Chromatography and the magic of mushrooms

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BACKGROUND

Psilocybin containing "magic" or psychedelic mushrooms affect perception, mood, behavior, and consciousness [1]. Their neuroplastic [2], immune modulation [3], and anti-inflammatory effects [4] are currently being explored in pre-clinical research and clinical trials for a range of conditions including depression, PTSD, anxiety, and substance abuse [5]. There is anecdotal evidence linking distinctive, subjective experiences with different psychedelic mushrooms and this may be due to variations in psilocybin content and effects of known, non-psychoactive tryptamines (baeocystin, aeruginascin, norpsilocin, and norbaeocystin) (Fig. 1) [6] This phenomenon, the entourage effect, may be the result of the pharmacological interaction between psilocybin and the non-psychoactive tryptamines [7]. This study aimed to establish the concentration of these tryptamines in a variety of popular psychedelic mushrooms that are associated with a variety of subjective experiences in the non-clinical setting. The correlation of the subjective experience with the tryptamine composition supports the identification of psychedelic mushroom-derived molecules with utility for the treatment of specific conditions.

METHODS

Mushroom Cultivation & Preparation - *Psilocybe subtropicalis*, *Panaeolus bisporus*, *Psilocybe tampanensis*, *Psilocybe cubensis* - "Enigma", *Psilocybe natalensis*, *Panaeolus cyanescens*, *Psilocybe cubensis* - American Mystic (PE) were cultivated from spores. *Psilocybe stunzii, Psilocybe ovoideocystidiata*, and *Psilocybe azurescens* were obtained from field collections. Spores were sourced through the mycological community and germinated on light malt extract agar in a petri dish. Agar-cultured colonies were transferred to sterilized grain jars (rye, white millet, oats). The colonized grain was transferred into pasteurized bulk substrate bags. The mushrooms were fruited and harvested after 14-28 days. The biomass was dried at 65°C for 2 hours. The fresh mushrooms were picked in the region of origin, transported overnight, and air-dried upon receipt.

Extraction - Samples of dried ground mushrooms were extracted at a ratio of 1 g of biomass to 20 mL of methanol for 24 hours at room temperature in inverted, magnetically stirred 50 mL falcon tubes, centrifuged at 2000 RPM for 15 minutes after which the supernatant was filtered through a 0.22 µm syringe filter and diluted in LC-MS grade water for injection into the UHPLC-MS/MS.

Chromatography - Tryptamines were quantified using a Waters Acquity H-Class UPLC and Xevo TQ-S Micro MS (Waters Corporation, Milford, MA, USA). UHPLC was run in reverse phase on a Waters HSS 1.8 µm T3 column (2.1 mm x 50 mm). Mobile phases were water with 0.1% formic acid for C and acetonitrile with 0.1% formic acid for D at a flow rate of 0.6 mL/min. Initial conditions were 99% C 1% D, immediately ramping to 23% D over 2.5 min before a 1 min ramp to 90% D and held isocratically for 1.5 min to flush the column, before returning to initial conditions over 0.5 min and re-equilibrating to initial conditions for 3 minutes. The MS was used in positive ion mode with a desolvation gas flow of 1000 L/hr and cone gas flow of 75 L/hr. Desolvation temperature was 600°C, source temperature was 150°C, and capillary voltage was 1 kV. Multiple reaction monitoring (MRM) was optimized (Table 1) and a standard curve was produced using standards produced by Cerilliant (Round Rock, TX, USA) (psilocybin and psilocin) and Usona (Madison, WI, USA) (norpsilocin, baeocystin, norbaeocystin, and aeruginascin). Original stock concentrations were 1 mg/mL in 50:50 water:acetonitrile or prepared from solid to 1 mg/mL in water. The standard range was from 0.01 to 1 µg/mL, diluted in LC-MS grade water.

Figure 1: *(a) Psilocybe cubensis* – Enigma, *(b) Panaeolus bisporus, (c) Psilocybe subtrolicalis,* and *(d) Psilocybe cubensis* - American Mystic PE

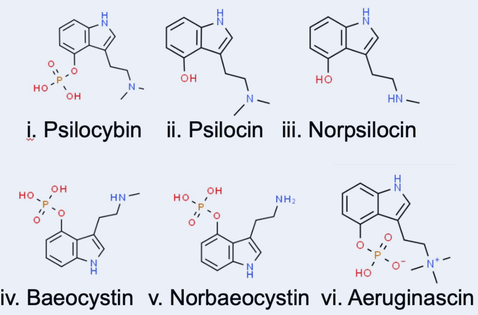

Figure 2: Tryptamines investigated in this study.

Table 1: Reactions selected and the conditions optimized for by Intellistart for each of the selected tryptamines.

Tryptamine	Parent (m/z)	Daughter (m/z)	Cone (V)	Collision Energy (V)
Psilocybin	285	115.11	50	16
		205.07		44
Psilocin	206.16	115.11	10	36
		132.16		24
Norpsilocin	191.14	160.04	18	14
		115.04		30
Baeocystin	271.05	115.04	10	44
		239.97		18
Norbaeocystin	257.03	198.95	46	8
		115.1		46
Aeruginascin	299.08	240.04	26	20
		115.1		50

Table 2: Dry wt. % average ± SD (n = 3) of analysed tryptamines for each of the 10 mushrooms extracts.

	% Dry wt.					
	Psilocybin	Psilocin	Norpsilocin	Baeocystin	Norbaeocystin	Aeruginascin
Psilocybe cubensis - Enigma	0.29 ± 0.099	0.267 ± 0.123	0.026 ± 0.027	0.011 ± 0.002	0.001 ± 0.002	0.011 ± 0.005
Psilocybe subtropicalis	1.793 ± 0.141	0.054 ± 0.055	0.008 ± 0.014	0.079 ± 0.002	0.033 ± 0.025	0.014 ± 0.005
Panaeolus bisporus	0.792 ± 0.033	0.224 ± 0.021	0.035 ± 0.018	0.022 ± 0.003	0.007 ± 0.006	0.014 ± 0.005
Psilocybe natalensis	0.346 ± 0.002	0.039 ± 0.046	0.009 ± 0.015	0.015 ± 0.004	0.004 ± 0.004	0.008 ± 0.005
Panaeolus cyanescens	0.120 ± 0.004	0.375 ± 0.026	0.030 ± 0.033	0.017 ± 0.005	0.002 ± 0.004	0.009 ± 0.008
Psilocybe tampanensis fruit	0.479 ± 0.004	0.017 ± 0.029	0.030 ± 0.027	0.014 ± 0.003	0.003 ± 0.005	0.009 ± 0.003
Psilocybe ovoideocystidiata	0.026 ± 0.002	0.054 ± 0.047	0.008 ± 0.014	0.002 ± 0.003	0.000 ± 0.000	0.009 ± 0.007
Psilocybe cubensis - PE	0.204 ± 0.004	0.102 ± 0.139	0.010 ± 0.017	0.015 ± 0.004	0.002 ± 0.002	0.004 ± 0.004
Psilocybe azurescens	0.024 ± 0.004	0.182 ± 0.086	0.008 ± 0.014	0.003 ± 0.004	0.002 ± 0.002	0.008 ± 0.007
Psilocybe stuntzii	0.088 ± 0.005	0.056 ± 0.030	0.027 ± 0.027	0.016 ± 0.002	0.001 ± 0.002	0.002 ± 0.002

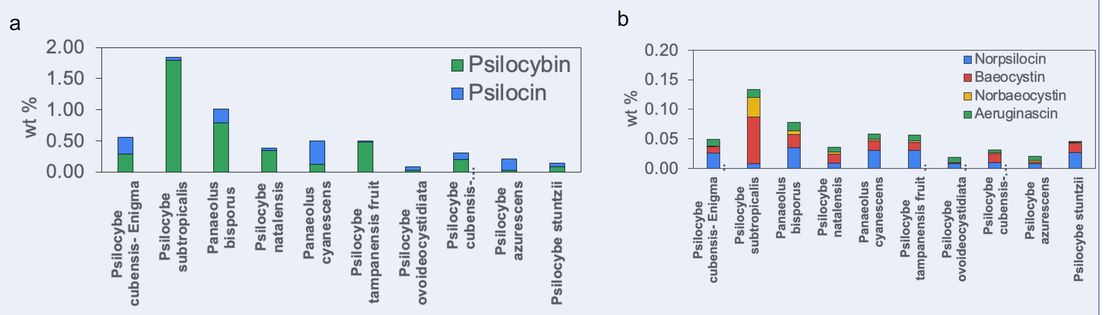

Figure 3: Wt. % average of analysed tryptamines for each of the 10 mushrooms extracts for (a) psilocybin and psilocin and (b) norpsilocin, baeocystin, norbaeocystin, and aeruginascin.

RESULTS & DISCUSSION

Psilocybin, the most abundant component, ranged from 0.03% (*Psilocybe ovoideocystidiata*) to 1.79% (*Psilocybe subtropicalis*) dry wt. % (Table 2, Fig. 2). The total content of other tryptamines was proportional to the combined psilocybin and psilocin content. These findings are consistent with the current literature [8,9]. Reports from the non-clinical setting describe different visual and physical effects from different mushrooms, which are attributed to the "entourage molecules". "Enigma", *Psilocybe subtropicalis*, and *Psilocybe azurensis*, are described as producing intense visual and physical experiences. *Psilocybe tampanesis* evokes intense physical, but minimal visual effects. *Psilocybe ovoideocystidiata* ranks lowest in terms of visual and physical effects. No obvious correlation was discerned between the non-psychoactive tryptamine content and the anecdotal descriptions of the physical effects. The differences in subjective experience after consuming different mushrooms could be attributed to the activation of pathways by other classes of molecules such as *β*-carbolines that are not extracted in methanol, or the natural variability in the composition of mushrooms and even within parts of the same mushroom [9].

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