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An Attempt to authenticate herbal tinctures using DNA Barcoding

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ABSTRACT

According to the National Medicinal Plant Board of India, of the 6000-7000 medicinal plants, 960 are traded with 178 being of high trade volume of more than 100 metric tonnes per annum. Many of them have become rare due to overexploitation. Consequently, instances of substitution/adulteration by look-alike substitutes have increased. Thus, it becomes necessary to authenticate herbal market samples, a task difficult to accomplish with the current taxonomic methods. DNA barcoding, which requires a minute amount of tissue or even its DNA for the identification of species, could be a potent tool for deciphering botanical identities of herbal samples, available in fragmented or powdered form. Tinctures are alcoholic extract of plant or animal material dissolved in ethanol. The solvent concentration ranges of which ranges from 15-90% but the most common being 20% in the case of herbal medicines.

Keywords: DNA Barcoding, adulteration, tinctures, ITS2.

I. INTRODUCTION

In recent decades, there has been a notable global resurgence in the utilization of medicinal plants for herbal-based healthcare. This resurgence has, however, come with its own set of challenges, primarily stemming from the increased consumption of medicinal plants and their derivatives (Verma and Biswas, 2020). The major issue at hand is the widespread adulteration of herbal products within the industry. Adulteration occurs when spurious and unauthorized raw materials are introduced into herbal products, severely compromising their quality and effectiveness (Poornima, 2010, Parveen et al. 2016, Mahima et al. 2022). To counter this problem, recent advances in molecular plant identification, particularly through the use of DNA barcoding (Hebert et al. 2003), have provided a robust methodology for identifying and authenticating the plant species present in herbal samples (Urumarudappa et al. 2016, Urumarudappa et al. 2020, Antil et al. 2022). This approach has proven to be reliable and accurate, ensuring that the herbal products on the market are indeed made from the correct plant materials and meet the expected standards of quality and efficacy. Consequently, the rapid and accurate identification of medicinal plants through DNA barcoding has become the key to success for the herbal industry, safeguarding both its reputation and the well-being of its consumers. In case of plants, there is no universal barcode loci that is species specific. Out of the most common barcode loci used, such as ITS, ITS2, *matK* and *rbcL* (Hollingsworth et al 2009, Singh et al. 2012), ITS2 has been chosen for the present study because of its short size (Chen et al 2010) as the DNA obtained from powdered or processed materials such as tinctures is often degraded.

A tincture is a type of herbal or medicinal extract that is typically created by dissolving plant or animal material in ethanol, which is also known as ethyl alcohol. The solvent concentration in tinctures can vary, with common ranges falling between 25% to 60%. However, in some cases, tinctures may have a much higher ethanol concentration, going as high as 90%. Tinctures are valued for their ability to effectively extract and preserve the beneficial compounds found in herbs or other organic materials, making them a popular form of herbal preparation and alternative medicine (Kazi et al. 2013). The objective of this study is to test whether ITS2 locus can be an effective barcode for authentication of tinctures of medicinal plants sold in markets of India.

II. MATERIALS AND METHODS

a. *In silico* analysis

A database of barcode quality ITS2 sequences of 20 species of the 960 medicinal plants traded in India was made by checking the availability and species-specificity of these on NCBI (National Center for Biotechnology Information (nih.gov). If the downloaded sequence exhibited 100% similarity as well as query coverage only with its own, the sequence was considered to be species-specific.

b. Experimental Analysis

Twenty tincture samples of 20 species belonging to 16 families were procured through online mode. Genomic DNA was isolated from each sample using a protocol followed by Kazi et al. 2013. The quantitative and qualitative checks of the genomic DNA was done by nanodrop biospectrometer and agarose (0.8%) gel electrophoresis. ITS2 locus of each sample was amplified by forward and reverse primers as described by Chen et al. 2010. Single band amplicons recovered from the samples were sequenced on ABI 3730 DNA sequencer. Botanical identities of the samples were determined by BLAST search of the retrieved sequences on NCBI Genbank and ITS2 database.

III. RESULTS AND DISCUSSION

a. *In silico* Analysis

Species-specific ITS2 sequences of the 20 species tested, were thus used as reference barcodes for the authentication of tinctures.

b. Experimental Analysis

In the qualitative analysis of genomic DNA, no bands were observed. Also, in the quantitative analysis, the concentration of DNA was quite low. This is because the DNA present in processed herbal materials such as, capsules, tablets or tinctures are generally present in highly degraded state, that is, the DNA is highly fragmented (Parveen et al. 2016). The amplification and sequencing success rates for the 20 tincture samples were 50% and 45%, respectively. This may be due to the fact that DNA dissolved in higher concentrations (70-100%) of ethanol is less likely to be detected whereas if dissolved in lower concentrations (25-60%) of ethanol can be traceable (Kazi et al. 2013). Most of the tincture samples purchased for this study were dissolved in 80-90% ethanol. On performing nucleotide BLAST analysis of the nine ITS2 sequences retrieved from the tinctures, ITS2 sequences of the samples, 1T and 6T depicted maximum similarity with the ITS2 sequences of *Pseudomonas aeruginosa* whereas the remaining seven were identified as *Abutilon pannosum* (Table 1). The two tinctures identified as *Pseudomonas putida* may be a case of contamination. ITS2 sequences of the remaining tincture samples showed maximum resemblance with *Abutilon pannosum*, also called as 'Kanghibunti', a medicinal plant species used to treat ulcer, urinary tract infection, diabetes, anaemia and haemorrhoids (Arbat 2012).

Table 1: List of tinctures with their barcode availability on NCBI.

S.no.	Sample ID	Name of the plant	Species-specific ITS2 availability
1.	1T	*Azadirachta indica*	SS
2.	2T	*Swertia chirayita*	SS
3.	3T	*Acorus calamus*	SS
4.	4T	*Justicia adhatoda*	SS
5.	5T	*Terminalia arjuna*	Annotated ITS2
6.	6T	*Withania somnifera*	SS
7.	7T	*Rauvolfia serpentina*	SS
8.	8T	*Alstonia scholaris*	SS
9.	9T	*Carum carvi*	SS
10.	10T	*Terminalia chebula*	Annotated ITS2
11.	11T	*Hemidesmus indicus*	SS
12.	12T	*Syzygium cumini*	SS
13.	13T	*Digitalis purpurea*	Annotated ITS2
14.	14T	*Cichorium intybus*	SS
15.	15T	*Aconitum ferox*	Annotated ITS2
16.	16T	*Achyranthes aspera*	Annotated ITS2
17.	17T	*Santalum album*	SS
18.	18T	*Juniperus communis*	SS
19.	19T	*Glycyrrhiza glabra*	SS
20.	20T	*Croton tiglium*	SS

Table 2: Identification of herbal tinctures using BLAST1 on NCBI

S.no.	Sample ID	Name of the Tincture	BLAST identification (query coverage) (% identity)
1.	1T	*Azadirachta indica*	*Psuedomonas aeruginosa (88) (96.38)*
2.	2T	*Swertia chirayita*	*Abutilon pannosum (99) (99.8)*
3.	3T	*Acorus calamus*	*Abutilon pannosum (100) (99.79)*
4.	5T	*Terminalia arjuna*	*Abutilon pannosum (100) (99.79)*
5.	6T	*Withania somnifera*	*Psuedomonas aeruginosa (87) (98.22)*
6.	7T	*Rauvolfia serpentina*	*Abutilon pannosum (100) (99.79)*
7.	8T	*Alstonia scholaris*	*Abutilon pannosum (100) (99.79)*
8.	9T	*Carum carvi*	*Abutilon pannosum (100) (99.79)*
9.	16T	*Achyranthes aspera*	*Abutilon pannosum (74) (97.44)*

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IV. CONCLUSION

DNA barcoding could be used as an efficient tool for identification of tincture samples of medicinal plants to some extent. ITS2 region, after establishing species-specificity, can be used as a standard DNA barcode to identify medicinal plants and their substitutes. Short length of ITS2 locus makes it easy for amplification from tincture samples of medicinal plants.

Better DNA isolation methods are required for isolating DNA from tinctures that are dissolved in varying ethanol concentrations. Focus should be on developing smaller barcodes, often called as 'minibarcodes' for amplifying DNA retrieved from degraded samples.

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