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The Screening of Mushrooms Found in Trinidad to Determine the Presence of the Psychoactive Substances Psilocin and Psilocybin

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ABSTRACT
The questions of whether psilocybe type mushrooms exist in Trinidad, and if they exist, whether they contain psychoactive drugs namely psilocin and psilocybin were addressed in this paper. To answer these questions naturally-growing mushrooms were collected and classified by trained mycologists. Thin layer chromatography and high-pressure liquid chromatography techniques were employed to screen the samples for the psychoactive drugs. One of the samples found in Santa Flora, South Trinidad was identified as *Psilocybe caerulescens*. The sample contained 0.01% and 0.003% of psilocybin and psilocin respectively.

Key words: Psilocybe, psilocybin, psilocin, mushroom.

INTRODUCTION

Psilocybin and psilocin are the active ingredients present in a number of psychedelic mushrooms found in Mexico, the United States, South America, South-East Asia and Europe. These mushrooms were used in Mexican and pre-Columbian American religious ceremonies dating back to 1000 BC (Furst 1976). These two psychedelic substances are found in over 75 species in the genera: *Psilocybe, Panaeolus, Stropharia* and *Conocybe* (Shultes and Hoffman 1980). So far there have been no reported findings in literature on psychedelic mushroom distribution in the Lesser Antilles.

There have been many studies on the analysis of psilocin and psilocybin using paper chromatography (Hofmann *et al.* 1976; Benedict *et al.* 1962), thin layer chromatography (Heim *et al.* 1966; Repke *et al.* 1977), and classical liquid chromatography (Leung *et al.* 1965; Koike *et al*. 1981). Today, the analysis of hallucinogenic mushrooms usually employs high performance liquid chromatography (HPLC) using ultraviolet (UV) detection (Christiansen *et al.* 1981; Thompson 1980). Gas chromatography/mass spectroscopy (GC/MS) can be used in the analysis of the hallucinogenic mushrooms. However, in the inlet system of the gas chromatograph, thermal dephosporylation of psilocybin occur readily converting psilocybin to psilocin. Hence, it is not possible to determine if the starting material contains psilocin, psilocybin or a mixture of both drugs (Gross 2000).

This investigation utilizes thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques using ultraviolet (UV) detection method to determine the presence, if any, of psilocin and psilocybin in mushrooms found in Trinidad.

EXPERIMENTAL

Collection and Classification of Mushrooms

Mushroom specimens were collected from various geographical locations in Trinidad. Specimens were placed individually in small brown paper bags. Dr. M. Alkins-Koo, Dr. M. D. Oatham and Ms. Doreen Jodhan of the Life Sciences Unit, University of the West Indies, St. Augustine, Trinidad, classified the specimens.

Sample Preparation

The mushroom samples were allowed to dry at ambient for 3-4 days. The samples were homogenized using a mortar and pestle. The powdered material was transferred to a Petri dish and further dried to constant weight at 100ºC.

A known mass of this prepared sample was allowed to soak in 10 ml methanol (analytical grade, Fisher) for two hours. The mixture was then shaken on a Gallenkamp orbital shaker for one hour (30 rev/min).

The methanolic extracts were evaporated using a stream of hot air to near dryness (1 ml). All the methanolic extracts were stored in darkened sample bottles in a freezer.

Thin Layer Chromatography

Chromatographic separation was carried out using thin layer chromatography on activated silica gel G support on 10 cm x 10 cm glass plates (Fluka); support coating (0.25 mm) thickness contained a fluorescing additive which fluoresces at 254 nm.

The presence of psilocin and psilocybin standards (Lipomed) in methanolic sample extracts were identified by simultaneously running standards on each plate. The plates were developed to 6 cm in a development tank using two

Table 1. Results of the Classification of mushroom samples.

Sample #	Mushroom sample
1	*Leucoprinus* sp.
2	*Cookeina* sp.
3	*Schizophyllum* sp.
4	*Pleurotus* sp.
5	*Pleurotus* sp.
6	*Macroleptiota molybdites* sp.
7	*Polypore* sp.
8	*Ascomycete* sp.
9	*Auricalaria* sp.
10	*Lentinus* sp.
11	*Coprinaceae* sp.
12	*Psilocybe* sp.
13	*Lentinus* sp.
14	*Trametes* sp.
15	*Daldinia* sp.
16	*Trametes* sp.
17	*Lenzites* sp.

(2) solvent systems: Solvent System 1 (n – butanol, acetic acid, water 20:10:10 ml). Solvent System 2 (methanol, concentrated ammonia 100:1.5 ml).

Upon completion of the runs, the bands on plates were visualised using initially UV light at short wavelength (254 nm) and followed by Ehrlich reagent. Ehrlich reagent consist of 1g of p-dimethylaminobenzaldehyde in 10 ml methanol, to which is added 10 ml concentrated ortho-phosphoric acid. The colours of all the spots obtained by the specimens were noted along with their R_f values.

The lower detection limits of the psilocin and psilocybin standards were determined by serial dilutions of the respective standards and separated by chromatography until the spots were not seen.

High Pressure Liquid Chromatography

The model used was a Hewlett Packard Series 1050 liquid Chromatograph equipped with a variable wavelength UV detector, a Valco model AH 60 injection valve (10 µl by loop) and a HP series II integrator. The separation was performed on a 250 mm x 4.6 mm I.D. column, packed with sperisorb 5 µm ODS –1. Solvent A was water containing 0.3M ammonium acetate and buffered to pH 8 with ammonia, solvent B was methanol containing 0.3M ammonia acetate. The solvents were filtered using a 0.45 µm nylon membrane filter (Supelco) and degassed using helium. The mobile flow composition was 75% solvent A and 25% solvent B carried out at room temperature.

RESULTS AND DISCUSSION

Table 1 shows the results of the classification of seventeen samples collected in the study. Sample 12 was collected on a playing field at Santa Flora, Southern Trinidad. The mushroom was further classified to be the species *Psilocybe caerulescens*.

Psilocybe caerulescens has been found in Alabama, Northern Florida and Mexico, but until now has not been known to occur in Trinidad.

The TLC results indicated that sample 12 contains both psilocin and psilocybin. Table 2 shows the R_f values obtained for psilocin and psilocybin present in sample 12 using both solvent systems.

Table 2. Results of Thin Layer Chromatography on sample 12.

Solvent System	A	B
Retention time (Rf) of Psilocybin Standard	40	15
Retention time (Rf) of Psilocin Standard	70	38
Retention time (Rf) of the components of sample 12 extract	40, 70	15, 38

The lower detection limit of psilocin and psilocybin was determined to be approximately 0.05 mg/ml.

The methanolic extract of sample 12 was efficiently separated by HPLC using the 5 μm spherical C18 packing support material, isocratic water/methanol solvent system containing ammonium acetate buffer. Psilocin and psilocybin were identified. The retention times were 2.88 minutes and 4.22 minutes respectively.

Quantitation of psilocin and psilocybin was done using an internal standard calibration method, i.e. addition of 80 µg/ml internal standard solution to standard solutions of psilocin and psilocybin in the concentration range 9 - 75 µg/ml. The data was analysed using linear regression. A linear relationship of ($r>0.999$) was found at 269 nm.

The amount of psilocybin and psilocin contained in the *Psilocybe caerulescens* mushroom was found to be

0.01% and 0.003% respectively.

CONCLUSION

A mushroom collected on a playing field in South Trinidad has been identified as *Psilocybe caerulescens* and found to contain 0.01% psilocybin and 0.003% psilocin. This is the first record of a psychedelic mushroom in Trinidad.

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