A Research Note

SPECTROPHOTOMETRIC DETERMINATION OF CAFFEINE IN NIGERIAN KOLA NUTS

INTRODUCTION

ALTHOUGH kola nuts contain xanthine stimulants like caffeine, theophylline and theobromine (Fleischer, 1956; Kamp, 1960; Rafal and Tadeusz, 1966; Van Pinnxteren and Schallenberg-Heertjes, 1953), there are no reports on the presence of these stimulants in Nigerian kola nuts. Most Nigerians chew kola nuts because it produces effects such as sleeplessness, prolonged capacity for work and increased mental efficiency when chewed in moderate quantities. Similar pharmacological effects have been reported for caffeine (Bowman et al., 1971). In Nigeria, it is commonly believed that the stimulating effects of the common edible kola nuts decrease in the order stated: Cola acuminata, Cola nitida rubra and Cola nitida alba. A study on the caffeine contents of the two commonly edible species of Nigerian kola nuts—C. acuminata and C. nitida—as well as a wild species, C. verticillata, which because of its slimy taste is not eaten by most Nigerians, is reported in this paper. This investigation not only provides some information about the caffeine contents of Nigerian kola nuts, it also provides a scientific basis for the acceptance of the local belief on the comparative stimulating effects of Nigerian kola nuts.

EXPERIMENTAL

Grinding of kola nuts

The samples of kola nuts (purchased from Ebute-Ero market, Lagos) were separately ground into coarse particles, dried to a constant weight in an oven at 105°C and ground to a fine powder.

Extraction

The procedure of extraction was a modification of the method reported by Van Pinnxteren and Schallenberg-Heertjes (1953). A 100g sample of kola nut powder was shaken vigorously in a 2-liter flask with 1 liter of chloroform and 100 ml of 25% aqueous ammonia, for 1 hr. The mixture was magnetically stirred for 24 hr and filtered on a 32.0 cm Whatman Grade 1 filter paper. The residue was washed thoroughly with about 250 ml of chloroform and the washing added to the filtrate. The filtrate was gently evaporated to dryness, so as not to sublime the ingredients present.

Preparation of calibration curve for caffeine in chloroform

The absorption peak for pure caffeine (BDH Chemical Ltd., England) in spectroscopic chloroform (BDH Chemical Ltd., England) was determined by observing the ultraviolet spectrum recorded with a Unicam SP 800 Ultraviolet spectrophotometer. It was found to be 276 mp.

Very pure caffeine was obtained by placing several grams of the crystalline commercial caffeine in an evaporating dish covered by a watch glass and heating over a hot plate. The sublimed caffeine was collected from the watch glass and dried at 110°C for 1 hr. Chloroform solutions containing from 5–25 mg per liter of the pure sublimed caffeine were examined with a Uni cam SP 800 Series 2 Ultraviolet and Visible spectrophotometer. Readings were made at 276 mp. A plot of absorbancy vs. concentration gave a straight line showing excellent conformity with Beer’s Law.

Purification and spectrophotometric determination of caffeine in the kola nut extracts

The procedure used for purification and spectrophotometric determination of caffeine was essentially that reported by Shingler and Carlton (1959) with slight modifications.

An air bubble free 70 x 1 cm column of silica gel was prepared and washed with spectroscopic chloroform until no absorbance was registered by the eluate at 276 μm using a Unicam SP 500 Series 2 Ultraviolet and Visible spectrophotometer. 250 mg of the kola nut extract was chromatographed on the column at an elution rate of 100 ml/hr using spectroscopic chloroform as the eluent. Several 50 ml fractions were collected until all the caffeine had passed from the column. The caffeine in each fraction was spectrophotometrically determined by measuring the difference in absorbance at 310 μm and 276 μm using either spectroscopic chloroform or the first 50 ml fraction, since both show no absorbance, as the reference.

Recovery studies

Fractions with optical density values greater than 0.025 (corresponding to 0.5 mg/liter) were combined and gently evaporated to dryness, to avoid sublimation of the ingredients. The dry crystalline material obtained was washed with small amounts of petroleum ether (60–80°C), 96% ethanol; benzene; chloroform; ether 100–120°C, (20:80 v/v) mixture, to remove colored impurities which contaminated it, then recrystallized from benzene/petroleum ether 100°C, (20:80 v/v) mixture, weighed and its melting point determined.

Thin layer chromatography

Silica gel G Merck (Brinkman Co., New York) was used to prepare the thin layer chromatographic plates according to the procedure outlined by Randerath (1966). The thin layer chromatography plates were spotted with chloroform solution of the recovered material and eluted separately with petroleum ether (60–80°C); 96% ethanol; benzene; chloroform; and 96% ethanol/chloroform (1:9 v/v) mixture. The plates were dried and developed in a tank of iodine vapor.

Spectral studies

The ultraviolet spectrum of the recovered material was recorded in water using a Unicam SP 800 Ultraviolet spectrophotometer. The infra red spectrum was examined as a mull, using Nujol as the mulling oil. The spectrum was recorded with a Unicam SP 1200 Infra-red spectrophotometer. The nuclear magnetic resonance spectrum of caffeine dissolved in deutero-chloroform was recorded with a Varian T60 NMR spectrophotometer.

RESULTS & DISCUSSION

THE RESULTS summarized in Table 1 prove satisfactory when recovery studies were carried out. The homogeneity of the recovered and recrystallized material was
CONCLUSION

This investigation shows that Nigerian kola nuts vary in caffeine content. The variation not only occurs from one species to another but also between varieties of the same species as exemplified by the differences in caffeine contents of *C. nitida rubra* and *C. alba*.

REFERENCES


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