Extraction of Caffeine from Native Kola-nut (cola - acuminate) using Swiss Water Process

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Abstract
Caffeine is a stimulant that may cause irritability and insomnia, and a major raw material in the Pharmaceutical Industries for production of drugs. Its inability to be produced locally in Nigeria except by importation has been a serious problem that gave rise to this work. Caffeine is extracted from Kola-nut using Swiss Water Process and the crude Caffeine-extract obtained was purified to get Caffeine whose properties compares very well with those of pure Caffeine in the literatures. Native Kola-nut gave average percentage yields of crude Caffeine and purified Caffeine of about 14% and 12% respectively. The obtained average percentage yield of crude Caffeine and purified Caffeine from native Kola-nut are higher than the percentage yield of Caffeine from other sources (e.g.5% Caffeine from Coffee, 3.34% Caffeine from black Tea and 2.24% Caffeine from green Tea). The optimum percentage yield of crude Caffeine from native Kola-nut using the Swiss Water Process is 15.10% corresponding to 200g of Kola-nut and 4 liters of flavour-charged water at constant temperature and atmospheric pressure. The corresponding optimum percentage yield of purified Caffeine at the same extraction condition is 14.00%. Finally, this work has shown the use of Kola-nut as source of Caffeine production in Nigeria, which diversify the economic use of Kola-nut other than for consumption as food.

Keywords: *Caffeine; Flavour Charged Water; Kola - nut and Swiss Water Process.*

Introduction

Caffeine is a stimulant that may cause irritability and insomnia, and a major raw material in the Pharmaceutical Industries for production of drugs and it is obtained using decaffeination processes such as solvent extraction, Swiss water process and others (Abdul *et al.*, 2006). It is used as a stimulant of the Central Nervous System, and also as a diuretic although its action as a diuretic is weaker than that of theophylline. It is also used in so called caffeine tablet to produce prolong wakefulness and also used in the popup kola beverages because of the mild “Pick up” that it affords(Rubin,2008). Caffeine prevent sleep and tiredness and allows for a rapid and clearer flow of thought (Aeschbachar *et al.*, 1975). Caffeine is used to restore mental alertness when unusual weakness or drowsiness occurs (Ivy et al., 1979). Global consumption of caffeine had been estimated at 120,000 tons per annum making it the world’s most popular psychoactive substance (Reeling, 1999).

The world’s primary source of caffeine is the bean of the Coffee plant, from which Coffee is brewed. Caffeine content in Coffee varies widely depending on the variety of Coffee-bean and the method of preparation used, but in general one-serving of Coffee ranges from about 40mg for a single shot of expresso to about 100mg for strong drip of Coffee. Generally, dark roasted Coffee has less caffeine than lighter roasts since the roasting process reduces caffeine content of the bean (Peker *et al.*, 1992).

Tea is another common source of caffeine in many cultures. Tea generally contains less caffeine per serving than Coffee, usually about half as much depending on the strength of the brew, though certain type of Tea such as black and Oolong, contains more caffeine than most other Tea. Tea contains small amounts of theobromine and slightly higher level of theophylline than Coffee (Adulmumin *et al.*, 2006).

Kola –nut is mentioned as a major source of Caffeine and Nigeria is recorded as the highest producer of kola –nut with over 130,000 tones production per year, but 70% of it consumed in Nigeria and other neighbouring countries (Reeling, 1999). Analysis showed the various chemical component of Kola-nut as caffeine, theobromine, theophylline, catechine, epicatechine, D-catechine, pheriolics, phlobaphens, betaine, starch, fat, thiamine, riboflavin, niacin, ascorbic acid, sugar, gum, cellulose, water, calcium, potassium, iron, beta-carotene and tannic acid (Reeling, 1999).

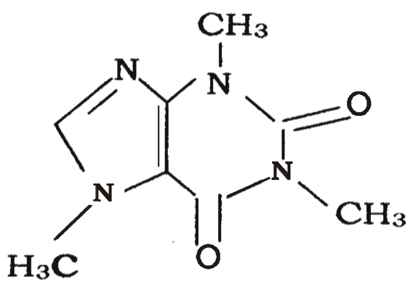


Fig. 1: Structural Formula of Caffeine (1, 3, 5, tri methyl xanthine)

Caffeine is also a common ingredient of soft drinks such as cola, originally prepared from Kola-nut (Essayam, 2001). Soft drinks typically contain about 10 to 50 milligrams of Caffeine per serving. By contrast, energy drinks such as Red Bull contains as much as 80 milligrams of Caffeine per serving. The Caffeine in these drinks either originates from the ingredient used or is an additive derived from the product of decaffeination or from chemical synthesis. Guarana, a prime ingredient of energy drink, contains large amount of Caffeine with small amount of theophylline in a naturally occurring slow release excipient (Essayam, 2001).

Materials and Methods

Drying of Kola-nut

3.5kg of fresh Kola nut was weighed into an evaporating dish using a weighing balance, which was then transferred into a thermostatic Oven maintained at 80^{o}C for 48 hours. At the end of 48 hours, it was removed and reweighed to obtain the dry weight of the Kola-nut as 1.86kg.

Grinding of dry Kola-nut.

The 1.86kg of dry Kola-nut was transferred into a Mortar and grounded with the Mortar pestle to reduce the size to fine particles.

Production of Caffeine-Free Water (Flavour-Charged Water)

Six litres of distilled water was heated in a Kettle to a temperature of 98°C. The hot water was then transferred into a plastic bucket and 780g of ground Kola-nut was soaked in it to release Caffeine and flavour ingredient into the hot water. (The use of hot water instead of cold water in soaking the ground kola-nut was to ensure effective extraction of Caffeine from the Kola-nut). Thereafter, the caffeine-rich water was passed through 75g of activated charcoal placed on a filter cloth which trapped the Caffeine molecules, but allowed the filtrate (which consists of water and flavour ingredients) to pass through the filter cloth into a second bucket. This was confirmed by carrying out Tannic Acid Test and Murexide Colour Reaction Test on the filtrate to ensure that the filtrate is free of Caffeine (Bownman & West, 1975).

Extraction of Caffeine using Flavour-Charged Water (Swiss Water Process)

One litre of the flavour – charged water was put into a separation funnel whose base – exit was blocked or locked. Thereafter, 50g of the ground Kola-nut was added to it and shaken for proper mixing and kept for six hours. Since the flavour-charged water is already saturated with flavour ingredients, only Caffeine is expected to be extracted from the Kola-nut into the flavour – charged water. After six hours, the mixture in the separating funnel separated into two layers with a low density brownish flavour-charged water as the top layer and high-density paste-like material as the bottom layer. The locked base-exit of the separating funnel was then gradually opened to remove the paste-like substance which was dried in an oven at a temperature of 60°C for about one hour ensuring that the paste is not charred. The dried paste was then removed from the oven and cooled to room temperature, weighed and transferred into a storage bottle. Various tests such as melting point, specific gravity, vapour pressure, pH, etc were then carried out on dried paste to confirm that the paste-like substance was caffeine.

The above experiment procedure for extraction of caffeine was repeated with 100g, 150g, 200g, and 250g of ground Kola-nut, with the volume of flavour-charged water increased by the same percentage.

Purification of Crude Caffeine Extract Obtained from Swiss Water Process

This involves application of recrystallization method (Van Atta, 1979) to the crude-caffeine extract obtained from the Swiss Water Process to produce a purified caffeine extract other than the extract obtained from the Swiss Water Process. In the purification of the crude-caffeine extract, 3 grams of crude-caffeine extract obtained from Swiss Water Process was dissolved in 10ml of boiling benzene. Hot Petroleum ether at 79°C was added to the boiling mixture drop – wise until the mixture became cloudy showing on-set of precipitation. More of the hot benzene was then added until the cloudiness clears. The clear solution was filtered hot into a beaker and cooled, during which brown-whitish crystals were obtained. The liquid was decanted, and the crystals were scrapped from the beaker using a scrapper. The obtained crystals were then dried in an oven at a constant temperature of 80^{0}C to release or evaporate any benzene left on the crystals and reweighed to obtain 2.45grams.

Tests for Identification of Caffeine.

The following tests for identification of Caffeine were carried out on the paste-like material obtained to confirm that it was Caffeine.

Murexide Colour Reaction Test

2 grams of crude-caffeine extract obtained from Swiss Water Process and 2 grams of the caffeine crystals obtained from purification processes were placed on porcelain dishes, and

1ml of concentrated hydrochloric acid and 5mg of potassium chlorate were added to the samples in each porcelain dish. Each of the resulting mixture was evaporated to dryness in a boiling water bath. After cooling to room temperature, the resulting residue from each porcelain dish was subjected to ammonia vapour. The two samples displayed intensive purple colour which disappeared on further treatment with the ammonia vapour indicating that the tested samples contain Caffeine. We note that the intensive purple colour disappeared faster in the sample obtained from Purification Process than the sample obtained from Swiss Water Process.

Tannic Acid Test

Standard solution of the Caffeine-extracts obtained from both methods were prepared in two beakers and each was treated with tannic acid drop by drop and a precipitate was obtained from both samples. On further treatment with excess of the Tannic Acid solution, the precipitate from the Purification Process disappeared faster while the Precipitate from Swiss Water Process disappeared slowly. The disappearance indicates the presence of Caffeine in the mixture, while the fast disappearance shows the presence of low impurities in the Caffeine obtained from the Purification Process compared to the Swiss Water Process.

Melting Point

The melting point of the Caffeine extract from the two processes were determined using a digital melting point Apparatus (Electrothermal, Model I A 9100).

Specific Gravity

The specific gravity of Caffeine samples from both methods were determined using specific gravity test (Model ASTMD 792) by CRT Laboratories Inc.

Vapour Pressure

The vapour pressures of Caffeine samples from both methods were determined using an Automated Detector (Model DSC 27 HP).

pH

pH meter (Model 600 UPG) was used to determine the pH of the Caffeine-extract from both the Swiss Water Process and the Purification Process.

Sublimation Point

Caffeine samples obtained from both methods of extraction were placed in different test tubes, which were clamped on a retort stand and heated with a Bunsen burner. The Sublimation temperature of Caffeine from each sample was recorded using a Thermometer.

Refractive Index or Value

Using thin layer chromatography (Whatman, 250μm layer, 20 x 20cm),Caffeine samples from both methods of extraction were prepared using chloroform as mobile phase and visualized under UV-Lamp. The refractive index of each sample was measured and recorded.

Wavelength

A UV- absorption spectrum of crude caffeine- extract and purified caffeine were prepared at different absorbance against different wavelength using a UV-absorption spectrophotometer (Model: UV-1601, SHIMAD 2U Corporation, Japan) and the maximum wavelength for both samples were recorded.

Determination of Rate of Dissolution of Caffeine in Water at Different Temperatures

12ml of water at $10^{O}C$, $20^{O}C$, $30^{O}C$, $40^{O}C$ and $50^{O}C$ were placed in different flasks and 10g of Caffeine extract was added to each flask. The time taken for the Caffeine extract in each flask to dissolve was recorded for the crude caffeine-extract obtained from the Swiss Water Process and the Purification Process.

Percentage Yield of Caffeine.

The percentage yield of purified or crude caffeine-extract obtained from the Swiss Water Process is calculated as follows:

$$= \frac{Massof\ Crude\ or\ purifiedcaffeine}{Massof\ kolanut}\ x\ 100 \qquad (1)$$

Results and Discussion

Table 1 shows the results of the various test carried out to determine the physical and chemical properties of crude caffeine and purified caffeine extracted from kola-nut using the Swiss Water Process.

Table 1*: Tests for identification of caffeine extracted from Kola- nut*.

Test	Crude Caffeine	Purified Caffeine	Pure Caffeine (Peker et al., 1992)
Tannic Acid Test	Precipitate disappeared slowly	Precipitate disappeared faster	Disappearance of precipitate
Melting point	236°C - 241°C	235°C - 237°C	237°C
Murexide colour reaction test	Intensive purple colour disappeared slowly on treatment with Ammonia vapour	Intensive purple colour disappeared faster on treatment with Ammonia vapour	Disappearance of intensive purple colour with ammonia vapour
Specific gravity	1.6	1.2	1.2
Vapour pressure	800 mm Hg at 178°C	760 mm Hg at 178°C	760 mm Hg at 178°C
pH	8.0	6.9	6.9
Sublimation point	180°C	178°C	178°C
Refraction Index	0.65	0.63	0.63
Wave length (maximum)	279nm	275nm	275nm

It is obvious from Table1 that, the results of Caffeine from the Purification process are better than those of the crude Caffeine- extract obtained from the Swiss Water Process, as the former are closer to the properties of pure Caffeine obtained from the literature than the latter. Thus, the fast disappearance of the precipitate in the Tannic Acid Test when purified Caffeine is used than with crude Caffeine-extract, and the fast disappearance of colour in the Murexide Colour Reaction test when purified Caffeine is used than with crude Caffeine-extract, may be as a result of the presence of impurities in the crude Caffeine-extract.

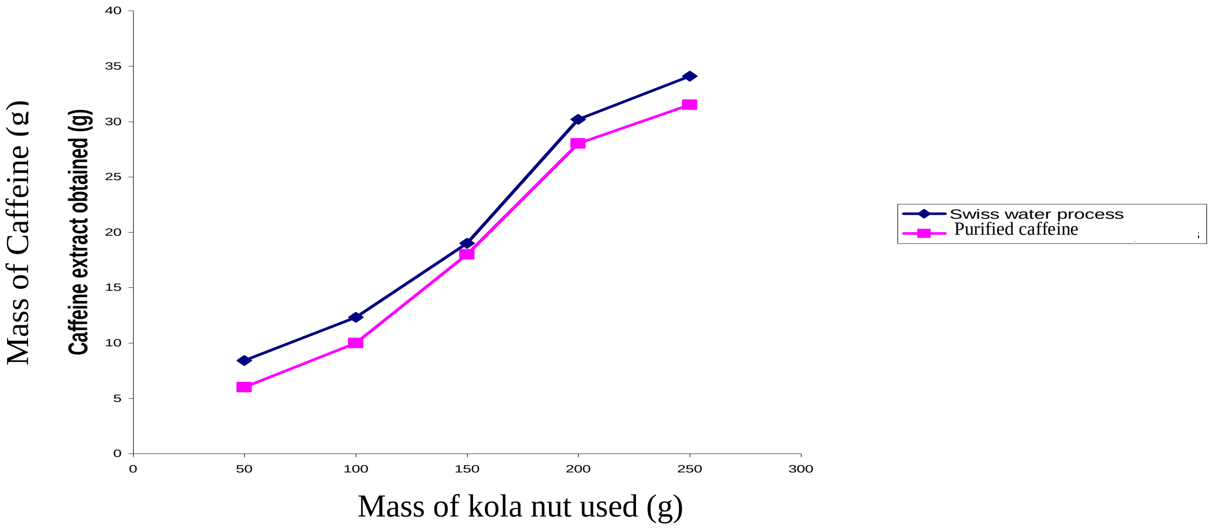


Fig. 2: ***Plot of Mass of Caffeine in dry form against Mass of Kola-nut used***

Figure 2 shows the variation of the mass of crude Caffeine-extract obtained from the Swiss Water Process with mass of Kola-nut used, as well as the variation of the mass of purified Caffeine with mass of Kola-nut used. Figure 2, indicated that the mass of crude Caffeine-extract obtained from the Swiss Water Process is higher than that of the purified caffeine. This may be attributed to impurities in the crude Caffeine-extract. Also, the mass of crude Caffeine-extract and the purified Caffeine increases as the mass of Kola-nut increases.
Table 2 shows the percentage yield of crude Caffeine-extract obtained from the Swiss Water Process, and percentage yield of purified Caffeine while percentage yield of Caffeine is calculated and presented as follows,

Table 2***: Percentage yield of crude Caffeine and purified Caffeine*****.**

Wt of kola-nut (g)	**Mass of Caffeine (Swiss Process) (g)**	**Crude-extract Water**	**% yield of crude Caffeine (%)**	**Mass of purified Caffeine (g)**	**% yield of purified caffeine (%)**
50	8.40		16.80	6.00	12.00
100	12.30		12.30	10.00	10.00
150	19.00		12.67	18.00	12.00
200	30.20		15.10	28.00	14.00
250	34.10		13.64	31.50	12.60
			Average =14.10%		Average =12.12%

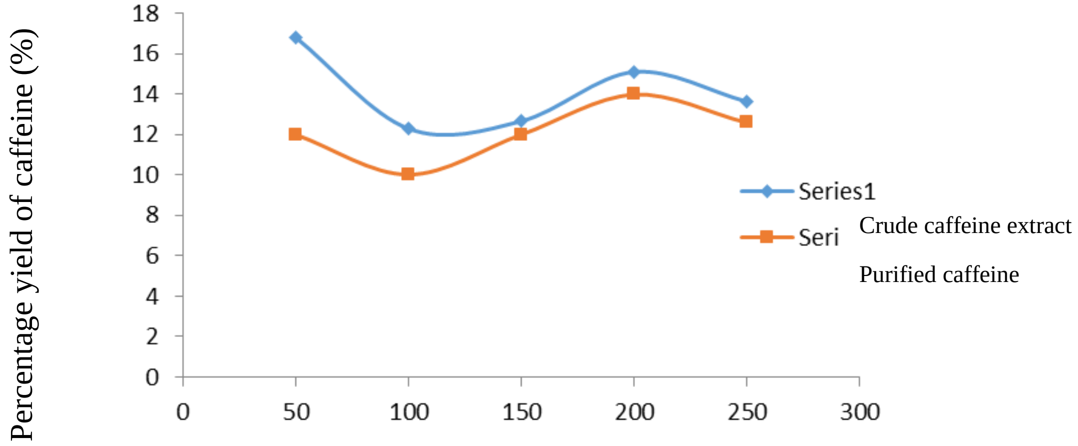
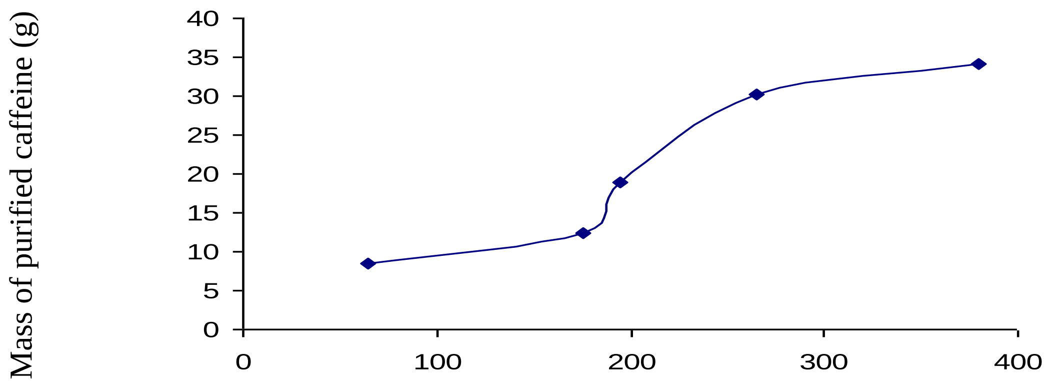


Fig. 2. *Plot of percentage yield of caffeine against mass of kola-nut*
Mass of Kola-nut (g)

Fig.3: ***Graph of Percentage Yield of Caffeine against Mass of Kola-nut***
Figure 3 shows the graph of percentage yield of Caffeine against mass of Kola-nut. It may be seen from the graph that the percentage yield of crude Caffeine initially decreases to a minimum value of 12.30% as the mass of Kola-nut increases to a corresponding value of 100g. Thereafter, the percentage yield of crude Caffeine increases to a maximum value of 15.10% as the mass of Kola-nut increases to a corresponding value of 200g. Beyond 200g of Kola-nut, the percentage yield of crude Caffeine decreases continuously as the mass of Kola-nut increases. A similar trend is obtained for the purified Caffeine. Thus, the optimum percentage yield of crude Caffeine from Kola-nut using the Swiss Water Process is 15.10% corresponding to 200g of Kola-nut and 4 liters of Flavour- Charged Water at constant temperature. The corresponding optimum yield of purified Caffeine at the same extraction condition is 14.0%. The results above show that Native kola-nut has higher yield of crude Caffeine compared to other sources of Caffeine (e.g. 5.20% caffeine from coffee, 3.34% caffeine from black tea, and 2.24% caffeine from green tea). The higher percentage yield of crude Caffeine than the percentage yield of purified caffeine indicates that the amount of impurities in the purified Caffeine is lower, which is consistent with Figure 2.



Time (sec.)

Fig. 4: ***Graph of Caffeine Extract against Time for increasing Mass of Kola-nut***

Figure 4 shows a plot of mass of crude Caffeine-extract against time for increasing mass of Kola-nut. It may be seen from Figure 4 that, the mass of Caffeine-extract obtained at the end of the extraction process increases with time as the mass of Kola-nut increases. Thus, more time is required to extract Caffeine as the mass of Kola-nut increases.

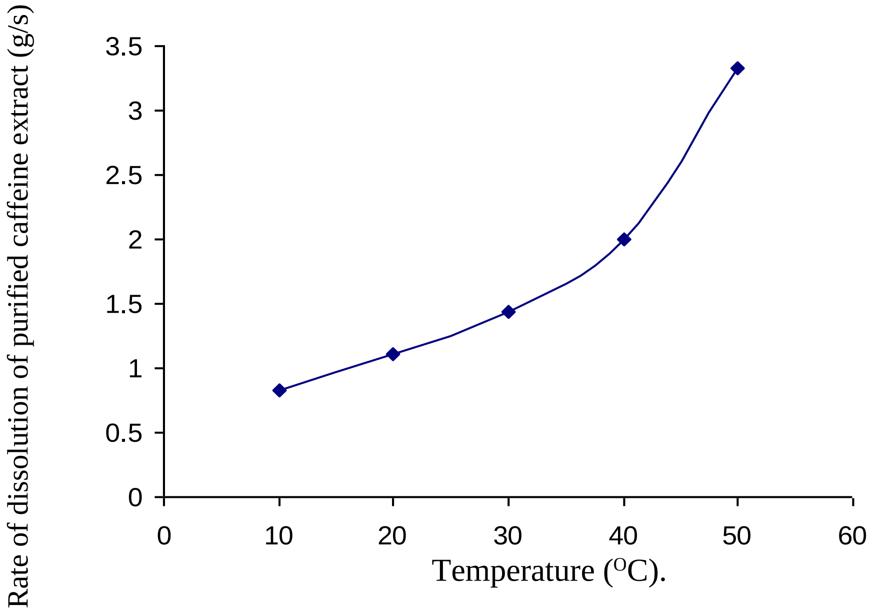


Fig.5: ***Rate of dissolution of a known Mass of Purified caffeine in Water at different Temperatures.***

Figure 5 shows the rate of dissolution of purified Caffeine in water at different temperatures. It may be seen from Figure 5 that the rate of dissolution of purified caffeine in water increases with temperature, which is consistent with the effect of temperature on the rate of chemical reaction (Murray and Hansen, 1995).

Table 3: ***Content of Benzene in purified caffeine***.

Mass of Purified Caffeine (g)	Benezene content (ppb)
6.0	0.16
10.0	0.17
18.0	0.16
28.0	0.18
31.5	0.15

Table 3 shows the content of benzene in different masses of the purified Caffeine determined using Gas-chromatograph (Hewlett-Packard, Model 5830 A). The allowable limits for benzene in food substances are 10 ppb (World Health Organization), 10 ppb (Republic of Korea), 5 ppb (Canada), 5 ppb (United States of America), and 1 ppb (European Union) (USFDA, 2007). Comparing the benzene contents in the Caffeine obtained in this work with the above allowable limits, indicates that the contents of benzene in the purified Caffeine is negligible and does not pose health risk to human being.

Conclusion

This study has shown that Caffeine can be obtained locally from native Kola-nut (cola–acuminate), especially in Nigeria which is the largest producer of Kola-nut with production rate of over 130,000 tons per annum. The purified caffeine in the study is adequate and compares very well with properties of pure caffeine in the literatures. The use of Kola-nut as source of Caffeine production will diversify the economic use of Kola-nut other than for consumption as food.

References

Abdul M., Kazi F.A., Zainal, A. and Zakir, H. (2006). Determination of Caffeine by solid phase Extraction and High Performance Liquid Chromatography. *Malaysian Journal of Chemistry*, 9,45-51

Adulmumin, M.D., Kazi F. A., Zainal, A. M. & Zakir, H. (2006). Determination and Characterization of Caffeine on Tea, Coffee and soft Drink by solid phase Chromatography (SPE - HPLC). *Malysian Journal of Chemistr. 8*, 065 – 075.

Aeschbacher H.V., Graham, R. & Bradbrook, J. (1975). The effect of Caffeine on Barbiturate sleeping Time and Brain Level. *Journal of Pharmacology and Experimental therapeutics*, 196,635

Bownman, W.C.R. and West, G. B. (1975). *Text Book of Pharmacology*, London Blackwell Scientific Publication. pp. 256-258

Essayam, D. M. (2001). Cyclic Nucleotide Phosphodiesterase, *Journal of Allergy Clinical Immunol*, 108, 671 – 680

Murray, D. S. & Hansen P. J. (1995). Extraction of Caffeine from Tea leave. *Journal of Chemical Education*, 72, 851

Reeling, J. (1999). Therapeutic of Kola Nut. *Journal of the American Medical Association*,5, 6-10.

Rubin, R. (2008). New Study, different outcomes on Caffeine, "Pregnancy" *Journal of Clinical pharmacology*, 41, 35 – 41

USFDA, (2007). United State Food Drug and Administration. Question and Answer on the occurrence of Benzene in soft drinks and other beverages. *www.cfsan.fda.gov/rdms/benzga.html*, 16

Van Atta, R.E (1979). Ultraviolet Spectro Photometric Determination of Caffeine in Cola Drinks: An analytical experiment. *Journal of Chemical Education*, 12, 56-66