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Integrative Review on *Cannabis sativa L.* Origin Traceability

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Abstract. Marijuana, dried and ground *Cannabis*, is the most consumed illicit drug in the world. Many undesirable and risky effects to human health are caused by its use. As *Cannabis* derived drugs and products gain popularity across the world, there has been more and more reports on mislabeled cannabinoid content of *Cannabis* plants, concentrates and edibles, as well as the number of health issues and deaths caused by their use. In spite of that, many countries have been making their laws more flexible as to marijuana purchase, sale, possession and consumption. Therefore, the development of methodologies capable of tracing the geographical origin of the seized samples, whether for quality control or forensic purposes, is increasingly necessary. Thus, our objective was to perform an integrative review on *Cannabis* traceability studies to assess the research being dedicated to this issue. We have found only 20 published scientific papers, using the databases and keywords employed, and several of these papers were published over a decade ago. We conclude that the efforts to trace *Cannabis* are not keeping up with the rapid changes and flexibilization in the

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legislation of many countries regarding legalization of the medicinal and/or recreational use of *Cannabis*.

Keywords: Sourcing; Marijuana; Geographical origin; Quality control; Forensics.

1. Introduction

Well-established traceability systems exist in many countries for a wide array of products and processes, usually aiming to verify the provenance and/or characterize products. The traceability of *Cannabis* and its derivatives, such as marijuana, medicines, concentrates, edibles and fiber, is a key approach that can be used for quality control or forensic purposes. This is particularly true considering that increasingly more countries have legalized the recreational and/or medicinal use of *Cannabis* in recent years, in spite of the fact that *Cannabis* use may cause harmful and undesired effects, such as modifications in the brain morphology[1], paranoia[2], ischaemic strokes[3], and death resulting from the association between psychiatric disturbances and *Cannabis* use[4], for instance.

Alarmingly, the legal *Cannabis* market in the state of Washington, USA, is currently dominated by high-tetrahydrocannabinol (THC) *Cannabis* flower[5]. Furthermore, in a study where 84 cannabidiol (CBD) products from different companies were tested, 42.85% of the products were underlabeled with respect to CBD, 26.19% were overlabeled and 30.95% were accurately labeled; other cannabinoids were also present but unlabeled, such as THC, that was detected in 21.43% of the products up to 6.43 mg/mL, cannabidiolic acid (up to 55.73 mg/mL) in 15.48% of the samples, and cannabigerol (up to 4.67 mg/mL) in 2.38% [6].

Over the years, legal and illegal *Cannabis* growers have been adding all sort of chemicals to their crops, from harmless products to plant growth regulators, pesticides and some fertilizers and supplements containing unlisted dangerous substances. According to a study where individual pesticides in *Cannabis* were quantified, the mean levels for most pesticides were between 1,000 and 5,000 ppb; also, 12% of the samples exceeded 10,000 ppb[7]. Pesticide contents in cannabinoid concentrates, extraction products that are very popular in the *Cannabis* market, were approximately 10 times higher than those found in the plant’s flowers. The data used for determining the limits for residual tolerance levels of pesticides on crops are based on oral ingestion exposure, although inhalation exposure through smoking can generate many kinds of pyrolysis compounds, even toxic ones, which are commonly present in the body in much higher concentrations than the orally ingested chemicals[7].

It is clear that quantitative and qualitative analyses are of great importance for quality, provenance and authenticity verification of *Cannabis* products (including *Cannabis*-based medicines) and consequently for human safety, since the increasing expansion of the trade and use of counterfeit medicines offers serious risks to public health worldwide[8]. Additionally, data resulting from these analyses can also be used for forensic purposes to identify and trace the geographical origin of *Cannabis* plants and derived drugs and products. Molecular markers, such as short tandem repeat markers, have been mostly used to recognize the presence of *Cannabis* in materials[9].

Taking all the aforementioned factors and the importance of *Cannabis* traceability into account, a research on the literature about means to achieve *Cannabis* sourcing was made.

2. Methods

The literature database consulted were SciELO, Scholar Google, ScienceDirect, and the following keywords were employed: ‘geographic origin cannabis’, ‘sourcing cannabis’, ‘fingerprinting cannabis’, ‘fingerprint cannabis’, and ‘origin cannabis drug’. This last keyword obtained a result that pointed to a new approach for tracking *Cannabis*: the entomology. Therefore, a new keyword (‘origin entomology cannabis’) was added in the research.

In this kind of review it is common to establish some criteria (e.g. year of publication, number of samples, country/region of samples) when choosing the papers to be analyzed, in order to form a homogeneous set of information and making comparisons more feasible. Many articles found in the databases using the aforementioned keywords were studies about *Cannabis* but not about its traceability, which falls off our objective with the present review, and therefore, these articles were not considered. Since the number of *Cannabis* traceability papers found in the results is quite small and having many criteria such as number of samples or year of publication, would not make the information more uniform in this case and would unnecessarily reduce greatly the number of results, therefore, the only criterion employed was that the paper must have analyzed *Cannabis* samples to trace their geographical origin. It is important to highlight that we only considered to further analysis the results from the researches performed using the aforementioned databases, no articles were added; also, other researches on the traceability of *Cannabis* may exist in addition to the ones showed in the present review, but the keywords employed were not able to find them. The information collected were (1) the paper identification: authors' names, year of publication and journal, (2) which technique was employed and its type, and (3) the study scope.

3. Results

The three categories of information collected are summarized in Table 1.

Table 1. Summary of *Cannabis* traceability studies indicating authors, year of publication, journal, technique used to trace the origin of *Cannabis*, study scope and sample type and country of origin.

Authors	Year	Journal	Technique	Scope	Sample type and country of origin	
					***Cannabis* or marijuana**	**Hashish**
10	1996	Forensic Science International	RAPD[a] - Genetic	International	Australia and Papa New Guinea	
11	2007	Forensic Science International	SNP[b] - Genetic	International	USA, Lebanon, Netherlands, Afghanistan, Swaziland, South Africa, Nepal, Jamaica, Mexico, Zimbabwe, Sierra Leone, Thailand, Uganda, Australia, Former Czechoslavakia, Italy, Former USSR, France, Former East Germany, Hungary, Japan, Turkey, China, Korea, India, Romania and	

					Canada	
12	2009	Anal Bioanal Chem	STR[c] - Genetic	National	USA	
13	2009	Electronic Journal of Biotechnology	RAPD[a] - Genetic	National	Turkey	
14	2006	Forensic Science International	$\delta^{13}C$ and $\delta^{15}N$ - Isotopic	National	Brazil	
15	2007	Journal of the Brazilian Chemical Society	HR-ICP-MS[d] - Isotopic and Analytical	National	Brazil	
16	2010	Forensic Science International	$\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and δD - Isotopic	Regional	Alaska, USA	
17	2010a	International Journal of Drug Policy	$\delta^{13}C$ and $\delta^{2}H$ – Isotopic	National	USA	
18	2010b	Science and Justice	$\delta^{13}C$ and $\delta^{2}H$ – Isotopic	National	USA	
19	2009	Journal of Forensic Science	$^{87}Sr/^{86}Sr$ – Isotopic	National	USA	
20	1973	Forensic Science	GC[e] – Analytical	International	Jamaica, South Africa, Burma and Nigeria	Pakistan, Morocco, Lebanon and Afghanistan
21	1973	Journal of Pharmaceutical Sciences	GLC[f], TLC[g], GLC/MS[h] – Analytical	International	India, Nepal, Pakistan, South Africa, Afghanistan, Brazil, Chile, Canary Islands, Czechoslovakia, Ethiopia, France, Ghana, Iran, Jamaica, Japan, Korea, Kenya, Mexico, Mauritius, Morocco, Manchuria, Nigeria, Peru, Poland, Senegal, Sierra Leone and Sudan	
22	1990	Forensic Science International	GC[e] - Analytical	International		Morocco, Lebanon, Iran, India, Pakistan and Afghanistan
23	1998	Journal of	LA-ICP-MS[i] -	Unspecified		

		Analytical Atomic Spectrometry	Analytical			
24	2007	Marijuana and the Cannabinoids (book)	GC/MS[j] - Analytical	International	Unspecified regions in the USA; California, Hawaii and Tennessee, USA; Colombia, Mexico, Jamaica, and Thailand	Afghanistan, Colombia, India, Lebanon, and Pakistan
25	2014	Australian Journal of Forensic Sciences	GC/MS[j] – Analytical	Unspecified		
26	1975	Agronomy Journal	Several physical and chemical analyses	Unspecified		
27	1980	Bulletin on narcotics	TLC[g] and physical analyses	International	Ghana, India, Jamaica, Kenya, Morocco, Nigeria, South Africa, Thailand and Zambia	India, Lebanon, Morocco, Nepal, Pakistan and Turkey
28	1986	Forensic Science Society	Microscopy, Duquenois test, TLC[g], GLC/FID[k] - Entomological	International	Burma, Thailand and Malaysia	
29	2013	Revista Brasileira de Entomologia	Magnifying glass, stereoscopic microscopy - Entomological	Unspecified		

[a] Random Amplified Polymorphic DNA; [b] Single Nucleotide Polymorphisms; [c] Short Tandem Repeat; [d] High Resolution Inductively Coupled Plasma Mass Spectrometry; [e] Gas Chromatography; [f] Gas-Liquid Chromatography; [g] Thin-layer Chromatography; [h] Gas-Liquid Chromatography coupled Mass Spectrometry; [l] Laser Ablation Inductively Coupled Plasma Mass Spectrometry; [j] Gas Chromatography coupled Mass Spectrometry; [k] Gas Liquid Chromatography-Flame Ionization Detector.

Figure 1 shows the countries of origin of the *Cannabis*, marijuana and hashish samples analyzed by the studies presented in this review (Table 1). The studies altogether comprehend countries from all continents, although for most countries only one study was performed, which is not sufficient to represent the entire continent. *Cannabis* or marijuana samples from the United States of America and from Jamaica were the most analyzed ones, followed by

South Africa, Nigeria, Morocco, Brazil, India, Thailand, Mexico, and Afghanistan; as for hashish samples, the ones from Pakistan and Lebanon were the most analyzed.

It is noticeable that in most cases, there is only one research published per year and the publications are quite rare; from 2006 onwards, the number and frequency of publications increased, although they did not surpass three publications per year (Table 1).

A comparison between the approaches above shows that genetic analyses are capable of discriminating outdoor and indoor cultivation and might even have a 100% correct classification as for the geographical origin of *Cannabis* in large territories at least, as is the case of [12]. On the other hand, some genetic analyses may fail to distinguish samples and their origin if they are clones.

The isotopic approaches may indicate indoor cultivation, the use of inorganic fertilizers, the geography, and the bedrock, which can be used to differentiate spatially distant samples grown in places with different isotopic profiles. The analytical methods (Table1) seem to be more sensitive in detecting subtle differences between samples, being adequate to be applied in the analysis of samples from close geographical origins, in contrast with the other approaches.

Finally, the entomological techniques provide unique data that are useful to complement the profile of samples and are helpful to contrast environments with very different fauna; although, since entomology is a very specialized science, its techniques are not suitable to be used in a laboratory routine for they are time consuming and require a team of trained professionals. Different approaches used to trace the geographical origin of other materials rather than *Cannabis*, therefore not discussed in the present review, should be investigated and their applicability to source *Cannabis* should be tested.

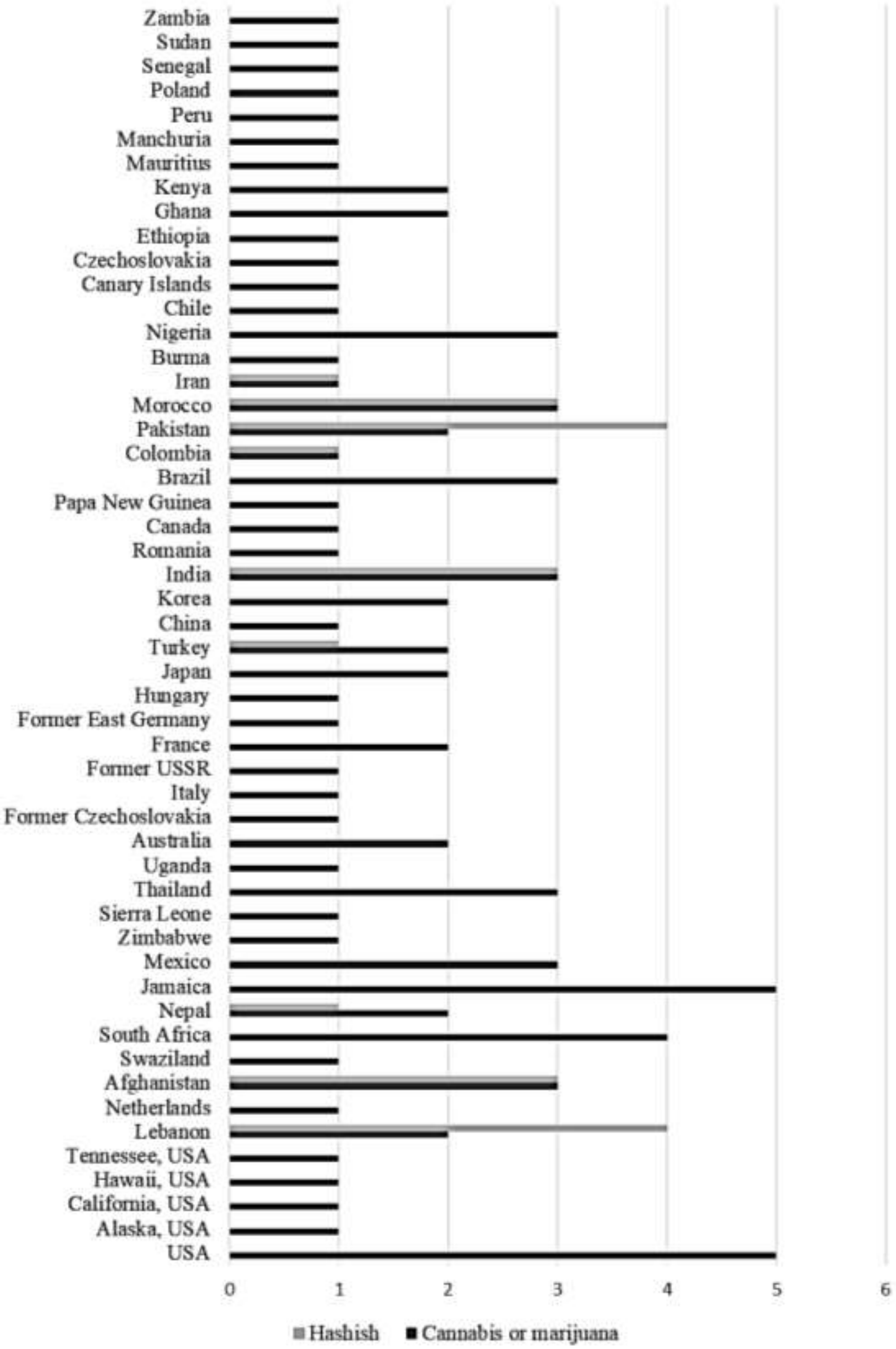

Figure 1. Number of articles analyzed according to country of origin of the *Cannabis* or marijuana, or hashish samples.

To sum up, only 20 scientific publications on *Cannabis* sourcing were found with the databases and keywords used. It is possible, although unlikely, that the design of this study diminished the quantity of articles found, since the keywords employed may have missed a few entries, but we believe this was not the case; a lack of interest from the scientific community for this subject seems to be the reason for so few studies made. It is also worth mentioning that the majority of the papers were published over a decade ago, however, there are several recent papers on the plant characterization[30,31], the discrimination between plant samples[32,33], biochemical processes[34.35], development of new techniques for cannabinoid analysis[36,37], factors that influence the plant's growth and development[38,39,40] the marijuana use impact in human health[41,42,43,44]. This points us to the conclusion that there has been progressively less scientific effort to trace the geographical origin of *Cannabis*, whether for forensic or quality purposes, over the last decades. Considering that, in general, the potency of *Cannabis* has been increasing over time in many countries[45,46], which may lead to public health problems, this lack of traceability systems development is an alarming issue.

4. Conclusion

Considering that several countries have been legalizing the medicinal and/or recreational use of *Cannabis* and *Cannabis*-based medicines and other products such as edibles and concentrates in the past few years, and that are many cases of mislabeled *Cannabis* products[6] and even death caused by their consumption[4], the number of papers published about *Cannabis* traceability is alarmingly small. The traceability of *Cannabis*, whether related to quality control or forensics, ought to be taken seriously.

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