Annals of Phytomedicine 7(1): 131-139, 2018 Ann. Phytomed., 7(1): 131-139 (2018)
DOI: 10.21276/ap.2018.7.1.16; Print ISSN : 2278-9839 and Online ISSN : 2393-9885

Original article

Authentication of polyherbal formulations using PCR technique

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Received January 10, 2018: Revised February 27, 2018: Accepted March 10, 2018: Published online June 30, 2018

Abstract

Unprecedented growth in popularity of complementary medicines raised concerns about their quality and safety. So, there is need to develop methods for their sensitive, specific and accurate analysis. Authenticity and similarity of medicinal nutrients and strict procedures of material handling are significant features to maintain the quality of herbal preparations. Genome based methods to authenticate these plants revolutionized the authentication process. Developing DNA molecular markers by sequencing a standard zone of the DNA is the best technique to identify the adulterants as well as to authenticate the required species of medicinal plants. Application of molecular biological technique serves as one of the very consistent system for authentication of natural herbal materials. The progress of authentic analytical methods is a major challenge to scientists as natural products are optimized as drug like molecules.

This research work is based on the application of PCR technique for authentication of *Glycyrrhiza glabra* L. in its crude form as well as in final dosage form, *i.e.*, Hamdard's Joshanda, Marhaba's Joshanda, GNC herbal supplement and Joshaba Sadar (Chest tea) available in market, provided isolated DNA from dried roots of the sample was used as templates in PCR. All the products gave the desired results except one. It proved to be a complementary tool to control quality of herbal materials alone and in different marketable herbal product having *Glycyrrhiza* spp. as their active ingredient.

Key words: *Glycyrrhiza*, authentication, cGMP, chloroplast DNA, herbal medicine, polymerase chain reaction, species specific primers

1. Introduction

Pharmaceutical quality of phytopharmaceuticals is highly necessitated for the commercialization of formulations. The quality of herbal drugs must be as high as that of other medicinal preparations. In the finished herbal medicinal products, quality is difficult to achieve and is more complex than for other pharmaceuticals (WHO, 2003; Vishwa *et al*., 2016; Rais-ur-Rahman, 2017). Increased cases of poisoning are demanding to identify the risks and set standards for the safety of drugs of natural origin.

Authentication means to prove that material is true in itself, it involves many parameters like morphology, microscopy, chemical analysis and DNA fingerprinting. Previously, regulatory principles most of the times emphasized upon just quantifiable analysis of formulations (Li *et al.*, 2008). Currently, there is marked increase in natural products investigation that leads to search for new leads for drug development, biotechnological applications for pharmaceuticals, nutraceuticals, and authenticationof old-fashioned prescriptions.

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Many people consider products stamped "natural" are always safe which is not essentially true (Zhou *et al*., 2004). Passionate work of pharmacognosists played a vital role in introduction of new technologies like microscopy, TLC, GC HPLC, MS, and NMR to check the quality of pharmaceutical preparations (Carles *et al.*, 2005).

Herbal materials cause intrinsic reactions (predictable toxicity or idiosyncratic reaction, *i.e*., type A or type B) due to active medicine and extrinsic reactions (contaminants, *e.g*., microbes, pesticides or heavy metals) due to inappropriate handling or failure of GMP (Drew and Myers, 1997; Bensoussan and Myers, 1996).

Previously, the amount of herbal extract in preparation conforms to claim on label but the suppliers these days provide standardized extracts, to achieve target strength. If, the selective marker is present in high concentration or a non-standardized pharmacologically active component is present, it will not contribute appreciably to the herb activity. The plants are variable raw materials as many factors contribute to this variation (Tilton *et al.*, 2010). Such variants contribute considerably in many batches of a product to modify its efficacy. Defining the authenticity and quality of drugs of natural origin remain as much a frontier as it is a vital science in guaranteeing clinical use of herbal drugs (Zhao *et al*., 2006).

It is essential to investigate current market of drugs of natural origin and find reasons for various confusions not solved by

conventional methods of authentication (Choo *et al.*, 2009). Real and consistent identification of herbal materials can only be attained by exercising advance molecular genetic tools (Ali *et al.*, 2014; Ganie *et al.*, 2015; Heuble, 2010; Hollingsworth *et al*., 2011; Shucher and Carles, 2008; Zhao *et al*., 2006).

Advance practice of genomic authentication has extended the scope of pharmacognostical science (Heubl, 2010) to molecular level, so it should be added as a parameter to control quality of drugs of natural origin (Huang, 2012).

A genetic marker is a measurable character that can detect variation. Molecular markers are based on biochemical macromolecule deoxyribonucleic acid (DNA). These have revolutionized research activities in biological science. DNA based molecular markers act as efficient and versatile tool and complement classical strategies for the genetic analysis. Since, the development of DNA based molecular markers, they are constantly modified to increase their use and the automation of the process of genome analysis. The genetic markers present in plants are playing their role in genome based procedures, *e.g*., SNP (Hyten *et al.*, 2010; Myles *et al*., 2010; Arai-Kichise *et al*., 2011; Barbazuk and Schnable, 2011; Marroni *et al.*, 2011) and micro-satellites (SSR) (Csencsics *et al*., 2010; Buehler *et al.*, 2011; Delmas *et al*., 2011; Gardner *et al*., 2011; Michalczyk *et al*., 2011; Appleby *et al.*, 2009). The application of sequence independent array technology to determine genomic polymorphism, PCR for the amplification of a minute locus of genomic DNA (CBOL, 2009) and RT-PCR to quantify amplified markers is exclusive progress (Kumar, 2010). As current DNA based techniques, only show the presence of a target DNA. Simply verifying the presence of a species does not give any indication of its quantity in the mixture. It is a limitation of PCR based DNA identification technique.

In this research PCR technique is applied on the species identification of *G. glabra* targeting one gene loci. One standard sample was obtained from Botanical garden of GC University, Lahore and was compared with licorice, sweet wood present in Pakistan's most famous herbal remedy "Johar Joshanda", Joshaba Sadar (Chest tea) and capsules of licorice root used for digestive problems (Le Roy *et al*., 2002). It is necessary to discern the false herb from the genuine herb in terms of their origins, distribution areas. Only in this way quality can be guaranteed (Cao *et al*., 1996; Cao *et al*., 1997; Cao *et al*.,2001).

Pakistan is the world's sixth largest country with an area of 796095 km^2 and population in millions. However , more than 70 millions live in urban areas and around 110 millions in rural areas (NIPS, 2016).

Due to this large number of population in rural areas with low daily income complementary medicine is taken as first line of treatment. As a direct result of this, the Unani and Homeopathic system, are practiced quite heavily, so there is need to develop a relationship between conventional allopathic physicians and complementary alternative medicine (Flink, 2002).

G. glabra, sweet wood commonly known as 'liquorice' is a potent and unique medicinal herb (Wang *et al.*, 2013), in Pakistan commonly known as "mulethi".

G. glabra is a perennial herb. Liquorice root consists of the dried unpeeled or peeled, whole or cut root of *G. glabra* and/or *Glycyrrhiza inflata* Bat. and/or *Glycyrrhiza uralensis* Fisch. (Dried drug) (European Pharmacopoeia, 2010).

Highly effective therapeutic agent of cough preparations, glycyrrhizin is important phytochemical present in liquorice. Besides this, unique herb liquorice is therapeutically active against other diseases like ulcer, constipation, inflammation **(**Armanini *et al*., 2002) and atopic dermatitis **(**Saeedi *et al.*, 2003; BHP, 1990; WHO, 1999).

2. Materials and Methods

Dried root samples of *G. glabra* (fresh sample, intact form, powdered form, crushed form, and capsule) for this study were acquired from botanical garden of GC University, local Dawakhana (Vohora, 1986). and Pharmacy store or local market of Lahore, Pakistan. The material was identified by a Professor of Taxonomy, Department of Botany and specimen with No. GC. Herb. Bot. 3780 has been deposited in the department for future reference. In order to shelter the producer's identity, sample formulations are named as fresh sample, intact form, powdered form, crushed form, and capsule.

Table 1: Dosage forms of the herbal formulations

Formulation	Targeted specie	Part used	Quantity	Dosage form	Therapeutic uses
Standard Material/Botanical garden of GC University	*G. glabra* (Mulethi)	Dried root and rhizome	3.00 g	Raw form	Anti-tussive expectorant
Hamdard's Joshanda	*G. glabra* (Mulethi)	Dried rhizome	3.00 g	Intact form	Natural cure for cough and cold relieve
Marhaba Joshanda	*G. glabra* (Mulethi)	Dried rhizome	3.00 g	Intact form	Natural cure for cough and cold relieve
Joshaba Sadar (Chest tea)	*G. glabra* (Mulethi)	Dried rhizome	1.80 g / 12.00 g	Crushed form	Chest tea
GNC herbal plus whole herb	*G. glabra* (Mulethi)	Licorice root herbal supplement	450 mg/ capsule	Powdered	Traditional digestive herb

2.1 Extraction of DNA

CTAB/chloroform-isoamyl alcohol DNA extraction protocol was used as mentioned by Cullings (1992), Doyle (1987), Doyle and Dickson (1987). The CTAB buffer was prepared by mixing CTAB, PVP and β-mercaptoethanol with the ratios as given below.

Table 2: Composition of different volumes of CTAB buffer

S.No.	CTAB	PVP-40	β-merc	Tris-Cl	EDTA	NaCl	H_2O
1	0.5 ml	0.02 g	2.5 µl	0.5 ml	0.25 ml	2 ml	1.15 ml
2	5 ml	0.2 g	25 µl	5 ml	2.5 ml	20 ml	11.5 ml
3	20 ml	0.8 g	100 µl	20 ml	10 ml	80 ml	46 ml

CTAB (cetyltrimethylammonium bromide), PVP (polyvinyl-pyrrolidone), β-merc (β-*Mercaptoethanol)*, Tris (tris (hydroxy-methyl) aminomethane) Cl, EDTA (pthylenediaminetetraacetic acid), NaCl (sodium chloride)

Applying modified CTAB method, fine powder sample (40-50 mg) was mixed in extraction buffer (as mentioned in Table 2) and incubated in water bath at 65°C for 20 min. 700 µl of chloroform-isoamyl alcohol (24:1) was mixed with sample and vortexed well to form an emulsion. Eppendorf tubes containing emulsion were centrifuged at 15000 rpm for 30 min., Pre-chilled (900 µl) isopropanol was added to the supernatant and incubated for 1 h. at RT followed by centrifugation at 14000 rpm for 30 min, so DNA can be precipitated. Treatment with RNaseA (10 mg/ml) was done to eliminate RNA from crude DNA.The quality and quantity of DNA can be confirmed by using NanoDrop™ by noticing the absorbance ratio at A260/280 (Porebski, *et al.,* 1997).

PCR reaction mixture containing 10 x reaction buffer 1.5 µl, dNTPs 0.3 µl, 50 mM $MgCl_2$ 0.45 µl, forward primer (ACGGGAATTGAAC CCGCGCA) 0.5 µl, reverse primer (CATATGACTTCACAATGT AAAATC) 0.5 µl, *Taq* polymerase 0.14 µl, water and template was used with following PCR cycling parameters: initial denaturation step of 30 sec. at 98°C; denaturation of 20 sec. at 98°C, annealing for 30 sec at 57°C and extension for 30 sec. at 72°C; and final extension period of 10 min at 72°C and with 35 times repeated cycles.

The entire process was optimized after several runs of PCR and gradient PCR for the right Tm of the primer range from 55ºC to 60ºC and that is found to be 57ºC and reactions without template DNA were utilized as controls.

Table 3: Primer sequence for *G. glabra*

Gene	Forward primer	Reverse primer	Product size (bp)	Annealing Temp. (Tm)°C
trnH-trnK1intergenic spacer region (Salimet al., 2007) cpDNA	ACGGGAATTGA ACCCGCGCA	CATATGACTT CACAATGTAA AATC	Around ± 220	57.3

+base pairs (bp)

Typically, PCR is a process of runs of 35 times repeated cycles involving 2-3 different stages of temperature.

The PCR products were electrophoresed on 1.8% (w/v) agarose gel, 0.5x Tea buffer and ethidium bromide stain at 80 V for ~30 min (Joseph Sambrook and Russel, 2001; Sambrook *et al.*, 1989; Sambrook and Russell, 2001).

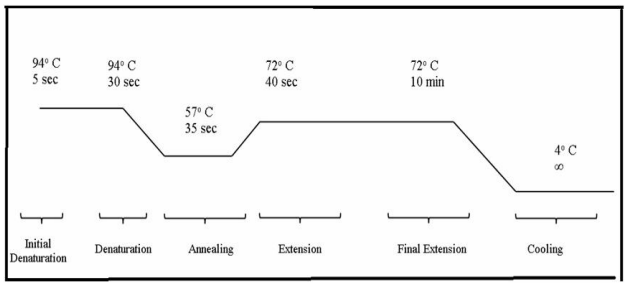

Figure 1: Polymerase chain reaction thermal cycle stages.

80	Minimum	Maximum
	30	45 min

Table 4: Voltage and time for the gel electrophoresis.

Electronic system called gel documentation system (Dolphin gel doc system) was used to visualize amplified DNA bands under UV light and the results were recorded in system for further analysis.

3. Results

This study determined the presence of *G. glabra*in herbal drugs by using PCR based molecular technique. Agarose separates DNA molecules of the same size which appear in the form of a clear band and the size can be determined by comparing with a DNA ladder and analyzed using Illuminator with a gel documentation system and GrabIt™ software.

Sample A: Standard sample of Botanical garden of GCU

Electrophoresis separated DNA fragments by size and it was found to be around ± 220 bp and band is shown in Figure 2 and was compared with other samples.

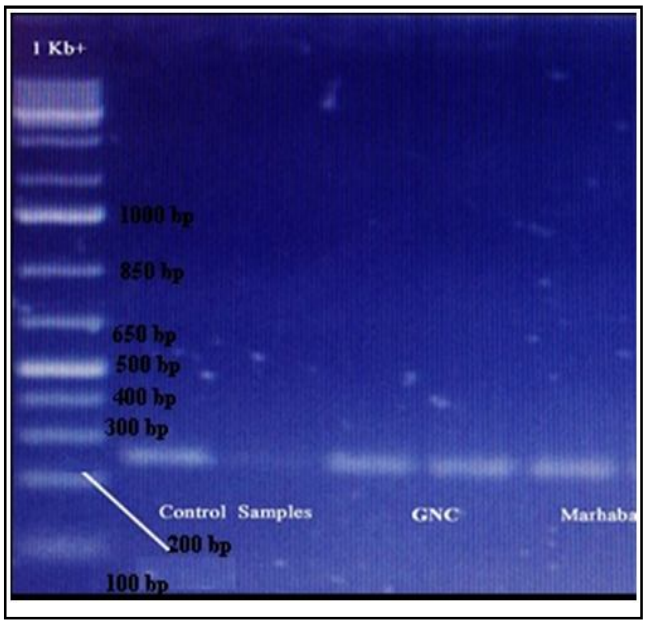

Figure 2: Standard sample, GNC herbal plus, Marhaba Johor Joshanda.

Sample B: Licorice root herbal supplement (GNC)

A full band of product size around ± 220 bp on agarose gel electrophoresis was observed under UV light shown in Figure 3.

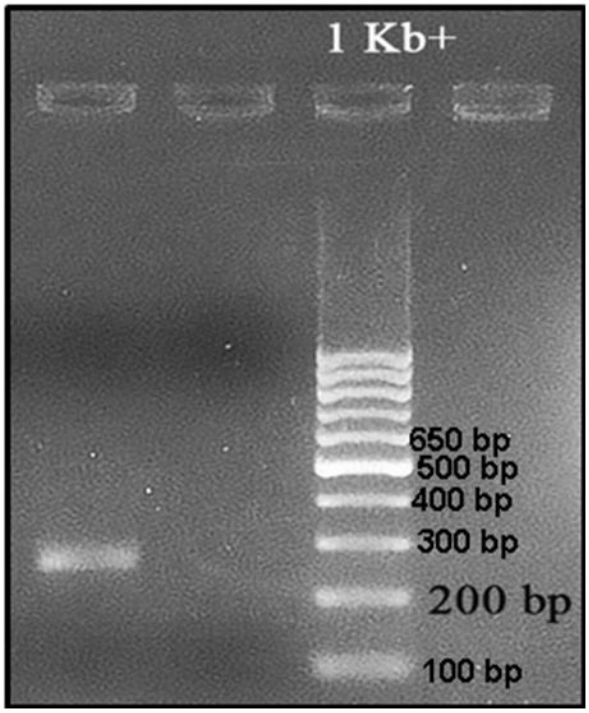

Figure 3: GNC herbal plus.

Sample c: Marhaba Joshanda (Marhaba Laboratories)

Product size of around ± 220 bp against DNA ladder of known fragments when visualized under UV light as shown in Figure 4.

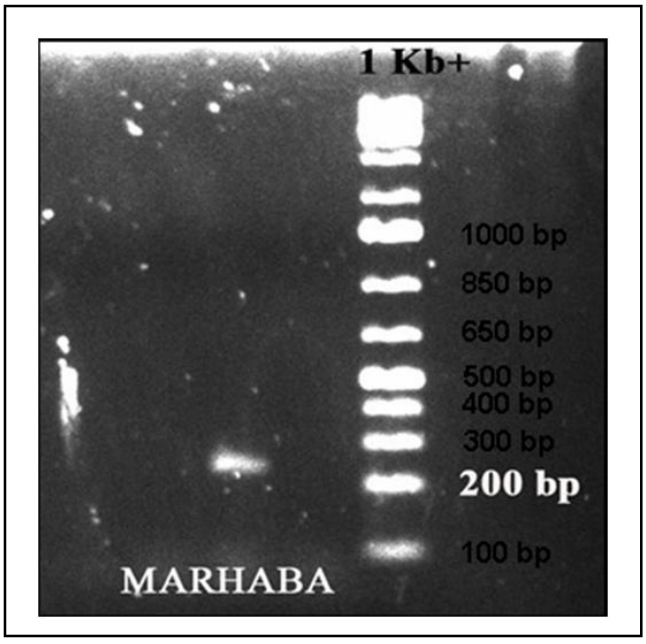

Figure 4: Marhaba's Joshanda.

Sample D: Hamdard's Joshanda (Hamdard's Laboratories)

Under UV light appearance of band is observed against DNA ladder and product size is measured that is found to be around ± 220 bp as shown in Figure 5.

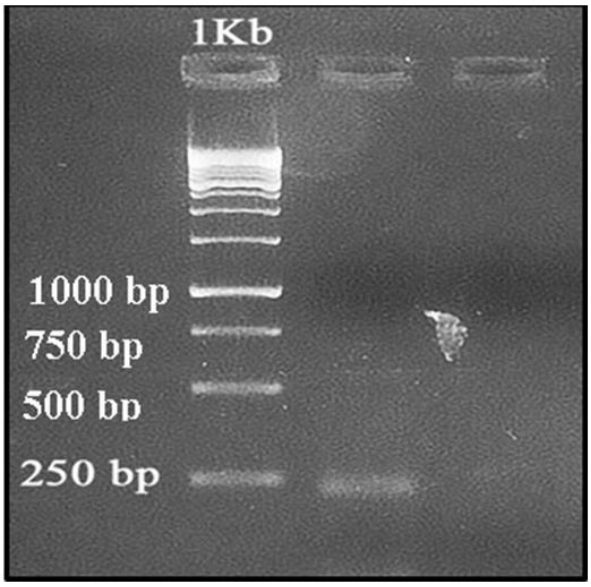

Figure 5: Hamdard's Joshanda.

Sample E: Joshaba Sadar (Chest tea)

The DNA extracted from this sample could not be identified with this primer, the sample is labeled as a mixture of plant materials.

There are various reasons for this, the amplified DNA might have come either from another specie or it might have been degraded (due to DNA shearing), and more than one herb increases the complexity and difficulty in analysis (Carr and Sean, 2012). Chemical analysis of such a mixed preparation containing many compounds from different species, produces a highly complex profile. However, other methods, *e.g.*, the multiplex plant ID system could potentially identify all different species present in the preparation and also test for species that are known to be used as adulterants (Caroline Howard *et al.*, 2012) and sequencing can be used to identify DNA of concerned species from highly degraded DNA.

4. Discussion

With the population growth in the developing world and increasing interest in the industrialized nations has greatly expanded the demand for medicinal plants themselves and the products derived from them. Even, the growth of the pharmaceutical industry and the unceasing development of new and more effective synthetic and biological medicinal products have not diminished the importance of medicinal plants in many societies. Herbs are no doubt precious natural source and economic crops due to their role in healthcare, *i.e.*, traditional and modern medicine.

In Pakistan, the total turnover of crude drugs is worth around in millions, although its cultivation is intermittent and grown as minor crops. Pakistan does not meet its total requirements and has to import major part of herbal materials from Nepal, Sri Lanka, India,

China, Kenya and Uganda. Pakistan annually spends millions on the import of raw herbal materials and medicinal/pharmaceutical products. So, it is required by higher authorities to monitor that how much plant material is collected /cultivated and actual quantity reaches to the market after passing through the process of refinements. The herbs are sold either dried or fresh to the local traders who sell them to wholesalers and ultimately to pharmaceutical concerns or exporters. Pakistan's major exporters of medicinal herbs and herbal medicines are Hamdard Laboratories (Pvt.) Ltd; Herbasian (Pvt.) Ltd; Hashmi Surma; Qarshi Industries (Pvt.) Ltd; Tayyebi Dawakana; Marhaba and Medics Laboratories out of them Hamdard Laboratories is one of the leading stake holders.

Table 5: DNA quantification of *G. glabra* by spectrophotometer

Samples	Manufacturers	Label claim (*G. glabra*)	Product description	Concentration ng/µl
A Standard crude sample	Botanical garden of GCU Lahore	**N/A**	Crude state	58.6
B Licorice root herbal supplement	GNC herbal plus whole herb	450 mg whole herb per capsule	Filled capsules with dried ground material	119.9
C Marhaba Joshanda	Marhaba Laboratories (Pvt.) Ltd	5 gm of pure herb per packet	Washed in crude form	67.6
D Hamdard's Joshanda	Hamdard's Laboratories (Pvt.) Ltd	5 gm of standardized herb	Washed in crude state	107.5
E Joshaba Sadar chest tea	(Hamdard laboratories (Pvt.) Ltd	1.8 gm/12 gm ground state mixed with other herbs	Ground mixed materials of all the herbs present in formulation	100 highly degraded and fragmented DNA was detected

Efforts should be made to explore the herbal product development. As safety is imperative, though herbal product's use has been embraced as complementary and alternative medicines but safety is conceded due to lack of suitable quality procedures, inappropriate labeling, adulteration, substitution and lack of patient data which has resulted in decline of faith in crude drug promotion (Dubey *et al.*, 2004). Safety of natural origin medicines has become a major concern to both national health authorities and the general public (Ekor, 2013). Therefore, regulatory policies on herbal medicines need to be standardized and strengthened on a global scale and with this adequate training regarding use of herbal products with prescription or non-prescription medicine to conform to standards of safety, quality, and efficacy are to be considered for public health. Regulations and WHO support is helping in the preparation of model guidelines in this field and the herbal medicines and phytonutrients or nutraceuticals continues to grow worldwide.

DNA based molecular markers, however, are important tool in quality assurance of medicinal plant species in the plant kingdom. This proved to be an additional analytical diagnostic tool for natural products from harvest to final commercial product. Recent era is the use of molecular biological techniques that helps in identification of species (Kondo, 2007). Even this tool is highly effective for authentication of specie specific constituents, *e.g*., three kinds glycoumarin, glabridin and licochalconeA (*G. uralensis*, *G. glabra*, *G. inflata*) but with the help of this technology, we can distinguish their kinds. In this study, species identification of licorice was done present in polyherbal formulation, because when licorice is used for medicinal purposes, the licorice species should be selected with recognition of even its constituent properties, *e.g*., *G. uralensis* mostly used as tonic, an antipyretic, treat acne, pimples, antidote while *G. glabra* to cure influenza, uterine complaints, *etc.* (Asha Roshan *et al.*, 2012) and it provides vital and absolute mean of authentication.

These days, herbal medicines are present in all forms, *i.e*., simple as well as in highly processed form mostly marketed as supplements for health augmentation but there are still concerns to be raised about the authenticity and efficacy of herbal medicines. Different techniques of molecular biotechnology can be utilized for the identification/authentication of herbal materials, in well-refined dosage forms such as tablets, capsules, troches, injections and oral liquids. Usually, PCR based method is applied to audit highly processed medicines and also assist in monitoring their quality and legality (Chong *et al*., 2005). A lot of work has been completed by researchers on Chinese highly processed patent drugs consisting of different kinds of plants or animals (Rong Chen *et al*., 2012). In this research, *G. glabra* is chosen to test the reliability of the quality control using PCR technique which is a widely growing herb in Pakistan due to its high medicinal value such as antioxidant, antibacterial, digestive, anti-tussive, cough and cold treatment, anti-inflammatory activities due to various constituents, *e.g*., flavonoids, coumarins, chalcons, glycyrrhetinic acid. Even synthetic antibiotic cannot withstand heat treatment at 100^{O}C but *G. glabra* after boiling at 100^{O}C shows antibiotic activity against *Staphylococus aureus.* Its roots, rhizomes and whole herb are used in different herbal medicines. There are different dosage forms available in market

containing *G. glabra*, capsules (root powder by GNC herbal plus), sachet (entire roots by Hamdard's Joshanda and Marhaba's Joshanda) and cough syrups, *e.g*., Inflo syrup, Myocid syrup, Ivy syrup (Herbasian Laboratories Pakistan (PVT.) Ltd).

G. glabra have different varieties depending on the habitat, *i.e*., *G. glabra* var. typica (Spanish liquorice) having purplish blue colored Papilionaceous flowers and large number of stolons, *G. glabra* var. glandulifera (Russian liquorice) which is a big root stock along with a number of elongated roots without stolons. *G. glabra* var. violacea (Persian liquorice) has violet flowers, various hybrids and a major adulterant, *Abrus precatorious*. The sweetish and mucilaginous roots of *Abrus precatorious* are similar in morphology with roots of *G. glabra*. On the basis of morphology and histology, it is very difficult to distinguish them. Herb authentication has presented a great challenge for people using them for medical purposes. Wrong herb usage can worsen the condition and even cause death (Bijoya Chatterjee *et al*., 2015; Jaya Preethi *et al*., 2014). So, intentional and unintentional adulteration need to be avoided. The objective of this research was to authenticate the presence of *G. glabra* in different formulations available in market, *i.e*., capsules (GNC Herbal Plus), sachet (Marhaba Joshanda, Hamdard's Joshanda) and (Joshaba Sadar Chest tea) and crude *G. glabra* (as standard by Herbasian Laboratories (Pvt.) Ltd). Each capsule of licorice root herbal supplement by GNC herbal plus contains 450 mg root powder per capsule, Marhaba's Johar Joshanda contains 5 gm of root per packet, Hamdard's Johar Joshanda contains 5 g of root herb per sachet and Joshaba Sadar Chest tea (OTC) contains 1.8 g / 12 g of *G. glabra*.

With the help of PCR, it becomes possible to detect DNA of specific herb in processed drugs which has undergone industrial extraction, filtration and sterilization. There is no doubt about the discriminatory power of DNA among different species (Del Serrone *et al*., 2007). To carry out this work, *G. glabra* pure sample was authenticated from Botany Department, GC University to compare with the commercial samples. DNA extraction protocol depends on the types of tissues, *e.g*., roots, seeds, leaves, *etc*., and concentration of DNA required. Using modified CTAB method by Salim *et al*. (2009) resulted in moderately degraded genomic DNA in concentrations detectable by agarose gel electrophoresis but was without purification and viscous which can't be amplified by PCR. There is a possibility the presence of pigments prohibit amplification by PCR. Later, in order to obtain the DNA of high quality and purity independent of secondary metabolites was extracted using Cullings (1992), Doyle (1987), Doyle and Dickson (1987) methods and resulted in successful PCR amplification, using specific primers. This work helped to attain a protocol for detecting all commercial licorice root/rhizome samples to species. Herbal samples used in this analysis were containing licorice root whole (Hamdard Joshanda, Marhaba Joshanda), chopped-up/comminuted (Joshaba Sadar, Chest tea) or powdered form (herbal supplement by GNC herbal plus). Polymerized agarose acts as a sieve and separates the DNA by size. Around ± 220 bp size was determined with specific primer *trnH-trnK1* in crude sample by crude authenticated standard sample of GCU, Marhaba Joshanda, Hamdard Joshanda and herbal supplement by GNC herbal plus confirmed the presence of *G. glabra* in the marketed formulations. Amusingly, among five investigated samples, Joshaba Sadar (Chest tea) was found to contradict the specie labeled by the manufacturer, signs of admixture were located. This is the basis of failure in crude drug marketing that results in altered efficacy (Dubey *et al*., 2004).

This technique proved to have specifically authenticated medicinal herb and have extensive applications in quality control of raw materials. The PCR based method can be carried out in any laboratory using unknown genomic DNA from any developmental stage and any part of herb (Kethidi *et al*., 2003). It was found that repeated thaw cycles did not affect the quality of DNA. These results obtained are highly reproducible and DNA extracted was also very stable even after keeping DNA at room temperature for one week produced the desired results (Schuster and Appleby, 1983).

Genome sizes in plants are remarkably diverse, with 2350 fold range from 63-149,000 Mb. Genome size is specificity of a species due to characteristic number of base pairs in its nuclei. Bennetlt and Leithch (2011) state the average genome size of angiosperm 5800 Mb. The advantage of this approach is rapidity, simplicity, reproducibility, discriminatory power and user friendliness. Major drawbacks of this approach are risk of contamination, the requirement of sequence information for the design of primers and it does not provide information about the presence of specie which is not the target of primer. An additional disadvantage is the need of performing electrophoresis to confirm amplification success of target sequences. However, by using nested PCR, the specificity and sensitivity of PCR can be enhanced. Real-time PCR is also known as quantitative PCR that has the capability to quantify the starting amount of specific DNA sequence in the sample utilizing flourcent technology.

Benefiting from PCR technique, DNA markers have become a powerful tool for authentication of plant, animal, fungal and botanical spp. (Kaplan *et al*., 2004). Contrary to chemical fingerprinting which are strongly influenced by the age of sample, physiological conditions, environmental factors and can be recovered from any state (fresh, dried , processed) besides, marker molecule is not tissue specific.

There is need to increase consumer confidence in herbal medicine. Along with traditional, this new innovative automated assays and specific tools DNA analysis are emerging and contribute to the next generation of technologies, *e.g*., mini-sequencing nano-scale DNA sequence, next generation sequencing NGS (Lerner and Fleischer, 2010). Further, extremely promising development is nanopore technology for identification of DNA bases and APEX (enzymatic genotyping to analyze variations of genome in single multiplexed reaction), OLA to detect highlypolymorphic gene. These will provide complete genome analysis, high multiplexing capacity and future taxon identification.

Recently, massive parallel sequencing technology produces millions of DNA sequence reads, *i.e*., giga base pairs in single run. These revolutionized research in medicine. However, there is no perfect DNA-typing method and the choice of a particular technique is often a compromise that depends on a number of factors, including: resources of the laboratory, financial constraints, available expertise, time limitations and more importantly,the research question pursued. All factors should be scrutinized to avoid an inappropriate choice.

5. Conclusion

Molecular techniques can be utilized to maintain the quality of herbal materials to ensure safety and efficacy. The developed

method is one of the effort to give guidelines for the identification and authentication of *G. glabra* as raw material as ingredient in different dosage forms. By using PCR technique, we can develop the methods for the authentication of other herbal materials. Scientific validation will prove the efficacy of nutraceuticals and worldwide acceptance of drugs obtained from natural sources that are with least side effects will grow. Biomolecules synthesized in living cells possess versatile therapeutic values and validation of marketed products using these biomolecules will enhance customers' confidence in them. Combining PCR with other advanced molecular methods for example RT-PCR, proteomics, metabolomics, transcriptomics and epigenomicsthechniques will revolutionize the world of phytomedicine.

Conflict of interest

We declare that we have no conflict of interest.

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