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Cannabis Sativa L.-II

Studies Of Samples Of Cannabis Sativa L. (Indian Hemp) In Ghana

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SUMMARY

Specificity of the chemical test-Ghamrawy and Duquenois-was evaluated with respect to eleven different local plant species. Some plants responded to colour spot tests similar to the response given by Cannabis sativa L. Thin Layer and Gas chromatographic methods were found to be specific for the identification of cannabinoids in *Cannabis sativa L.* extracts.

A local *Cannabis resin* and that from United Nations Laboratory were examined qualitatively with the U.V and I.R. Spectrophotometry. The I.R. spectra of the resins were used to evaluate the maturity of the samples.

INTRODUCTION

Seized samples of *Cannabis sativa L.* (Indian Hemp) sent for analysis by law enforcing bodies in Ghana comprised mainly of the flowering tops of the plant, and sometimes, the whole uprooted plant. These samples are seized under various conditions.

The Police may uncover a Cannabis farm which may be concealed amongst other crops like cassava, tomatoes and pepper. If the undergrowth of a farm is overgrown, other plant species might make it difficult for the law enforcing officer to recognize the Cannabis. The illegal dealer in Cannabis may adulterate wrappers of Cannabis with some other plant materials. Cases have been known of suspected Cannabis smokers chewing up partly smoked Cannabis wrappers, when apprehended by the Police.

Bits and pieces of the chewed up plant materials may be sent to a laboratory for analysis by the Police.

It is apparent that analytical methods used for the identification of seized *Cannabis sativa L.* should be selective and specific. It has been reported that some plant materials other than *Cannabis sativa L.* respond positively to some of the chemical tests for the *Cannabis sativa L.* notably Beams, Ghamwary and Duquenois tests (1,2,**3**).

The present studies seek to evaluate the specificity of the normal laboratory identification tests for *Cannabis sativa L.* vis a vis other local plant materials likely to be found with seized Cannabis samples. Spectrophotometry and Chromatography are also used to evaluate qualitatively seized samples of *Cannabis sativa L.* and compared with authentic samples.

EXPERIMENTAL

MATERIALS

(A) Apparatuses:

(i) Microscope (ERNSTLEITZ)

(ii) Thin layer Chromatography equipment (DESAGA) with plates 20"x 20"

(iii) U. V. Spectrophotometer-Beckman Model DK 2A with recorder.

(iv) I. R. Spectrophotometer-Beckman Model IR 33.

(v) Gas Chromatography- Varian Model 1700 with Flame Ionization Detector. Stainless steel column (5' x $^{1/8}$") Stationary Phase: 3 per cent SE. 30.

(B) Reagents:

(i) *Acid Chloral Reagent*

Dissolve 10G, Chloral hydrate in 100ml. of 20 per cent v/v aqueous hydrochloric acid.

(ii) *Ghamwary*

Dissolve 0.5 G of pdimethylamino benzaldehyde in 50ml. 95nper cent Ethanol. Add 2.8ml. conc. H_2SO_4

(iii) *Duquenois*

Dissolve 2G vanillin in 100 ml of 95 per cent ethanol. The stock solution may be kept indefinitely in a refrigerator. For immediate use, 1 ml of the vanillin solution is mixed with 3 drops of acetal dehyde.

(iv) *Beam*

Dissolve approximately 5G, KOH in 100 ml of ethanol (95%).

(v) *Brentamine*

(a) Fast blue salt (B) (Echtblausalz in water 1% w/v).

(vi) (b) Aqueous KOH (2N) Spray first with (a) followed by (b)

(vii) *Tetrazotised benzidine or toldine*

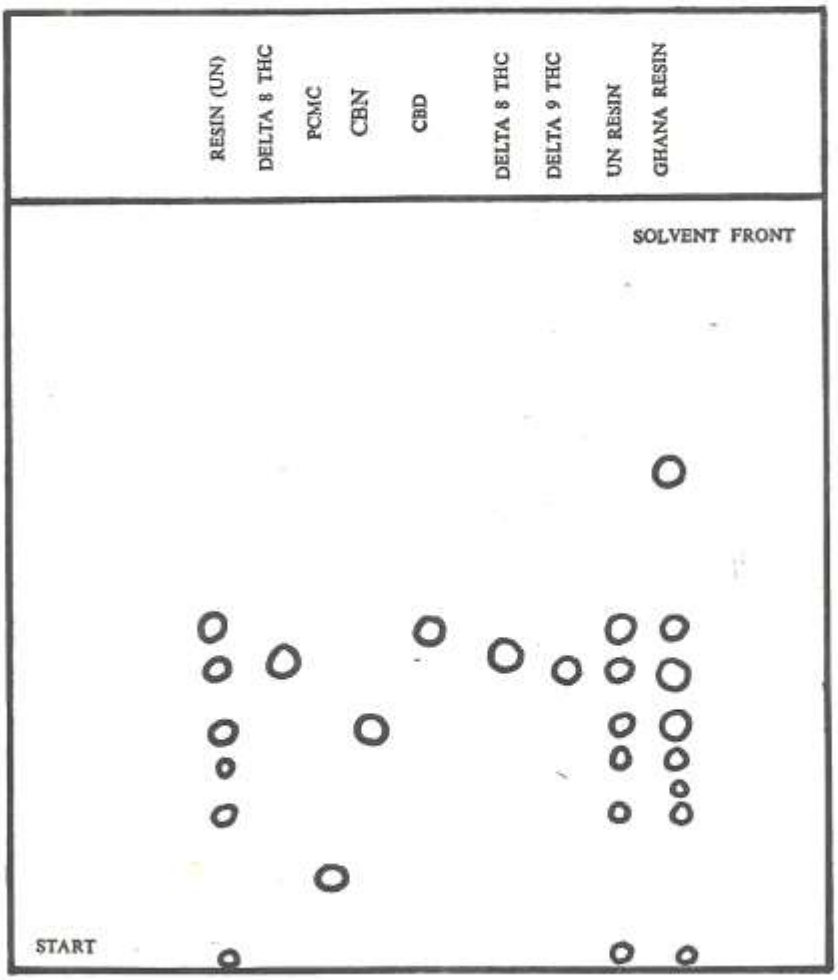
(a) 1ml. or 1g. of amine 3ml. conc. HCL made with water.

(b) 10 per cent Sodium nitrate. Mix equal volumes of (1) and (2) and stand for 1-2 minutes before spraying.

(C) PLANT MATERIALS:

The *Cannabis sativa L.* used in the investigation were taken from Police seizures from suspects.

FIG. 1 THIN LAYER CHROMATOGRAM OF CANNABIS RESIN



The other plant materials were samples from small farms around Accra, Aburi and Kumasi. The plants were identified by the Botany Department, University of Ghana, Legon. The selection of the non-Cannabis plants was based on their morphological resemblance to *Cannabis sativa L.* and their likelihood to be used to adulterate genuine *Cannabis sativa L.*

(D) REFERENCE

CANNABINOIDS

(i) Authentic resin (UN).

(ii) Cannabinol (CBN) (UN)

(iii) Cannabidiol (CBD) (UN).

(iv) 8- THC) (UN)
9- THC) (UN)

PROCEDURE

Macroscopy and Microscopy

The main morphorlogical features of the leaf structure and inflorescence of eleven non-Cannabis plants were examined. Powdered plant materials were cleared with chloral hydrate solution and the presence of cystolithic trichomes were checked with acid chloral solution. The details of examination are as indicated in Table I.

Colour Spot Tests

50 mg of the powdered plant materials (leaves and flowering tops) were extracted with 20 ml. petroleum ether (40-60^{o}) and filtered. The filtrate was evaporated to dryness and the reagent was applied to the residue.

The non-Cannabis plants were tested with Ghamrawy and Duquenois tests (1,2,3). Extended Duquenois test which involved extraction of the colour formed with the plant residue was also applied to each plant sample. The various colour reactions of the reagent with the plant extracts are as also shown in Table I.

Thin Layer Chromatography (T.L.C.)

About 0.5 to 1 G of the powdered leaf and flowering tops of the plant were extracted with 3x20ml, hot methanol. The methanolic extract was filtered through Whatman No.4 filterpaper and the filtrate was evaporated to dryness. The residue was then wetted with 0.5ml benzene and taken up with 0.5G florisil (60-100 mesh). 2G slurry of florisil was prepared with benzene and transferred to a column (12x1.2cm). The plant extract residue mixed with florisil was transferred to the column and eluted with 3x25ml. benzene. The benzene extract was evaporated to dryness and the residue was taken up in n-hexane or cyclohexane into 5ml. volumetric flask and made up to volume.

The final solution was then spotted (5ml) on 250mn layer of silica gel (GF254-Merck-Stahl) (air dried) on 20”x20” glass plates and run in a given solvent system.

The solvent system were:-

(a) 1.5 per cent v/v Absolute ethanol in petroleum ether and chloroform (1:1)

(b) Chloroform: benzene (50:50)

(c) Benzene: Diethylamine (100:1)

Plates impregnated with silver nitrate and solvent system (petroleum ether/chloroform 1:1) were used (4). Plates impregnated with dimethylformamide and developed with cyclohexane were also used (5).

The Benzene: Diethylamine (100:1) system and silica gel (GF254) were selected for the screening of cannabis and non-cannabis plant materials. Para-chlorometacresol was used as Relative Rf values (Rx) of Cannabinoids are as shown in Table II, T.L.C. chromatogram of cannabinoids is as shown in fig. I

U.V and I.R Absorption

U.V and IR absorption spectra of Cannabis resins from U.N and Ghana samples were recorded using Beckman IR 33 Spectrophotometer. For U.V absorption, n-hexane was used as a solvent and a thin film of the resin was used for the Infra Red Spectrophotometric examination. The Cannabis resins were extracted from plant materials as described under the method for thin layer chromatography. The U.V and IR spectra are as shown in figs 2 and 3 respectively.

FIG. 2 U.V. ABSORPTION SPECTRA OF CANNABIS RESINS

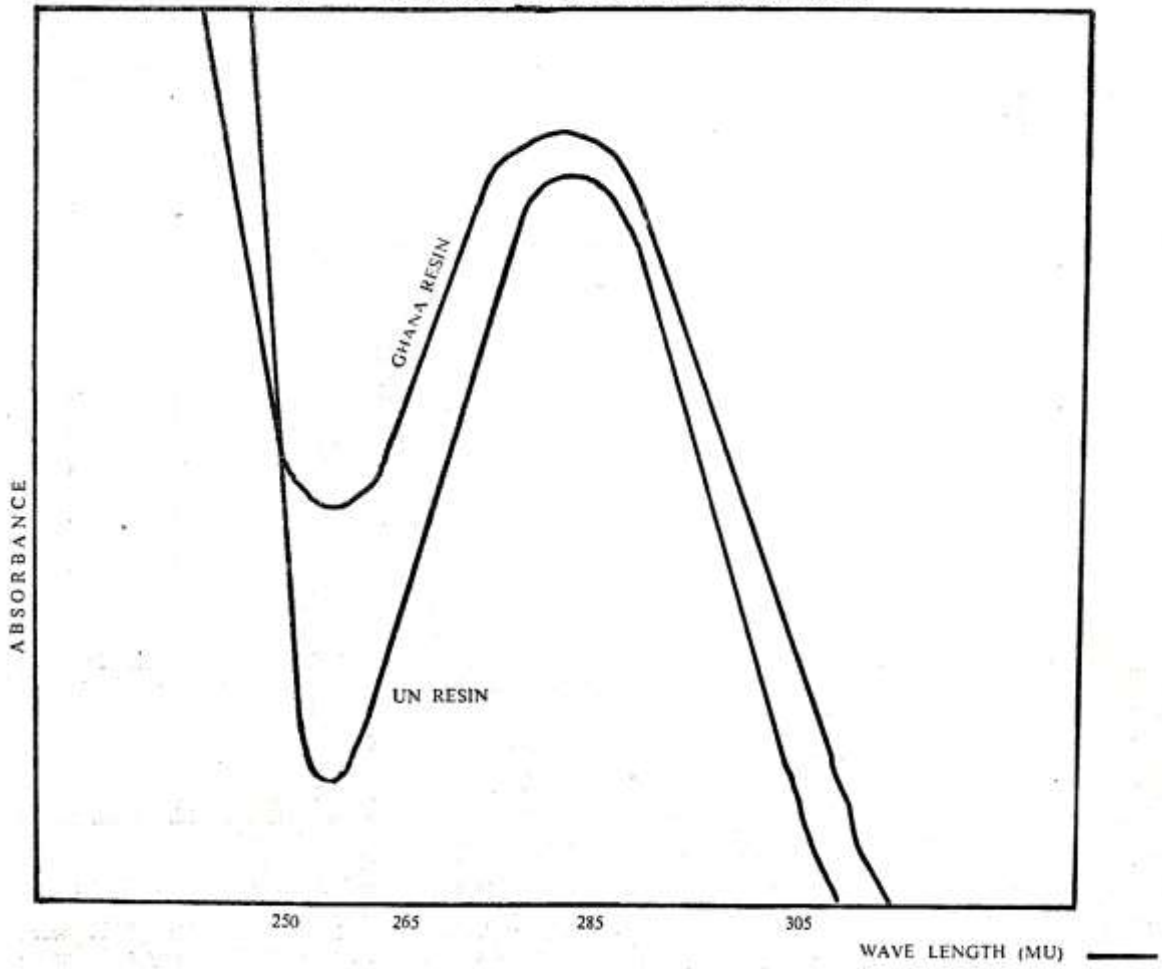


FIG. 3 INFRA RED ABSORPTION SPECTRA OF CANNABIS RESINS

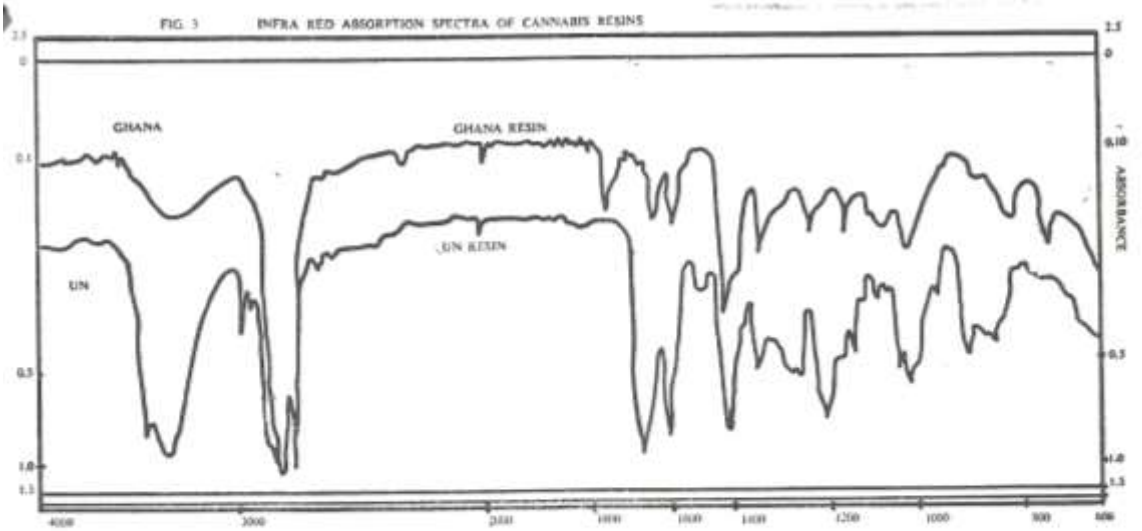


FIG. 4 GAS CHROMATOGRAM OF CANNABIS RESIN

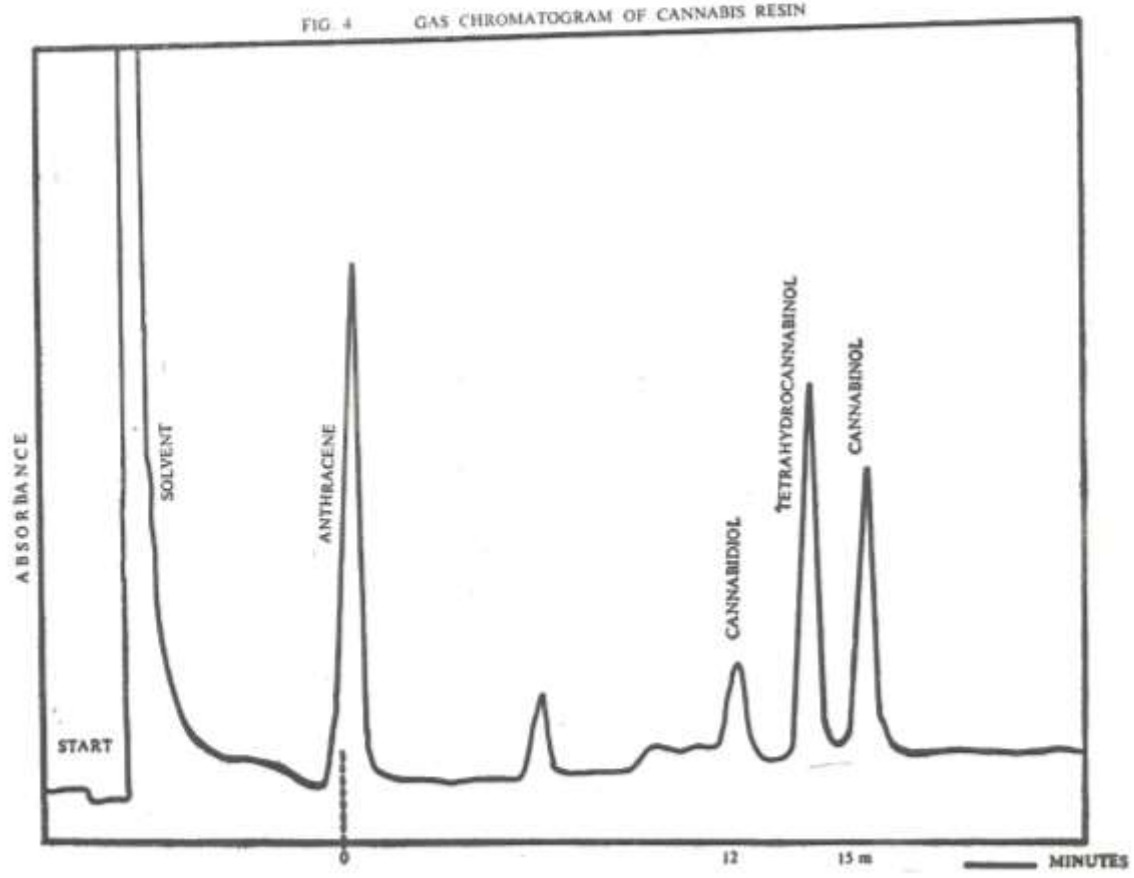


Table 1

Name of Plant	**Macroscopy-leaf Structure**	**Microscopy**	**Duquenois Test**	**Ghamrawy's Test**
Crotalari goreansis (Papillionaceae)	Simple trifoliate leaf. Retuse apex. entire margin glabrous and ovate-shaped	1)slender, unicellular, uniseriate trichomes with burning bases. 2) anisocytic Stomata observed.	Yellow with reagent. Yellow in chloroform extract.	Brown with reagent. Mauve with water.
Ficus religiosus (Moraceae)	Long-stalked simple leaf. Sinuate margin with a long apiculate apex, cordate-shaped.	1)idioblasts with yellow contents. (2) anomocytic stomata. (3) No hairs or covering trichomes observed	Yellow with reagent. Yellow in Chloroform extract.	Brown in reagent. Brown-mauve with water
Hibiscuc micranthus (Malvaceae)	Separate margin; Acuminate apex; pubescent surface	1)Abundant cluster crystals of calcium oxalate. 2) Abundant honey covering trichomes of varying sizes. Highly thickened Mostly unicellular and inuseriate.	Yellow with reagent. Yellow in chloroform extract.	Green with reagent. Mauve with water.
Solanum lycopersium (solanceae)	Assymetric base of the leaflets. Fleshy-texture; acute apex.	1)Abundant compound trichomes mostly unicellular. 2) Cluster crystals of calcium oxalate. 3)Glandular	Yellow with reagent. Yellow with chloroform extract.	Brown with reagent. Dirty blue with water.

		hairs not observed.		
Spigalia anthelmia	Lanceolate Leaflet; accuminate apex	1)Paracytic stomata 2) No hairs observed	Deep yellow with reagent. Light yellow in chloroform extract	Brownish-red with reagent. Green with water.
Hyphis Suaveolens	Very pubescent, thin stalks, Apiculate apex; serrate margin.	1)Abundant covering trichomes, 3-5 celled. 2) Cluster crystals in the Mesophyll. (3) gladular hairs.	Pale yellow with reagent. Colourless after shaking with chloroform.	Black after boiling with reagent. Mauve with water.
Carica papaya (caricaeae)	Palmatid incision, Palmate simple leaf.	Very few trichomes observed. Uniseriate and unicellular.	Yellow with reagent. Yellow in chloroform extract.	Purple after boiling with reagent. Light blue with water.
Croton lobatus (Fuphobiaceae)	Hairy, simple trifoliate leaf; apiculate apex; serrate margin	Abundant trichomes, unicellular slender, uniseriate, highly thickened. Few compound trichomes	Yellow with reagent. Yellow in chloroform extract.	Mauve with reagent. Light violet with H_2O.
Clausena anisate (Rutaceae)	Papary texture, glabrous surface, paripinnate leaf.	Uniseriate covering trichomes with little thickening. 2) idioblasts with oil contents.	Yellow with reagent. Yellow in chloroform extract.	Black with reagent. Violet with water.

Splendens Red	Serrate margin, brittle texture acuminate apex	Idioblast containing brown pigments	Pale yellow with reagent. Pale yellow in chloroform extract	Violet with reagent. Blue with water.
Ocimum gratissimum	Pubescent, yellow flowered inflorescence, serrate margin.	1)Uniseriate covering trichomes of various sizes (3-6 celled) (2)Anomicytic stomata (3) Presence of Cicatrix	Yellow with reagent Yellow in chloroform extract.	Mauve with reagent. Light violet with water.

GAS CHROMATOGRAPHY

Gas chromatography was used to evaluate qualitatively the contents of samples of *Cannabis sativa L.* extract as obtained and compared with that from U.N. The final residues from 2.0G samples were dissolved in n-hexane as described under T.L.C. procedure. Stainless steel column (5' x 1/8") with stationary phase 3 per SE 30 on Porapak Q (80- 100 mesh) was used. The following conditions were used:

(i) Temperature: programmed temperature from 170°-230°C at the rate of 6°C/min. detector temperature:- 250°C. Injection temperature:- 270°C.

(ii) Carrier gas:- Nitrogen-flow-rate: 30ml/min.

(iii) Detector: Flame Ionization Detector: Hydrogen (30 ml/ min) Air- 300ml/ min.

(iv) Attention $8x10^{-10}$.

Anthracene (0.2% w/v in n-hexane) was used as internal standard.

RESULTS AND DISCUSSION

Macroscopic and microscopic examination of the non-Cannabis plant species revealed that none of the plants examined resembled *Cannabis sativa L.* However, some of the plants had limited resemblance to Cannabis when specific features like trichomes and stomata were considered. As shown in Table I, covering trichomes from *Crotalaria gereansis, Hibiscus micranthus* and *Clausena anisate* might confuse inexperienced worker especially when the materials are powdered. Anomocytic stomata of *Ficus religiosus* and *Ocimum gratissimum* could also be wrongly taken as those from *Cannabis sativa L.*

Application of colour spot tests to the non-cannabis plants revealed that some of them could give false weak positive tests to Ghamrawy test- *Croton lobatus, Ocimum gratissimum* and *Carica papaya*. None of the plants examined responded positively to Duquenois and extended Duquenois tests, although certain plants are known to give positive reactions (2, 6). Report in U.N. Document 1969 (2) indicated that mace oil and nutmeg oil could give false positive reactions to the extended Duquenois tests. These plant materials are not likely to be found in Cannabis wrappers but could probably interfere with Duquenois test when applied to a mouth wash from suspected Cannabis smoker.

None of the non-cannabis plant species examined with thin layer chromatography gave RF values and colour reactions of spots to spray reagents like those given by *Cannabis sativa L.* extract. Thus, the combination of colour spot tests, microscopic and the thin layer chromatographic techniques offers useful means for the identification of Cannabis samples. The benzene: diethylamine (100:1) solvent system gave the best separation of the cannabinoids. (fig 1)

(Table 1)

The U.V and I.R spectra of U.N sample and Ghana sample are as shown in Figs. 2 and 3 respectively. There was no significant difference between the two U.V. spectra. However, the I.R spectra of the two samples showed some differences especially around 1500 cm^{-1} , 1760cm^{-1}, 900cm^{-1} and 3080cm^{-1} . It has been established by other workers (7, 8) that only CBN exhibits a band at 815cm^{-1} . this is attributed to the 1, 2, 4- trisubstituted benzene ring.

Thus intensity of I.R. band at 815cm^{-1} could be an indication that the cannabis extract is from 'Overripe' sample. Hence, the cannabis extract from Ghana examined could be said to be from an 'Unripe' sample since the band at 815 cm-1 is weak. The relatively weak bands at 1130 and 1160 cm^{-1} of the resin from Ghana as compared with the UN sample confirms the earlier observation that the resin from Ghana was relatively unripe plant. The bands at 1130 and 160 cm^{-1} are attributed to the skeletal frequencies for ($CH_3)_2C$-group present in THC and CBN which replaces the CH_3 CH-CH_2 group for CBDA and CBD. The absorption band at 1265 cm^{-1} is found mostly in "unripe" Cannabis (9). This is present in the I.R. spectrum of the resin from Ghana whist absent from the U.N. sample, thus confirming the "unripe" stage of the Ghana resin.

A typical Gas Chromatography of Cannabis resin is shown in fig. 4 Retention times relative to Anthracene were; CBD:- 12 min ; THC:- 14 min. and CBN:- 15 min. The G.L.C. provides a specific method for the identification of Cannabis samples for forensic purposes. It has been found in our laboratory routine work to be particularly useful when a suspected hemp smoker chews up his wrapper when apprehended by the police.

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TABLE II

THIN LAYER CHROMATOGRAPHY OF EXTRACTS OF CANNABIS SATIVA LEAVES

Constituent		Rf Value		RX Value
	Mean	Range	Mean	Range
CBN	0.42	0.39-0.43	2.51	2.42-2.70
CBD	0.53	0.50-056	3.31	3.0-3.63
Delta 8THC	0.53	0.51-055.	3.25	3.2-3.48
Delta 9THC	0.45	0.43-0.47	2.88	2.48-3.32

N.B.- Internal Standard for Rx values; p-chlorometacresol

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