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Cannabis Sativa L.-I
Some Properties of Cannabis Sativa L. (Indian Hemp) useful in Forensic Science.

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SUMMARY

The smoking of *Cannabis Sativa L.* is now a world-wide problem and Ghana is no exception. This requires the law enforcing bodies to have detailed knowledge about the botanical and physico-chemical properties of the plant. The general characteristics, microscopic features and the chemistry of the *Cannabis sativa L.* are discussed. Identification methods used currently by law enforcing laboratories are mentioned and their limitations discussed.

INTRODUCTION

Cannabis sativa (Indian Hemp) is now found to be smuggled for smoking in many countries. Law enforcing bodies and scientists have been occupied in studying the problem being posed by the use of *Cannabis sativa L.*

Figures on police seizure of Indian Hemp (*Cannabis sativa L*) in Ghana are rising in recent years as indicated in Table I. This rise might be due to increase in efficiency of the police operations and more likely to increase in illegal cultivation of the plant. Observations made in Ghana revealed that about "25 per cent of 100 consecutive male admissions to Accra Mental Hospital were either active smokers of Indian Hemp or had at one time or other smoked it" (I).

In Ghana, the resin, "Hashish" of Cannabis is not known to be used.

Samples analysed comprise mostly of the flowering tops of the plant and occasionally, the police send whole plants uprooted from farms for identification.

The Cannabis plant is normally found cultivated in many parts of the country, especially in and around cities and towns with few incidences in Northern and Upper Regions. They are normally found grown among food crops. In remote areas, (from the cities and towns) cannabis is cultivated in small farms.

Cannabis is known by various in Ghana, Stuff, Kinshasha, Swala and Whisky-in-peppers (2). These names normally refers to the flowering tops and leaves of the plant in paper wrappers.

For Forensic purposes, the various characteristics of *Cannabis sativa L.* plant are used in the identification tests. When the plant is cultivated in a farm, the law enforcing officer need be acquainted with the general characteristic features of the *Cannabis sativa L.* in order to recognize it. knowledge of the microscopic features and the chemistry of the plant constituent is essential for the identification of the plant materials, especially when seized as powders in paper wrappers.

General Characteristics

Samples of *Cannabis sativa L.* usually consists of green leaves and flowering parts. The leaves are digitate with serrated margins, and the fruits are of achene type. Uprooted whole plants are 2ft-5ft high and mostly branched. The plant is dioecious and the male plant has a loosely branched many flowered in florescence which stands out from the leaves. The female inflorescences are compact, short and few-flowered and do not project beyond the leaves.

(see fig. I).

Microscopic Features

Microscopic features of *Cannabis sativa L.* of diagnostic importance are the trichomes. The trichomes are of two main types:- cystolithic inicellular trichomes and the grandular trichomeswith multicellular heads. The grandular trichomes may have a multicellular, multiseriate stalks. These trichomes may sometimes be sessile. The grandular trichomes secrete the Cannabis resin, although other parts of the plant,stem and roots are known to contain resin (3-7).

The stomata of *Cannabis sativa L.* are of the anomocytic type and they occur on the lower epidermis of the leaf.

Chemistry

The main components of the Cannabis resin are the Cannabinoids – Cannabidiolic Acid, Cannabidiol, Cannabinol and Tetrahydrocannabinol. Other constituents isolated from Cannabis resin include Cannabichromene, Cannabilinic acid and Cannabigerolic acid (3-6).

The tetrahydocannabinol (THC) is known to be physiologically active and the activity is attributed to 9-THC isomer (7-9).

The phytochemical interconversion of Cannabinols are shown in figure 2. From the phytochemical changes, the predominant cannabinol content in Cannabis and its resin can be used to classify the plant as Unripe, Intermediate, Ripe and Over Ripe as shown below:-

The cannabinoids exists in the fresh plant as the carboxylic acids but slowly decarboxylate on harvesting and storing. The acid forms of the THC are not physiologically very active. On smoking, the acid forms of the THC decarboxylate into active THC.

IDENTIFICATION TESTS

Botanical & Colour Tests

Microscopic examination and chemical tests (10, 11, 12) are mainly used in routine identification of Cannabis. The microscopic examination is useful in the identification of the flowering tops of the plant for forensic purposes. The colour tests indicate the presence of the Cannabis sample. None of these methods, are however, considered specific enough for unequivocal identification of cannabis. Nakamura (10) found that 64 out of 82 plant species examined had cystolithic trichomes resembling that of Cannabis.

The chemical tests commonly used for the routine analysis of Indian Hemp are the Beam, Duquenois and Ghamrawy tests. The reactions of these tests and other reagents for cannabinoids are shown in Table II. False positive reactions could be obtained when the reagents are applied to certain plant extracts (11-16). It is reported in a United Nations Document (1960) (15), that application of Beam's tests to extracts of 120 non-cannabis plant species, belonging to 28 different families gave faint positive tests on 2 plants. One plant gave a false negative response to Beam's test because of the absence of CBD in the sampleSimilar false positive response were also observed with Duquenois and Ghamrawy *tests. In 1966, Butler (17) reported that the modified Duquenois test, (colour formed with the reagent is extracted into a chloroform layer), enhanced the discriminating value of the test.

Chromatography

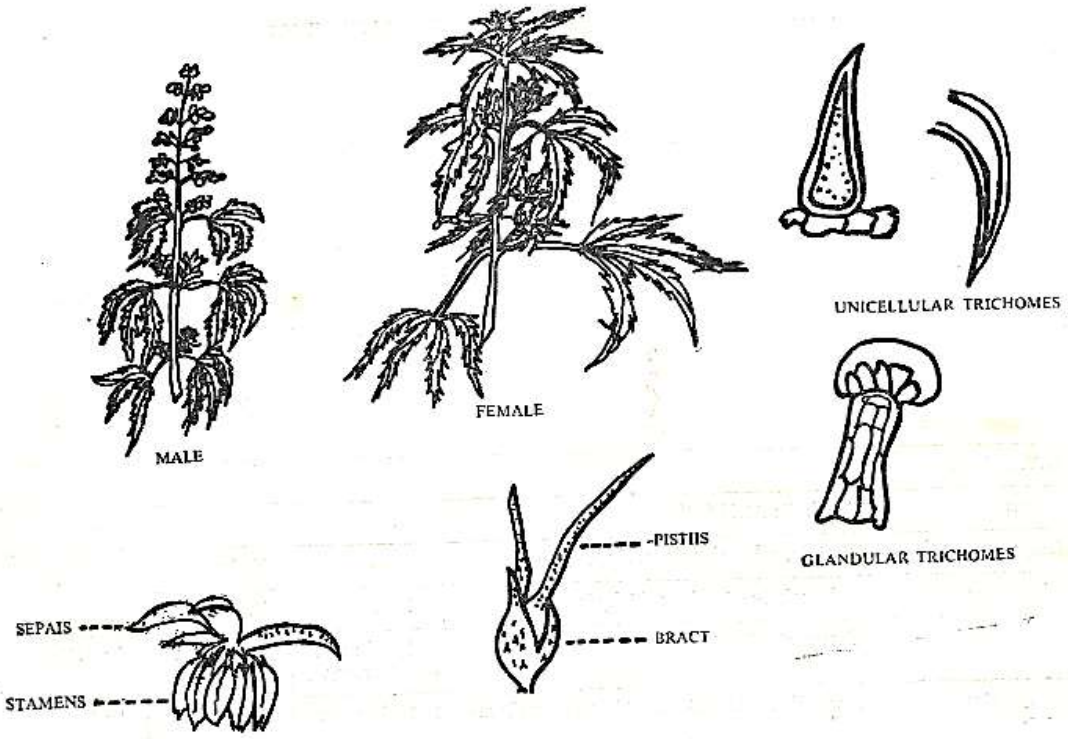
Considerable number of reports on the use of chromatography for the identification of cannabis and its resin have appeared in literature (18, 19). Column chromatography has been used for purification of the crude resin (Davis, et al, 1963) (20).

The use of paper chromatography is now rare, however, recently, Petersen and Stevens reported a 10 minute technique for the separation of CBD, THC, and CBN on Silver Nitrate-impregnated Whatman SG 81 paper (21).

The thin layer chromatography (TLC) is extensively used now for the isolation and identification of cannabinoids (Korte and Sieper, 1964 (22) (12, 23,24. 25). The technique is simple, relatively inexpensive and specific.

Gas chromatography is very useful for the identification and quantitative evaluation of Cannabis and its resin. Columns currently in use are SE-30.

FIG 1 MACROSCOPIC AND MICROSCOPIC FEATURES OF CANNABIS SATIVA (L)



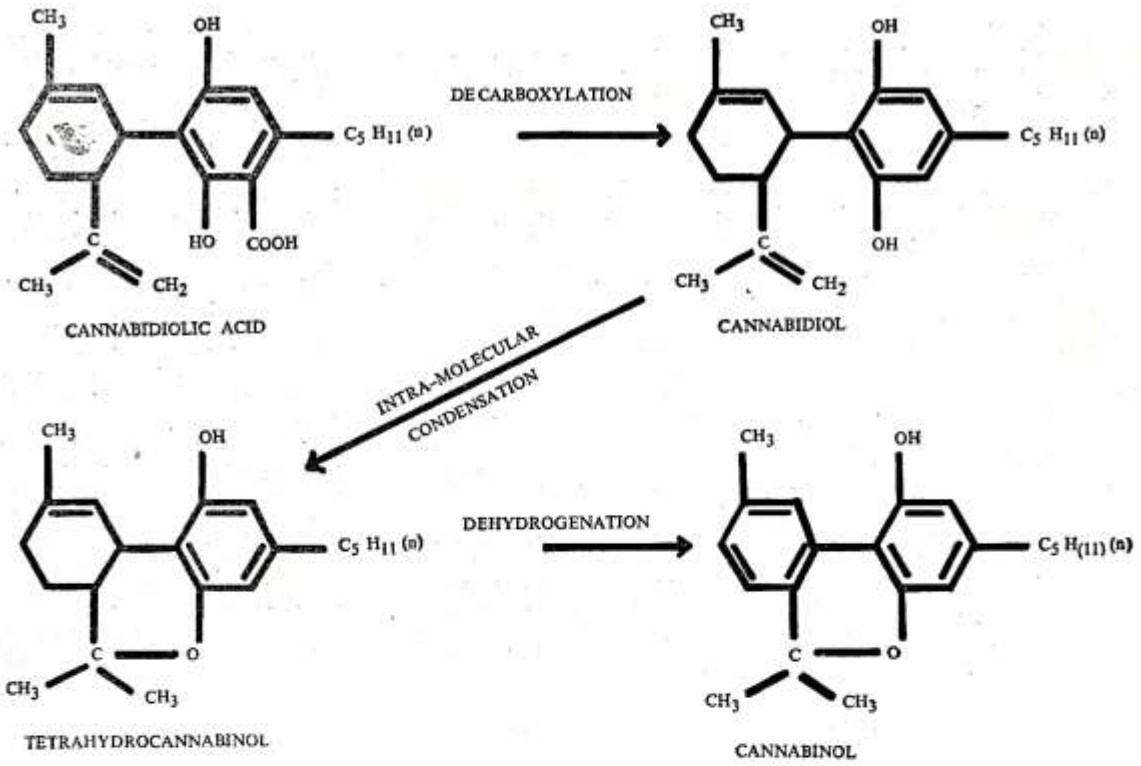


FIG. 2 PHYTOCHEMICAL INTERCONVERSION OF CANNABINOLS

TABLE I
SAMPLES OF CANNABIS SATIVA SEIZURES ANALYSED

Year	No. of Samples	Weight (KG)
1968	207	–29.00
1969	169	193.00
1970	229	— *
1971	544	529.39
1972	505	428.93

* **Figure Not available**

TABLE II
TESTS FOR DETECTING CANNABINOIDS
(SPOT TESTS AND TLC)

Name of Test	Reagent	Reactions With		
		CBD	THC	CBN
U.V. 254 mu		Dark	Dark	Dark
Beams	Alcoholic KOH (5%)	Blue	—	—
Duquenois	Vanillin, ethanol & Aceteldyde	—	Violet	Violet
Ghamrawy	p-dimethyl-amino benzaldehyde	Pink	Violet	Violet
	Tetrazotized tolidine	Red Brown (Orange	Red-Violet	Red (Mauve)
Brentamine	Fast Blue B. (di-o-anisidine-tetrazolium-chloride)	Red-Brown	Red Violet	Red

(26, 27), XE 60, (27) Carbowax 20M (29) OV-17 (20), and 2 per cent Ov-17 on chromosorb Q at 235^0C (31-33). Gas chromatography is a powerful tool in the study of the various constituents of *Cannabis sativa L.*

Spectrophotometry:

(a) *Ultra-violet spectra*

Attempts have been made to use ultra-violet spectra of cannabis extracts to differentiate between cannabinoids but with little success. (17)

(b) *Infra-Red Spectra*

Infra-red spectra of cannabis extracts have also been used to differentiate between cannabis samples of various ages and origins (de Ropp, 1960 Grlic 1965 (22) and Mechoulam Gaoni (1967((34).

(c) Mass and N.M.R. Spectroscopy have been used to elucidate the structures of various cannabinoids Claussen, 1966 (35) Schultz et al, 1958 (36), and Mechoulan and Gaoni 1964 (4).*

Ultra-violet and Infra-red spectroscopy are not normally required in the day to day analysis of Indian Hemp samples for forensic work. They serve, however, as good research tools especially when used in conjunction with Mass spectroscopy coupled with Gas Chromatography serves as powerful tool in cannabinoids in body fluids.

Conclusion

Knowledge of botanical and chemical properties of *Cannabis sativa L.* is reasonably adequate for the identification of Cannabis samples for most forensic cases. It is clear from the above discussion that more than one test need be applied to provide unequivocal proof of the identity of the plant material. Interference from plant materials other than *Cannabis sativa L.* need be evaluated so that false positive results will not be obtained for non-cannabinoid plants.

REFERENCES

(1) Amarquaye F. K., Ghana Medical Journal, **6** (4), 126 (1967)

(2) Sarpong K., An Introduction to the Identification of Cannabis in Ghana; U.N Seminar on Narcotics, 1971, Accra, Ghana.

(3) Mechoulaum, R. and Shvo, Y., Tetrahedron, **19**: 2073 (1963)

(4) Gaoni, Y. and Mechoulam R., Proc. Chem. Soc. 82 (1964).

(5) Claussen, U., von Spulak, F. and Korte, F., Tetrahedon, **22**:1477 (1966)

(6) Mechoulum, R., and Gaoni, Y., Tetrahedon letters, **21**: 1223 (1965)

(7) Joyce C.R.B and Curry S.H., The Botany and Chemistry of Cannabis; J. & A Churchill, London ; 1970. p 97-98

(8) Gaoni Y. and Mechoulaum R, Journal of the American Chemical Society **86,** 1646 (1964)

(9) Isbell H., et al Phychopharmacologia, **11**, 184 (1967).

(10) Nakamura, G.R.J of the Association of Official and Agricultural Chemists, **52,** 5 (1969)

(11) Sperling, A, Journal of Chromatographic Sciences, **10,** 268 (1972).

(12) Neumeyer, J.L and Shagoury, R.A.J. Pharm. Sci., **60** 1433 (1971).

(13) Grlic, L., Bulletin on Narcotics, **14** (3) 39 (1962).

(14) Fulton, C.C., ibid, XXII (2), 33 (1970).

(15) United Nations Secretariat Document ST/SOA/SER.S/I, (1960).

(16) Grlic, L. Bull Narcotics, **16** 29-38, (1964).

(17) United Nations Secretariat Document ST/SOA/SERS/18, (1969).

(18) Grlic, L. Bulletin on Narcotics, **14** (3), 39 (1972)

(19) Curry, A.S. Advances in Forensic and clinical Toxiocology. Cleveland, Ohio: Chemical Rubber Company Press (1972).

(20) Davis, T.W.M Farmilo C.G. et al., Anal., Chem. **35,** 751 (1963)

(21) Patterson, D.A., and Stevens, H.M.J. of Pharm. And Pharmacol. **21** 334 (1969)

(22) Korte, F. and Sieper, H.J. Chromatog **14.** 179 (1964)

(23) Caddy B. and Fish, F. ibid, **31** 584 (1967)

(24) Arainaki, 4, Tomiyasu N. et al Chem. Pharm Bull., **16,** 822 (1968).

(25) United Nations Secretariat Document, ST/SOA/SER.S/ 41 (1963)

(26) Davis T.W.M., and Farmilo C.G. and Osadchuk, Anal. Chem., **35** 751 (1963).

(27) Toffoli, F. et al., Bull. Narcotics, **20** (1) 55 (1968).

(28) Caddy B., Fish F. and Wilson W.D.C., J. Pharm. Pharmacol. **19,** 851 (1967).

(29) Heagsman, L. T., Walker E. A., and D.T. Lewis, Analyst (London) **92,** 450 (1967)

(30) Lerner, P., Bull Narcotics, **21** (3), 39 (1969).

(31) Gaoni, Y. and Mechoulam., J. Amer. Chem. Soc., **93,** 217 (1971).

(32) Ropp, R.S. de., J. Am. Pharm. Ass., **49,** (12), 756 (1960).

(33) Grlic, L., United Nations Document ST/SOA/SER.S/14, (1967).

(34) Mechoulam, R., and Gaoni, Y., Fortschrott, Chem. Org. Nat., **25** (1967).

(35) Claussen, U. et al. Tetrahedron **22,** 3535 (1966).

(36) Schultz, O. E. and Haftner, G., Tetrahedron, **21,** 1223 (1965).