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Research Article

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Review on Plants Fingerprinting and Tlc Fingerprinting of Two Aqueous Herbal Products

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Abstract

As herbal medicines have become commercialized, the safety, quality and efficacy of medicinal plants and herbal products have become of great concern. In view of this a review on Plant fingerprinting was carried out using systematic review and TLC fingerprinting was also carried out on two herbal products using simple Thin Layer Chromatography. The results revealed Morphological, Chemical and genetic methods are the major types studied. The High Performance Thin Layer Chromatography (HPTLC) is the most common type to chemical method utilized and most of the studies on Plants fingerprinting were carried out in Asia. Comparing the TLC profile of the two herbal preparations reveals that the two products are basically the same.

Keywords: Fingerprinting, Thin Layer Chromatography (TLC), Herbal

Introduction

Herbal preparations are finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. These are usually produced by extraction, fractionation, purification, concentration, or other physical or biological processes. Finished herbal products consist of herbal preparations made from one or more herbs which may contain excipients in addition to the active ingredient.

As commercialization of the herbal medicine increases, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. Herbal raw materials are prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic [1].

Also one of the major problems faced by herbal drug industry is unavailability of rigid quality control profile for herbal materials and their formulations. As per American Herbal Product Association, Standardization refers to the body of information and control necessary to obtain product material of reasonable consistency.

'Standardization' is a process of evaluating the quality and purity of herbal drug on the basis of various parameters like morphological, microscopical, physical, chemical & biological parameters [2].

Genetic Plant Fingerprinting

DNA fingerprints are also known as DNA typing, genetic fingerprinting and DNA profiling. DNA profiling is primarily used in plants for protection of biodiversity, identifying markers for traits, identification of gene diversity and variation etc [3].

DNA Fingerprinting Methods in plants involve the isolation of DNA from plant cell, quantification and quality assessment of isolation. The fingerprinting can further be done using polymerase chain reaction (PCR) methods such as ; Random amplification polymorphic DNA (RAPD), Inter simple sequence repeat (ISSR), Amplified fragment length polymorphism (AFLP), DNA amplification fingerprinting (DAF) and non-polymerase chain reaction methods like restriction fragment length polymerase (RFLP) [3].

Chemical Fingerprinting

Chemical fingerprinting is a process that determines the concentrations of a set of characteristic chemical substances in a plant using chemical markers [1]. It is also known as chromatographic fingerprinting; a chromatographic fingerprint of a plant or plant product is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and or chemical characteristics. This chromatographic profile should be featured by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the plant investigated [1,2,4,5].

Chromatographic fingerprinting can be carried out using techniques such as thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and other hyphenated techniques [1].

Morphological Fingerprints

Morphological markers are based on visually accessible or observable characteristics of plants such as flower color, seed shape, growth habits, and pigmentation. These marker traits are often susceptible to permanent physical changes; conversely, this allows assessment of diversity in the presence of environmental variation which cannot be neglected from the genotypic variation. These types of markers are still having advantage and they are mandatory for distinguishing the adult plants from their genetic contamination in the field, for example, spiny seeds, bristled panicle, and flower/leaf color variants [6].

Standardization of widely used herbal medicines is a key challenge facing WHO member states including Nigeria. Standardization is therefore a quality control tool whose aspects must be explored through research. Analytical methods and parameters that can be reliably used to authenticate the herbal formulations, assure the quality and profile the phytochemical composition of the products are not readily available and development of the methods is a major challenge to scientists yet a long overdue process. This study therefore addressed the development of specific quality control and standardization procedures for medicinal plants and products by exploring the literature for morphological, genetic and chemical (chromatographic) parameters in medicinal plants.

Standardization for widely used medicinal plants within West Africa and other parts of Africa are much needed in order guarantee the safety of commercialized products. This study also seek to demonstrate the utilization of TLC for differentiating two unidentified aqueous herbal preparations sold in Jos, Nigeria.

Materials and Method

Literature Review

Journal articles on Plant fingerprinting published between 2005-2020 were selected for review

Sample Collection and Description

Samples used are aqueous preparation collected from Jos North open market with claims of being used to cure erectile dysfunctions without the identity of the Plant.

Phytochemical Screening

The aqueous preparation was evaluated for Phytochemical constituents using standard procedure as discussed in Trease and Evans.

Results

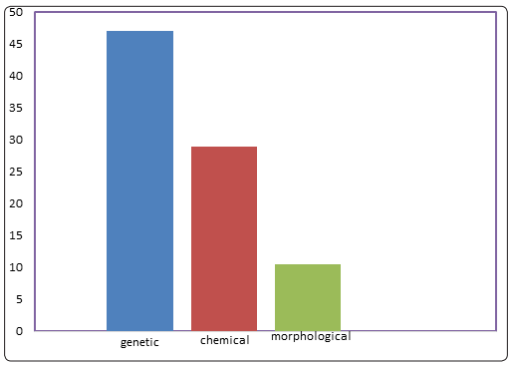

Figure1: Frequency of fingerprinting study types

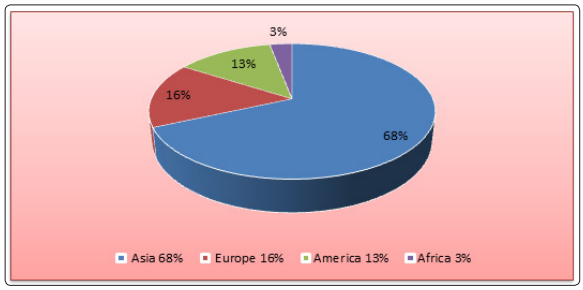

Figure 2: Regions or location of Fingerprinting Studies

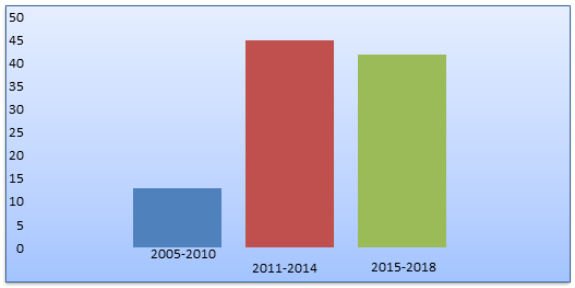

Figure 3: Years of studies on plant fingerprinting

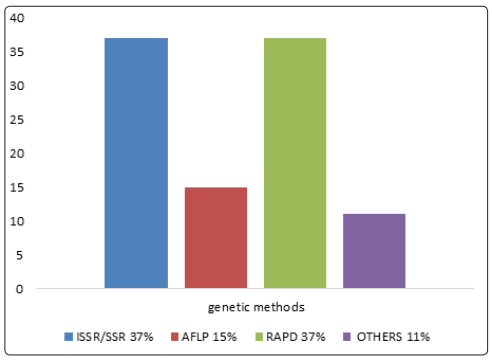

Figure 4: Genetic Fingerprints Methods

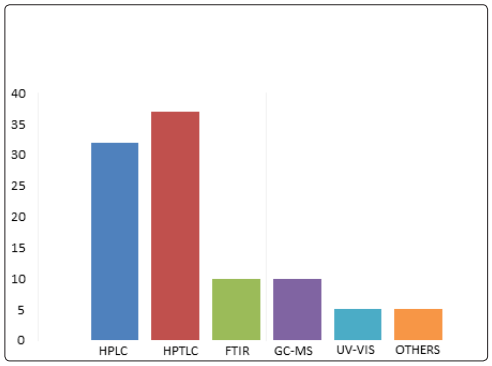

Figure 5: Chemical Fingerprinting Methods

Table 1: Phytochemical Screening

S/N	PHYTOCHEMICALS	SAMPLE D1	SAMPLE D2
1	Alkaloids	+++	++
2	Saponins	++	++
3	Tannins	+	+
4	Flavonoid	-	-
Keys: (+) Present (+++)Abundant (-)Absent			

TABLE 2: RETENTION FACTORS FOR AQUEOUS SAMPLE D1

S/N	MOBILE PHASE	WHITE LIGHT	UV-254 nm	UV-366 nm	Spraying	Inference
1	Toluene, ethyl acetate(95:5)	No spot	No spot	No Spot	No Spot	Essential oil or non-polar comp.
2	Chloroform, methanol, water(70:30:4)	No spot	1 spot RF-0.76	No spot	1spot RF-0.76	Saponin and lignans.
3	Ethyl acetate, acetic acid, formic acid, water(100:11:11:27)	No spots	No spot	No spot	No spot	Flavonoid
4	Acetonitrile, water, formic acid (30:8:2)	No spots	Absolute fluorescence	Absolute fluorescence	No spots	Amino acid
5	1-Buthanol, acetic acid, water(7:1:2)	No spot	No spot	No spot	No spot	Polar comp.

Table 3: Retention Factors for Aqueous Sample D2

S/N	MOBILE PHASE	WHITE LIGHT	UV-254 nm	UV-366 nm	Spraying	Inference
1	Toluene, ethyl acetate(95:5)	No spot	No spot	No Spot	No Spot	Essential oil or non-polar comp.
2	Chloroform, methanol, ater(70:30:4)	No spot	1 spot RF-0.73	No spot	1spot RF-0.73	Saponin and lignans.
3	Ethyl acetate, acetic acid, formic acid, water(100:11:11:27)	No spots	No spot	No spot	No spot	Flavonoid
	Acetonitrile, water, formic acid (30:8:2)	No spots	Absolute fluorescence	Absolute fluorescence	No spots	Amino acid
5	1-Buthanol, acetic acid, water(7:1:2)	No spot	No spot	No spot	No spot	Polar comp.

Discussion

The most widely genetic method explored method is inter simple sequence repeats target simple sequence repeats (microsatellites) that are abundant throughout the eukaryotic genome and evolve rapidly, but they do not require prior knowledge of DNA sequence for primer design, ISSR is one of the simplest, less expensive and widely used techniques, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction, this method is also fast and can separate closely related genotypes [7,8]. Though ISSR markers are dominant like RAPD, they are more stable and reproducible. Because of these properties ISSR markers have recently been found using extensively for finger printing, phylogenetic analysis, population structure analysis, varietal/line identification, genetic mapping, marker-assisted selection, etc.

Other commonly used genetic markers include; RFLP (or Restriction fragment length polymorphism), AFLP (Amplified fragment length polymorphism), RAPD (Random amplification of polymorphic DNA), VNTR (Variable number tandem repeat), Micro satellite polymorphism SNP (Single nucleotide polymorphism), STR (Short tandem repeat), SFP (Single feature polymorphism) [2].

Most commonly used chemical fingerprinting method used is the high performance thin layer chromatography is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC [9,10]. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions [2]. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation [11]. With this technique, authentication of various species of plant possible, as well as the evaluation of stability and consistency of their preparations from different manufactures [2]. Another commonly applied method in the review was the HPLC which is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Reversed- phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines.

Other commonly methods include; High performance liquid chromatography (HPLC), Gas chromatography (GC) and other hyphenated techniques such as Liquid chromatography- mass spectrometry (LC-MS), Liquid chromatography – nuclear magnetic resonance (LC-NMR), Gas Chromatography-Mass Spectroscopy (GC-MS), Gas Chromatography Fourier Transform Infrared spectrometry (GC-FTIR) [1].

Specific methods have been used especially chemically for plants with volatile constituents mostly the gas chromatography due to their sensitivity, stability and high efficiency. In general studies on plant fingerprinting have focused more on genetic fingerprinting, which does not have direct application in identifying the chemical markers that characterize drug chemical constituents, which implies that more needs to be done in optimizing chemical markers for medicinal plants and discovering active metabolites responsible for their medicinal properties [1].

Region or location of studies, most studies on plant fingerprinting has been carried out largely in the Asian regions of the world and other continents like Europe and America, least or less studies has been in places like Africa implying that more needs to be done in

Africa, review also indicated list of plants and metabolites found in them, among the plants species studied in the review the most common metabolites were the tannins, phenols, flavonoid, saponins and a number of primary metabolites.

TLC Fingerprints of Aqueous Herbal Products

Two herbal product aqueous in nature obtained from the open market having claims of curing erectile dysfunctions, samples D1 and D2 TLC fingerprint was carried out using different solvent Ssystems both samples gave a clear separation both under UV 256 and clearer spots with anisaldehyde spray reagent with similar retention factors when mobile phase 2 was used confirming the presence of saponins and lignans, both samples also fluoresce using mobile phase 4 on a reverse phase TLC plates indicating presence of amino acids, other mobile phases indicated no spots and no clear separation for the two samples. Phytochemical screening indicated presences of alkaloids, tannins and saponins.

Conclusion

This study provides valuable evidence or information suggesting that the concept of plant fingerprinting is an area of science that needs to be explored deeply as the usage or importance of medicinal plants cannot be over emphasized, thus justifying its importance in standardization and quality control of medicinal plants. The problem of quality assurance of medicinal plant can be resolved to a great extent with the help of genetic, chemical and morphological fingerprint analysis. TLC fingerprinting if well developed will provide a cheaper means of herbal products standardisation .The two aqueous products are basically the same base on the TLC profiles.

References

1. Kulkarni K M, Patil L S, Khanvilkar V V, Kadam V J (2014) Fingerprinting techniques in herbal standardization. Indo American Journal of Pharmaceutical Research 4: 1049-1062.
2. Nikam P H, Kareparamban J, Jadhav A, Kadam V (2012) Future Trends in Standardization of Herbal Drugs. Journal of Applied Pharmaceutical Science 2: 38-44.
3. Selvakumari E, Jenifer J, Priyadharshini S, Vinodhini R (2017) Application of DNA Fingerprinting for Plant Identification. Journal of Academia and Industrial Research 5: 149-152.
4. Djingova R, Kuleff I, Markert B (2004) Chemical fingerprinting of plants. Ecological Research. 19: 3-11.
5. Farooqui N A, Dey A, Singh G N, Easwari T S, Pandey M K (2014) Analytical Techniques In Quality Evaluation Of Herbal Drugs. Asian J. Pharm. Res 4: 112-117.
6. Verma N K, Kumar A, Deshwal R K, Tyagi V, Rana P (2013) A Current Review On Assessment Of Genetic Analysis System For Medicinal Plants With Microsatellites Ijcrr A Current Review On Assessment Of Genetic Analysis System For Medicinal Plants With Microsatellites. Int J Cur Res Rev 5: 1-11.
7. Jabbes N, Geoffriau E, Le Clerc V, Dridi B, Hannechi C (2011) Inter simple sequence repeat fingerprints for assesses genetic diversity of Tunisian garlic populations. Journal of Agricultural Science 3: 77-85.
8. Abdel-Mawgood A L (2012) 4 DNA Based Techniques for Studying Genetic Diversity 382.
9. Soni K Naved T (2010) HPTLC- Its applications in herbal drug Industry. The Pharma Review 112-117.
10. Jianga Y, David B, Tu P, Barbin Y (2010) Recent analytical approaches in quality control of traditional Chinese medicines-A review, Anal. Chim. Acta 657: 9-18.
11. Thoppil SO, Cardoza R.M, Amin PD (2011) Stability indicating HPTLC determination of Trimetazidine as bulk drug and in pharmaceutical formulations, J. Pharm. Biomed. Anal 25: 5-20.