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EXTRACTION, IDENTIFICATION AND DETERMINATION OF CAFFEINE AND TRACE METALS IN THREE TYPES OF TEA LEAVES

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A R T I C L E   I N F O

A B S T R A C T

Sufficient amounts of caffeine were extracted from Kabbos and Wazzah tea leaves, while traces of caffeine were found in green tea leaves which may be due to unriped tea leaves. Extracted caffeine was identified by UV and IR spectroscopy which offered spectra matching precisely the standard caffeine crystals which confirmed the purity of caffeine and the efficiency of extraction methods. Moderate amounts of sodium, potassium and calcium were found in the three types of tea leaves while lithium was found in traces. The results indicated the presence of necessary elements for the metabolic activities of tea plants and humans who take tea drink with their meals.

Introduction:

Caffeine is a naturally occuring alkaloid found in tea ,coffee, chocolate and several other common foods and beverages.(1) It acts as stimulant and diuretic delays fatigue and affects the central nervous system.(2)

It is rapidly absorbed through the stomach lining and reaches the blood stream within30 -45 minutes.(3)

It becomes equally distributed throughout the water of the body, being metabolized in the liver and expelled via the kidneys. (3) Doses of 100-200 mg result in increased alertness and wakefulness, faster and clearer flow of thoughts, increased focusing and better general body coordination. Sudden withdrawal of caffeine may cause headaches, drowsiness, irritability, nausea, vomiting and other symptoms.(4) Ingestion of caffeine results in wakefulness, thus it is used to counteract the hypnotic effect and is found in a number of analgesics.(5)

Well controlled laboratory studies involving running or cycling after taking 3-13 mg caffeine/kg body weight 1 hour before exercise have shown that caffeine improves endurance by prolonging time to exhaustion .(6,7)

Caffeine is an organic molecule and it is slightly polar due to the presence of two carbonyl groups and four nitrogens in its bicyclic structure.

While caffeine is rather soluble in water it is much soluble in organic solvents much closer to its own

polarity such as dichloromethane (mathylenchloride).

The aim of research due to these   significant characteristics and benefits of caffeine, black tea deserves much more research and identification to find out the accurate levels of caffeine in different types of tea leaves. This paper aimed also to determine of some trace metals in the tea leaves after ignition and dissolving in nitric acid.

Experimental:
Materials and instruments:

All reagents used in this research were of analytical grade supplied from Fluka and BDH. UV-Visible spectrophotometer model 6405 JENWAY was used to study the absorption of the extracted caffeine at UV-Vis region.

Pellets press designed for IR spectroscopic disc (20 tons pressure) were used for caffeine membrane casting.

IR spectra for caffeine were obtained using Philips IR spectrophotometer model PU 970, from Germany.

Flame photometer model CM63LB from JENWAY was used for determination of some alkaline and alkaline earth metals.

Double distilled water was used for all solutions preparation.

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Three types of high quality of tea leaves were purchased from market; Kabbos, Wazzah and Green leaves which were used in this research.

Procedures of extraction:(8)(1) Extraction by dichloromethane (CH2Cl2):

A sample of 5 gm of tea leaves was accurately weighed and transferred into conical flask. Calcium carbonate (4g) and 100ml of distilled water were added to the tea sample. The mixture was boiled for 15 minutes on hot plate with stirring and cooled to around 500C and then filtered using vacuum filtration through whatman No.54 paper. The filtrate was cooled to room temperature and transferred into a separating funnel. 15ml of dichloromethane was added to the funnel and shaked gently .Once the layers have been separated, the organic layer was drained into 50 ml conical flask. The extraction was repeated with another fresh 15 ml dichloromethane. The methylene dichloride solution was dried with anhydrous sodium sulphate. The mixture was swirled for several minutes to allow sufficient time for sodium sulphate to dehydrate the solution which was then filtered into dry preweighed 100 ml round bottom flask.

Eventually, the round – bottom flask was placed on a steam bath and the contents were evaporated to dryness at low – medium heat of water bath. The flask was weighed to find out the crude mass of caffeine.

(2)Extraction with chloroform:

10 g of tea leaves were transferred into 250 ml size conical flask and 100 ml of distilled water and 10 g of $CaCO_3$ were added and heated to boiling for 25 minutes with stirring by glass rod.

The mixture was filtered through Buchner funnel and the filtrate was transferred into separating funnel of 250 ml size. 80 ml of chloroform were added to the separating funnel and swirled gently for 5 minutes. Then, the mixture was left to settle in order for chloroform layer to be exhibited. The separating funnel stopper was opened and the chloroform was collected in beaker of 100 ml size and the chloroform was evaporated to dryness on water bath in the hood.

The produced crystals were recrystallized by ethanol (95%) where caffeine is soluble in the heated ethanol and the crystals were separated by cooling and filtering through Buchner funnel. The crystals were washed with 5 ml of ethanol. The caffeine crystals were transferred into a crucible and left in a desiccator for one day for drying and weighing.

Caffeine was distinguished by dissolving its several crystals in three drops of 0.1M HNO_3 in a porcelain crucible. The mixture was heated to dryness. The addition of five drops of 0.1M NH_4OH to the crucible content have revealed purple colour of solution an indication of caffeine.

Moderate concentrations of Sodium, Potassium and Calcium were found in the three types of tea leaves, while Lithium was found in traces (Table.3). Appropriate calibration curves were obtained for these metals by flame emission photometric technique, and the concentrations were picked up directly from these calibration curves (Fig.4).

The results compiled in table (3) shows the presence of necessary elements for the metabolic activities of the plants and humans who employ tea with their meals.

Ignition of tea leaves and preparation of their sample solution

10g of each type of tea leaves were weighed in porcelain crucible and ignited at 2500C for two hours in a Muffle Furnace (Carbalate, UK).

The crucible with its contents were left in a desiccator for cooling and weighed. The residue in the crucible was transferred into a beaker and dissolved in 200 ml of 0.1M HNO_3 for the three types of tea leaves.

Standard solutions for Sodium, Potassium, Lithium and Calcium were prepared from their corresponding salts in the range of 1–80ppm. Percentages of emissions of these standard solutions and samples solutions were measured by flame emission photometer at its highest sensitivity.

Results and discussion:
Identification of Caffeine:
 IR Spectroscopy:

Fig (1) show IR spectrum of extracted caffeine compared with its standard Spectrum at the same chart. Table (1) presents the absorption data of functional groups of extracted caffeine compared with the standard (in wave number, cm-1).

Data on table (1) show the closeness of the functional groups absorptions of the extracted caffeine with the standard one which confirms the correct structure of the extracted caffeine. The small shift in

some absorptions data is attributed to instrumental errors (shifting in instrumental measurements).

UV Spectrum (nm):

10g of extracted caffeine was dissolved in 100ml of 0.05M sulphuric acid in volumetric flask (which gives 0.1mg/ml). 2.5ml of this solution is transferred into 25ml volumetric flask and diluted to the mark with 0.05M sulphuric acid. UV spectrum was run for this solution and a maximum absorption of 275nm was obtained similar to the standard caffeine spectrum (Figs.2and3).

Purines give sharp absorption at 270nm, but in caffeine the absorption is shifted into 275nm, due to substitution of three methyl groups on three nitrogen atoms.

The appearance of absorption band at 275nm for caffeine is attributed to the electronic transition of $n \longrightarrow \sigma^*$ and $\pi \longrightarrow \pi^*$ where unpaired electrons are available in excess on nitrogen atoms and carbonyl groups in addition to double bonds. (9)

Some caffeine contents of the extracted caffeine are compiled on Table (2), which offer percentages of caffeine in Kabbos and Wazzah in agreement with literature value(10) while in Green tea caffeine was found in traces which may be due to unriped tea leaves. However, caffeine contents of various sources varies with soil conditions and climate

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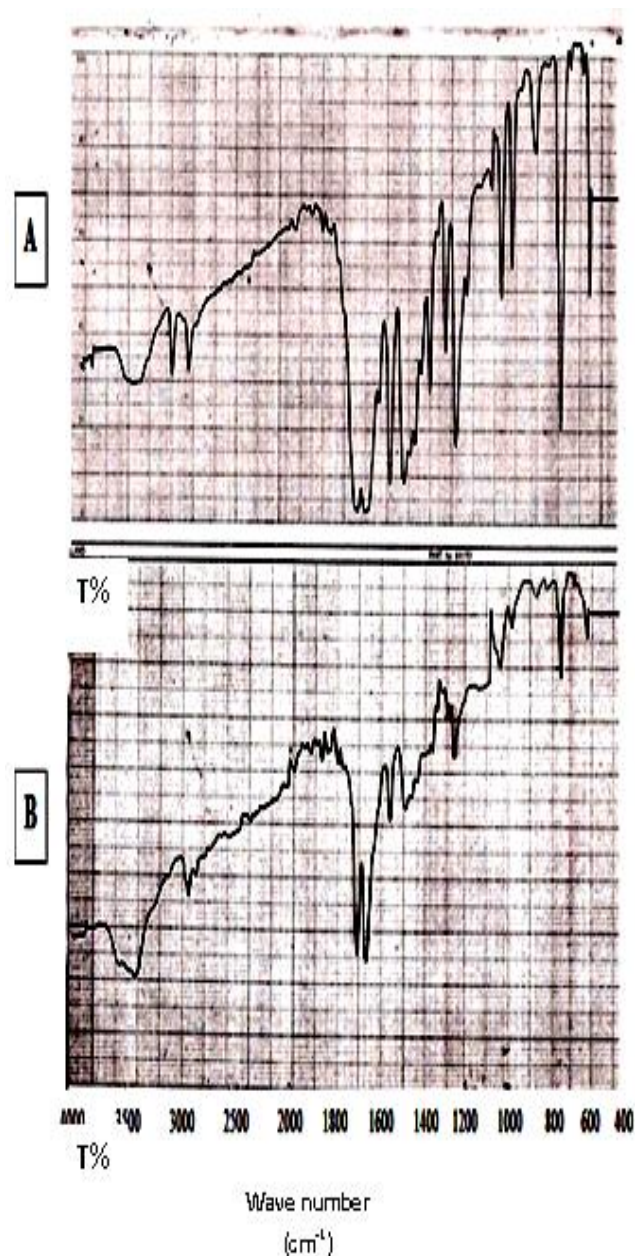
Fig (1): IR spectra of caffeine
A= IR spectrum of standard caffeine
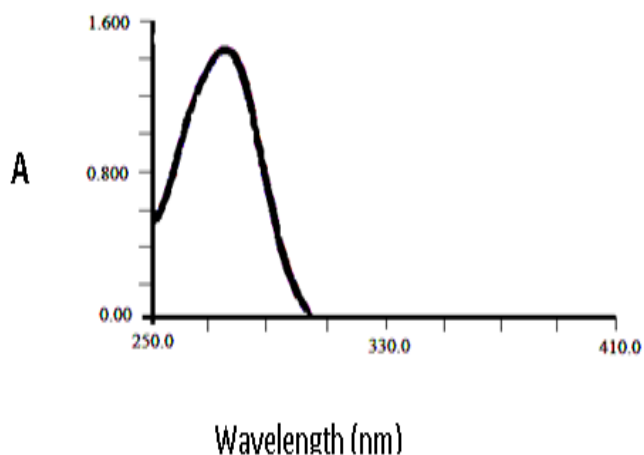B= IR spectrum of extracted caffeine
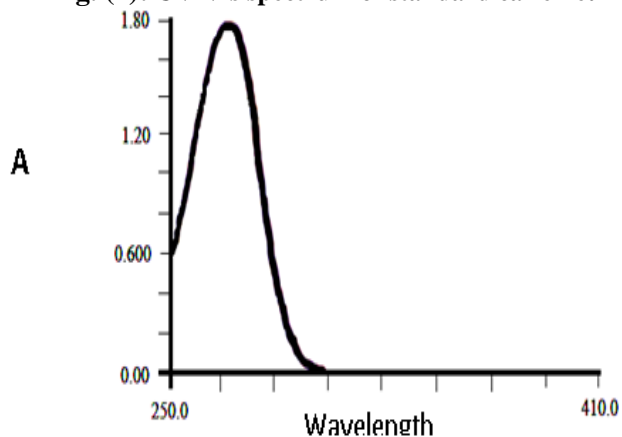
Fig. (2): UV-Vis spectrum of standard caffeine.
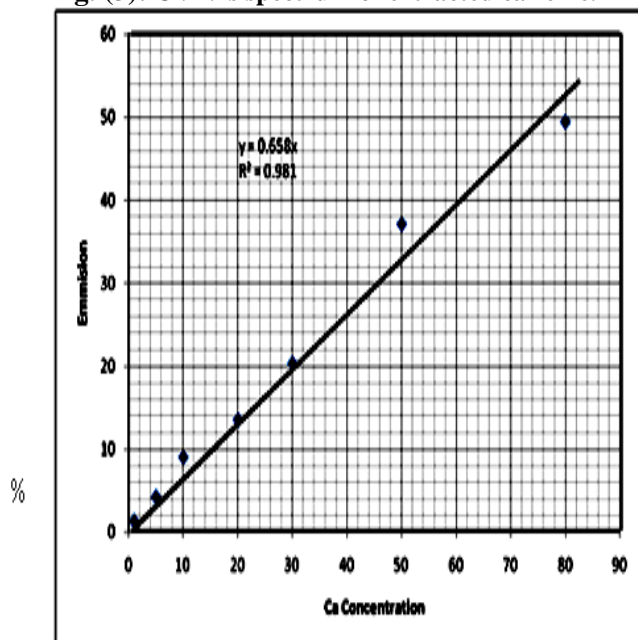

Fig. (3): UV-Vis spectrum of extracted caffeine.

Fig (4): Calibration curve for Calcium determination by flame emission photometry

Table (1): IR Identification of extracted caffeine

Functional group	Wave number (cm^{-1}) (Extracted caffeine)	Wave number (cm^{-1}) (Standard caffeine)
υ_{C-H}	3130	3134
υ_{C-CH3}	3855	3850
$\upsilon_{C=O}$	1692	1690
υ_{C-N}	1015,1190,1238	1020,1197,1240
$\upsilon_{C=N}$	1592	1590
Asym and sym CH$_3$ bending	1335	1358 - 1470
C –H deformation	737	740

Table (2): Caffeine contents of the three types of tea leaves.

Type of tea leaves	M.P.(C^0)	% Caffeine (CH$_2$Cl$_2$)	% Caffeine (CHCl$_3$)	% Ash	% Volatiale
Kabbos	233-235	2.9	2.6	35.9	64.1
Wazzah	233-235	2.2	2.1	17.6	82.4
Green tea	233-235	Traces	Traces	35.9	64.1

Table (3): Concentrations (ppm) of some alkaline and alkaline earth metals in the three samples of tea leaves.

Types of tea leaves	Sodium	Potassium	Lithium	Calcium
Kabbos	6.5	62.0	1.4	20.0
Wazzah	17.3	70.5	1.7	27.6
Green tea	7.5	57.0	1.3	20.7

استخلاص، تشخيص وتقدير الكافائين وبعض العناصر الفلزية في ثلاثة أنواع من أوراق الشاي

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الخلاصة:

تم استخلاص كميات مناسبة من الكافائين من أوراق شاي الكبوس وشاي ألوزه ، بينما تم الحصول فقط على تراكيز ضئيلة من الكافائين في أوراق الشاي الأخضر والذي قد يعزى إلى عدم نضج أوراق الشاي. وقد تم تشخيص الكافائين المستخلص بأطياف الاشعه فوق البنفسجية والأشعة تحت الحمراء والتي أعطت معلومات تضاهي المعلومات المنتقاة من الكافائين القياسي والتي أثبتت نقاوة الكافائين المستخلص وجدوى طريقة الاستخلاص. وتم الحصول على كميات معتدلة من فلزات الصوديوم والبوتاسيوم والكالسيوم في أوراق الشاي بأنواعه الثلاثة بينما كان الليثيوم بتراكيز ضئيلة. بينت هذه النتائج وجود أو توفر بعض العناصر الفلزية الضرورية للفعاليات الحيوية لنبات الشاي وللناس الذين يتناولون شراب الشاي مع وجباتهم الغذائية.