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# Quantitative Evaluation of Some Cannabis Sativa (Indian Hemp) Samples from Ghana.

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## Summary

The levels of three Cannabinoids- Cannabidol, (CBD) Delta-9- Tetrahydro Cannabinol (THC) and Cannabinol (CBN) in 24 samples from the various regions in the country have been determined using gas liquid chromatographic method. The levels of THC (the biological active cannabinoid) in the samples ranged from 0.039-1.549 per cent w/w with coefficient of variation of 2.45 per cent. Classification according to the phenotype ratio method put the Ghanaian samples into phenotype I. the content of CBD ranged from 0.116-0.620 per cent w/w whilst the levels of CBN fell between 0.049-1.069 per cent w/w.

## Introduction

The levels of Cannabinoids in Cannabis sativa (L) are known to be influenced by factors such as ecology, stages of development of the plant, genetics and seasonal changes 1-2. Various attempts have been made to classify Cannabis according to the geographical origin but not with much success (3-6). Difficulties encountered in such classification exercises have been attributed partly to the fact that there are a whole range of unauthenticated samples of Cannabis obtainable from unauthorized cultivations from all corners of the world. The problem is made more difficult by the unstable nature of Cannabis samples on storage. These difficulties in classification are not encountered with Opium where cultivation in most parts of the world is authorized and the Opium where samples tend to be more stable on storage than Cannabis.

Despite the above mentioned difficulties, some efforts have been made to classify various samples of Cannabis using other characteristics of the plant. Content of Cannabinoid acid sand phenols in Cannabis has been used to classify the plant into fibre and drug types[7]. Fetterman et al[8] recently also suggested another way of classifying Cannabis into chemical phenotypes: phenotype ratio= (% (-) $\Delta^9$ – trans -tetrahydro-cannabinol +% Cannabinol): %Cannabinol.

According to this method, Cannabis samples with phenotype ratios greater than 1.0 are classified as phenotype I. the phenotype I is regarded as biologically active and samples with ratios less than 1.0 are classified into phenotype II or the fibre-type Cannabis.

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This method of classification is used in classifying 24 samples of Cannabis randomly selected from Police seizures from the various regions in Ghana. Up-to-date no data on quantitative levels of Cannabinoid in Cannabis samples as found in Ghana have been reported. With complexing problems in classifying the various samples as found in many parts of the world are systematically studied. The present report is part of a programme initiated in our Laboratory to examine systematically samples of Cannabis in Ghana.

## EXPERIMENTAL

### Materials

*Cannabis Samples*

Random samples were taken from Police seizures of Cannabis from the various regions in Ghana. The samples were air-dried at room temperature (air-conditioned room- $22^0$-$25^0$ C) for three days. The dried plant materials (flowering tops) were powdered into fine powder and passed through No. 44 sieve (355 mm. aperture). The powdered materials were then stored in stoppered amber-coloured bottles and stored in a cold room ($4^0$C) up to the time of analysis. The storage time in each case did not exceed one month.

### Apparatus

Gas chromatograph – varian model 1700 with flame ionization detector. Column: Stainless steel column (5' X 9") with stationary phase three per cent SE 30 on varAport 30 (80-100 mesh) Column temperature was programmed from $170^0$ - $230^0$C at the rate of $6^0$C/min. Injector and Detector temperatures used were $270^0$C and $250^0$C respectively. Nitrogen gas was used as the carrier gas with the flow rate for the hydrogen gas and air were 30 ml/min and 300 ml/min respectively.

All the chemicals used were of analytic grade (B.D.H.).

United Nations Authentic samples of $\Delta^9$–THC, CBD and CBN were used as standard cannabinoids.

### Assay of Cannabinoids

Gas liquid chromatographic method as described by Binka and Bediako-Donkor [9] was used to evaluate quantitatively the content of CBD, $\Delta^9$–THC and CBN in the 24 samples of Cannabis with slight modifications. About 0.50G of the powdered plant material was accurately weighed and extracted with 3 X 20 ml, hot methanol. The methanolic extract was filtered through No. 4 Whatman paper and the filtrate evaporated to dryness using vacuum evaporator. The residue was wetted with 0.5 ml benzene and taken up with 0.5G florisil (60-100 mesh). A column chromatographic clean-up was set up with 2G slurry of florisil prepared with benzene. The column dimensions were 12 X1.2 cm and the plant ectract mixed with florisil was transferred to the column and eluted with 3 X 25 ml. benzene. The benzene extract was then evaporated to

dryness under vacuum and the residue was taken up in n-hexane, containing 0.2 per cent w/w of anthracene as the internal standard into 10 ml. volumetric flask and made up to volume.

The contents of CBD, CBN and THC were estimated by the method of peak areas. An extract from the Cannabis sample No.9 was analysed for its THC content eleven times and the results were statistically evaluated. Variations in THC content in the 24 samples analysed were also evaluated using the following formula: (2)

coefficient of variation =

N=number of pairs of assays and X 2 and 1 results from each pair.

## Results and Discussion

The mean values of CBD, CBN THC from duplicate results from the analysis of the 24 samples of Cannabis are as shown in Table I. The coefficient of variation from eleven repeated analysis for THC content in sample No. 9 was found to be 2.45 per cent with the mean value of 1.466 per cent w/w. coefficient of variation of THC content in all the 24 samples analysed was found to be 2.36 per cent. These results indicate that the prescribed procedure for the determination of the Cannabinoids was reasonably reproducible and they compared favourably with those reported by Fairbairn and Leibmann (1973)[2]. The retention times for the Cannabinoids relative to anthracene (o min) were as the following : -

Cannabidiol, 12 min. (-1 $\Delta^9$–Tetrahydrocannabinol- 14 min.

Cannabinol – 15 min.

Using the phenotype ratio method all the Ghanaian samples analysed could be classified as belonging to phenotype I group of Cannabis sativa (L). It could therefore be inferred that the samples were biologically active. One cannot say much about differences in the content of cannabinoids in samples from the various parts of the country. Realistic comparison could be made from Cannabis grown in the same season and harvested at the same time.

## Acknowledgement

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**TABLE I**
**QUANTITATIVE EVALUATION OF CANNABIS SAMPLES FROM GHANA**

| Sample | Region | CBD (%w/w) | THC (%w/w) | CBN (%w/w) | Phenotype Ratio |
|---|---|---|---|---|---|
| 1 | Greater-Accra … … | 0.241 | 0.523 | 0.516 | 2.518 |
| 2 | ,, … … …… | 0.420 | 1.032 | 0.681 | 4.079 |
| 3 | ,, … … … … | 0.620 | 2.307 | 0.158 | 3.976 |
| 4 | ,, … … … … | 0.214 | 1.308 | 0.158 | 7.690 |
| 5 | Western … … … | 0.116 | 0.629 | 0.539 | 7.690 |
| 6 | ,, … … … … | 0.548 | 1.011 | 0.502 | 9.870 |
| 7 | ,, … … … … | 0.258 | 1.466 | 0.639 | 8.159 |
| 8 | ,, … … … … | 0.164 | 0.921 | 0.172 | 6.665 |
| 9 | ,, … … … … | 0.200 | 0.829 | 0.176 | 6.128 |
| 10 | ,, … … … … | 0.198 | 0.699 | 0.536 | 6.207 |
| 11 | ,, … … … … | 0.298 | 0.507 | 1.069 | 5.289 |
| 12 | ,, … … … … | 0.494 | 0.636 | 0.372 | 2.242 |
| 13 | ,, … … … … | 0.394 | 1.431 | 0.498 | 5.741 |
| 14 | ,, … … … … | 0.336 | 1.549 | 0.236 | 5.313 |
| 15 | ,, … … … … | 0.272 | 1.093 | 0.274 | 5.026 |
| 16 | ,, … … … … | 0.422 | 1.062 | 0.274 | 3.166 |

| | | | | | |
|---|---|---|---|---|---|
| 17 | „ … … … … | 0.162 | 0.887 | 0.244 | 6.981 |
| 18 | „ … … … … | 0.234 | 1.331 | 0.302 | 6.265 |
| 19 | „ … … … … | 0.1851 | 0.836 | 0.272 | 5.449 |
| 20 | „ … … … … | 0.152 | 0.917 | 0.049 | 6.289 |
| 21 | Central … … … | 0.140 | 0.039 | 0.123 | 1.157 |
| 22 | Brong-Ahafo … … … | 0.186 | 0.928 | 0.803 | 9.306 |
| 23 | „ … … … … | 0.200 | 0.210 | 0.974 | 5.920 |
| 24 | Eastern … … … | 0.414 | 0.753 | 0.131 | 2.135 |