, and aqueous solubility = 2.17% (Unsal-Kacmaz et al., 2018).
Commercially, Caffeine increases the pharmaceuticals (Slade, 2017) and certain drinks such as coffee or tea. Usual tea bags comprise 1.95-2.05 grams tea leave having about 55 mg of Caffeine. By using appropriate abstraction approaches, Caffeine from a tea might possibly be extract to produce a solid product while weight of product reflects actual yield of Caffeine from tea.
In order to make ensure so, Caffeine must be introduced to a solvent which must be volatile and also water insoluble. An excellent variety of such solvent is Methylene Chloride (MC). Caffeine has a superior attraction for MC and will certainly soluble in MC than water. Though, it is not only an organic compound present in tea which is capable to react with MC (Mehta et al., 2017). Not only Caffeine, tea bags contain also other organic compounds which are called tannins (Khajeh et al., 2017), or also called Gallic Acid (GA). Both of Caffeine and GA are able to dissolve in the water (Lin et al., 2017). However, Caffeine has a strong affinity for water due to dipole-dipole interactions which result from stronger polarity of Caffeine and H-bonding formed in water and Caffeine. Hypothetically, intermolecular attractions in GA can be employed making a stronger dipole-dipole interface (Acree Jr, 2018). On introduction of any general salt i.e. Sodium Carbonate in its mixture, (Azevedo et al., 2018), GA could be return converted into phenolic salt (Figure.3): which is an inorganic polar molecule and insoluble in MC.

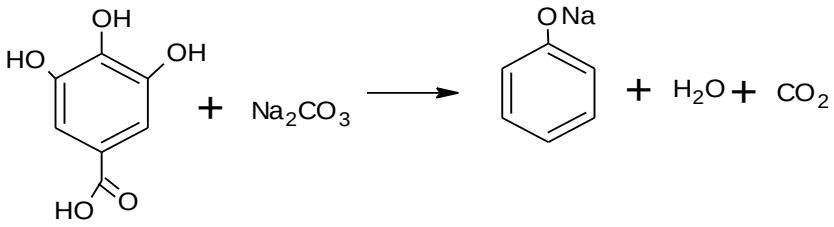

Fig. 3: Formation of Phenolic Salt.

In MC, Caffeine will have a strong affinity for organic solvents and H-bonds present in water and Caffeine will be destroyed. By use of Separatory Funnel, two insoluble solutions can be separated making two distinct layers, through isolation of Caffeine and new phenolic anion or phenolic salt from each other. Thicker layer of MC might then be escaped from Separatory Funnel (SF) to purify a pure mixture of caffeine and MC. To certify that no water restricts with contact of Caffeine and MC, sodium sulfate (Na_2SO_4) may mixed to fascinate any additional moisture escaped from tea solution. If heat that solution, MC may rapidly have evaporated due to low b.p. While residual solid may be the pure Caffeine.
Caffeine has actually originated to work as receptor antagonists through hindering the influence of adenosine. Adenosine is actually a modulator regulating the sleep and also other neural sensations in humans (Cortés et al., 2017). This hindering influence of Caffeine sorts it as a central nervous system stimulant. Greater dosages of caffeine in a mature human can be lethal. The serious fatal dosage of the caffeine in adult human has been likely to be 10grams per person (Faber et al., 2017).

2. Medical aspects of caffeine

Caffeine is one of interesting chemical natural compounds which are found in different drinking products like tea & coffee, guarana, cola, and mate etc. Caffeine is most generally used as mind stimulant as well as some other different applications especially in medical fields. Caffeine can be administrated orally combining with pain killers like aspirin as well as acetaminophen and also can be taken along with a chemical; ergotamine for migraine headache treatment (Malenka et al., 2009).
Different pain killers used for treatment of usual headache and the headache after epidural anesthesia also have Caffeine in their chemical composition which also prevent for further pain and health issues. It also have other medical applications in treatment of different diseases

like gall-bladder problems, Attention Deficit Hyperactivity Disorder (ADHD), short breathe problems in new born babies, low blood pressure, asthma, weight loss, and diabetes Type-II.
It is also used as illegal drug in very extreme doses combining with ephedrine. Sports mans and other athletes use this natural product as stimulant (Castrén, 2013). It is allowed within permissible limits recommended by National Collegiate Athletic Association.
Concentrations of urine more than 15 mcg/mL are banned (Pohler, 2010) and to achieve this concentration, numerous people take approximated 8 cups of coffee which may give 100 mg/cup of caffeine (Goddard, 2016). Its creams are applied over the skin to diminish the skin redness, itching in dermatitis and other skin problems. Healthcare workers occasionally offer the Caffeine to treat the headache intravenously (Budney & Emond, 2014; Griffiths & Woodson, 1988) and also to enhance the flow of urine (Gorsane et al., 2016). It is also used as food ingredient in different food products like beverages, energy and soft drinks. The patients of sounds and voice problems, singers and other professionals use it to improve their voices (Budney & Emond, 2014). But, many research studies has provided the basis and also Health Care agencies that it is also very toxic and harmful as it can disturb their nervous system as well as the vocal cords making their voices unseen (Rainey, 1985). Some other studies also revealed that it is also used stimulant for CNS, Cardiac muscles, and commands the control centre of bloof pressure (Brooks, 2017).

3. Extraction principal

To extract the Caffeine from tea, several approaches and practices are applied. Firstly, to get solid Caffeine in MC liquid solvent, Solid-Liquid Extraction (SLE) practice is applied. SLE is applied with the apparatus set comprising of Soxhelet extractor. SLE also can be applied by simple distillation of a tea cup. Secondly, liquid-liquid extraction (LLE) approach is applied to separate the other chemical compounds from natural Caffeine. LLE approach may be of two different types; Acid-Base LLE (AB-LLE) and Neutral LLE (N-LEE). AB-LLE is applied to separate the Caffeine from tea. This approach entertains a mixture of two distinct layers: an organic layer and an aqueous layer. In AB-LLE, ideal solvent with very low boiling point, have no interaction with solute or other solvent, not toxic or extremely flammable, not miscible with aqueous media, be inexpensive, and should easily dissolve natural Caffeine at 25ºC.
To extract the Caffeine from tea, a solvent pair water-DCM is used. Because of presence of water in mixture, inorganic compounds are isolated from organic compounds which are water insoluble and immiscible. On mixing the solvent pair with tea extract, density of solvent pair deduced which one of solvent will be in upper layer and which one will be in bottom layer in SF. Caffeine may present in upper layer. After separation of both layers, collected product still comprises the numerous other impurities. Sublimation process can be done for decontamination of product because Caffeine has capability to be sublimate.

4. Experimental

4.1. Chemicals/apparatus used

Black Tea bags (Lipton™ Black Tea Brand), Dichloromethane (DCM), Sodium Carbonate; Na_2CO_3 (1-2g), Sodium Chloride; NaCl, Distilled Water, UV-Lamp, TLC set, SF

4.2. Mobile phase for TLC

1% acetic acid in Acetone

5. Method

Weighed out two tea bags of Lipton™ Black Tea Brand. Placed the tea bags in a beaker and added 40 mL distilled water. Covered the beaker with watch glass or china dish and started to heat it to the gentle boiling for 5 min. After heating, poured the mixture in second beaker and added 20mL distilled water to previous beaker and boiled it again for 5 min. Poured this mixture in that second beaker. Removed tea bags from first beaker and wasted them. Added about 1g of Sodium Carbonate, dissolved in it and allowed the mixture to cool down to 25ºC. Transferred the mixture to 150 mL SF. In addition, 10 mL DCM was added in SF, shaked well, and allowed the mixture to separate (Figure.4). Collected the organic layer (Upper Layer). Added another 10 mL DCM in that SF, shake well, and kept the mixture to separate. Again, obtained the organic, upper layer. Discarded the aqueous layer, evaporated the DCM and dried the caffeine. Recorded the Mass of Caffeine.

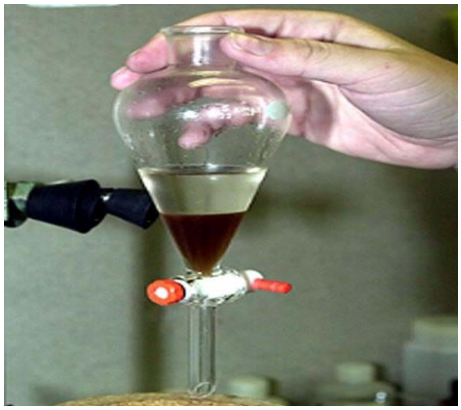

Fig. 4: Separated Layers in SF.

5.1. TLC Analysis

To take TLC of product, separated very small amount of caffeine. Took TLC with Mobile Phase and observed under UV-Lamp, encircled the spots, and measured the Rf-Value of caffeine. Also Calculated the percentage yield of caffeine.

6. Results and discussion

6.1. Extraction

Caffeine has capability to experience sublimation process under the diverse environments after which the impurities can therefore be separated from main desired product. Sequence of practices have been applied to abstract the pure Caffeine from tea. The percent error as well as percent recovery have been calculated to evaluate the amount of pure Caffeine achieved, and to excuse for the errors that might have happened that directed to a loss of product Caffeine. As 4.10 g tea was used in first boiling step. On whole procedure, very small amount of caffeine was isolated which was only 0.05 g or 50 mg. So, percentage yield of caffeine was 1.22%. This percentage yield clarified that in this tea brand, very small caffeine is investigated which clearly showed that this quantity is not too much harmful for humans (Yalwa & Bello, 2017).

6.2. TLC result

The Rf value was measured which was 0.63 and it was realized that commercially available Caffeine have alike Rf value, most near about to reported Rf-value by other research studies (Mumin et al., 2006). This confirms the purity of caffeine.

6.3. IR analysis

The solid Caffeine product was allowed to be analysed through an IR Spectrophotometer for identification. IR spectra of photon energy having the peaks at about f=400-4000 Hz was obtained. Distinct spikes on the IR spectra indicated the bond frequencies of certain functional groups. Peak at f=3000Hz indicates the presence of $-NH_2$ and $-CONH_2$ groups both apparent in Caffeine. Other important peaks at f=1600 Hz and f=1750 Hz indicates the presence of alkene portion of the caffeine molecule. By these peaks obtained by IR spectroscopy the composition of final product was predicted. IR Spectrophotometer predicted the possibility that extracted sample was Caffeine at f= 869 out of 1000 nm. But this range is not a valid or proved as evidence for pure Caffeine. The functions, the Caffeine performs are being related to that of Caffeine's structure (Wang et al., 2011). Principally, Caffeine is a purine base with three different functional groups which are $-NH_2$, $-CONH_2$ and C=C groups. The fundamental property of Caffeine originates from electron lone pair which is found around N-atoms(s) in Caffeine. Caffeine is an achiral molecule i.e. no stereo-isomers the Caffeine have due to achirality. It is a polar molecule i.e. London-Dispersive forces, Dipole-Dipole forces, and H-bonding present when it comes in contact with H_2O. Caffeine also possesses the hydro-phobic nature due to presence of hydrophobic region in its structure. The N-atom present in structure of Caffeine is also very important as controls its solubility in different mediums. Caffeine is water soluble with following extents; 2.2 mg/ml approximately at normal room temperature, 180 mg/ml at 80° C, and 670 mg/ml at 100°C (Williamson, 2011). Caffeine is an organic molecule having base like properties due to presence of $-NH_2$ group (Tello et al., 2011). During extraction, temperature of water is always fixed at high just to increase solubility of Caffeine in water to extent of 670 mg/ml at 100°C. To prevent the bumping in solution, the boiling chips were mixed which also enabled the smooth formation of bubbles on gentle boiling. Later on, mixture was cooled to normal temperature to impart the solubility and also to minimize the attractive forces with water when mixture was in SF (Williamson, 2011). It has a density of 1.325 g/m (Dulloo et al., 1999). Possibly, 4.1g of Caffeine was extracted from two tea bags. The weight of the Caffeine extract was 0.05 g. The percent recovery was 1.2 % which was calculated. This deduces a significant loss of product throughout the procedure which are due to formation of emulsions and not due to washing thoroughly with DCM to extract maximum yield. It is also significant to be considered that reactions of precursor with solvent pair may not be completed, so 100% yield is not conceivable. Due to much transfers in all processes, this loss might be occurred. It is also revealed that as much water was added which decreased the concentration of Caffeine.

7. Conclusion

Caffeine from the tea and coffee was extracted by liquid-liquid extraction and interferences were removed by employing Liquid-Liquid extraction. The percent error and percent recovery were calculated to evaluate how much pure caffeine was achieved, and to excuse for errors that may have happened that directed to a loss of product caffeine. A pure product of 0.05 g caffeine was obtained giving the percentage yield or percent recovery of 1.22%. The calculated percent recovery was 1.2 %. This was the amount of Caffeine extracted from the tea bags. This demonstrates that there was a significant amount of product lost throughout the procedure. It is also important to consider that the reaction cannot go to completion, so 100% yield is not possible. This deduces a significant loss of product throughout the procedure which are due to formation of emulsions and not due to washing thoroughly with DCM to extract maximum yield. Due to much transfers in all processes, this loss might be occurred that's why repeated the process three times again. It is also significant to be considered that reactions of precursor with solvent pair may not be completed, so 100% yield is not conceivable. Due to much transfers in all processes, this loss might be occurred. It is also revealed that as much water was added which decreased the concentration of Caffeine. On analysis with IR, Peak at f=3000Hz indicates the presence of $-NH_2$ and $-CONH_2$ groups while the peaks at f=1600 Hz and f=1750 Hz indicates the presence of alkene portion of the caffeine molecule which concluded that Caffeine is a purine base.

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