



Received on 17 September, 2014; received in revised form, 19 November, 2014; accepted, 19 January, 2015; published 01 May, 2015

## APPLICATION AND COST-EFFECTIVENESS OF LAMP (LOOP-MEDIATED ISOTHERMAL AMPLIFICATION) TECHNOLOGY FOR AUTHENTICATION OF MEDICINAL PLANTS *PODOPHYLLUM HEXANDRUM* ROYLE

Waleed M. Al-Shaqha <sup>1</sup>, Mohsin Khan <sup>2</sup>, Arezki Azzi <sup>1</sup> and Anis Ahmad Chaudhary <sup>\*1</sup>

College of Medicine <sup>1</sup>, Al-Imam Muhammad Ibn Saud Islamic University (IMSIU), Riyadh-13317-7544, Kingdom of Saudi Arabia

Department of Energy and Environmental sciences <sup>2</sup>, Chaudhary Devi Lal University, Sirsa, Haryana-125055, India

### Keywords:

Loop-Mediated Isothermal Amplification, *Podophyllum hexandrum* Royle, Polymerase Chain Reaction, RAPD

### Correspondence to Author: Anis Ahmad Chaudhary

College of Medicine  
Al-Imam Muhammad Ibn Saud  
Islamic University (IMSIU), Riyadh-  
13317-7544, Kingdom of Saudi  
Arabia.


**E-mail:** anis.chaudhary@gmail.com

**ABSTRACT:** We have developed a novel LAMP-based molecular marker for easy and fast identification of *Podophyllum hexandrum* Royle (Indian May apple). It is an important medicinal plant valued all over the world. It has both anti-cancer (pro-oxidant ability) and radioprotective (antioxidant ability) effects respectively. The LAMP-based molecular marker was constructed based on genomic DNA of *P. hexandrum* that amplified by RAPD. A RAPD amplicon common to all accessions, was eluted, cloned and sequenced. Based on these sequences in-house specific LAMP primers were synthesized. This method employs by a *Bst* DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the *P. hexandrum* genomic DNA. In LAMP reaction the amplification was carried out in thermal cycler and water bath at 60°C for 60 min, which was followed by monitoring DNA amplification with the naked eye through color changes by standardizing SyBr Green<sup>®</sup> I nucleic acid stain. The data showed confirmatory results in both thermal cycler and water bath. Since, the assay method is sensitive and cost-effective; it is a feasible method for identifying and authentication of *P. hexandrum*. Due to its specificity and simplicity, the method could become a useful tool for identification and authentication medicinal plants.

**INTRODUCTION:** *Podophyllum hexandrum* Royle (Himalayan Mayapple), is an herb that grows at about 4000 meters of altitude in the Himalayan region. The rhizomes and roots of *P. hexandrum* contain anti-tumor (pro-oxidant ability) and radioprotective (antioxidant ability) lignans such as podophyllotoxin, 4'-demethyl podophyllotoxin and podophyllotoxin 4-O-glucoside <sup>1,2,3</sup>.

Among these lignans, podophyllotoxin is most important for its use in the synthesis of anti-cancer drugs etoposide, teniposide and etopos <sup>4</sup>. These compounds have been used for the treatment of long and testicular cancers as well as certain leukemias <sup>5,6</sup>.

Presently, *P. hexandrum* (an Indian species) and *P. peltatum* L. (an American species) are the commercial source of podophyllotoxin for the pharmaceutical industry. However, the Indian species *P. hexandrum* contain 3 times more podophyllotoxin than the American species *P. peltatum* <sup>7,8</sup> adding more to the importance of *P. hexandrum*. It also has numerous applications in

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.6(5).2035-41</p>
<p>Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a></p>	
<p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.6(5).2035-41">http://dx.doi.org/10.13040/IJPSR.0975-8232.6(5).2035-41</a></p>	

modern medicine by virtue of its free radical scavenging capacity. However, it commonly in physiology areas and it is difficult to distinguish them accurately through morphological and physiological features. Therefore, it would be very important to search for a simple and effective method to distinguish these commercial species of the superior type from other commercial species.

Genetic tools that use hybridization, polymerase chain reaction (PCR), and sequencing techniques provide more objective and reliable methods for authenticating herbal medicines<sup>9, 10</sup>. RAPD analysis has become one of the most effective methods for estimating genetic diversity in plant populations or cultivars because it can reveal high levels of polymorphism. However, it is less reproducible than other methods<sup>10, 11</sup> and also has many advantages, such as its high speed, low cost, and requirement of minute amounts of plant material<sup>12, 13, 14</sup> used RAPD analysis to develop SCAR markers, a more accurate and reliable technique.

This technique can be used to develop markers that authenticate herbal medicines by using specific PCR primers derived from RAPD or AFLP fragments. A SCAR marker was detected by PCR using specific oligonucleotide primers designed based on the sequence data of one RAPD or AFLP fragments and following PCR amplification under more stringent conditions. SCAR markers also need the analysis of gels, time-consuming and sophisticated PCR machine.

To overcome these problems associated with the discussed technique, isothermal amplification of DNA useful method. Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method developed by<sup>15</sup> relies on an autocycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment<sup>15, 16</sup>.

LAMP requires two specially designed inner and two outer primers to perform the amplification of the target gene, as such, LAMP amplifies DNA with high specificity, efficiency, and rapidity the amplification uses a single temperature step at 60–66 °C for about 30-60 min, simple incubators, such

as a water bath or block heater, are sufficient for the DNA amplification, and the amplification products have many types of structures in large amounts.

Thus, LAMP is faster and easier to perform than PCR, as well as being more specific. Furthermore, gel electrophoresis is not needed, LAMP products because the LAMP products can be detected indirectly by the turbidity that arises due a large amount of by-product, pyrophosphate ion, being produced, yielding an insoluble white precipitate of magnesium pyrophosphate in the reaction mixture<sup>17, 18</sup>.

### MATERIALS AND METHODS:

Fresh leafs of mature plants were collected from Uttarakhand (West Himalaya) state of India (**Table 1**). The leaf samples were frozen in liquid nitrogen and stored at –20°C until used for DNA isolation.

**TABLE 1: ALL THREE ACCESSIONS OF *P. HEXANDRUM***

S.N.	Accessions	Altitude (masl)	Locations
1.	P1	1500	Dohra Nalla, Kullu
2.	P2	1660	Upper Valley, Kullu
3.	P3	1675	Parbati Valley, Kullu

### DNA isolation:

DNA was isolated from fresh or frozen leaves using a modified CTAB method (cetyl trimethyl ammonium bromide,<sup>19</sup> Briefly, leaf samples (0.2–0.5 g) were ground to fine powder in liquid nitrogen and transferred to a centrifuge tube containing freshly prepared equal volume of extraction buffer (100 mmol/L Tris buffer, pH 8.0, 20 mmol/L Na<sub>2</sub>EDTA, 1.4 mol/L NaCl, 2% CTAB, 1% polyvinyl pyrrolidone).

The suspension was gently mixed and incubated at 60°C for 60 min with occasional mixing. The suspension was then cooled to room temperature and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The mixture was centrifuged at 13000 x g for 10 min. The clear upper aqueous phase was then transferred to a new tube containing 0.5 mL ice-cooled isopropanol and incubated at –20 °C for 30 min. The nucleic acid was collected by centrifuging at 13000 x g for 10

min. The resulting pellet was washed twice with 70% ethanol containing 10mmol/L ammonium acetate. The pellet was air-dried under a sterile laminar hood and the nucleic acid was dissolved in TE (10 mmol/L Tris buffer, pH 8.0, 1 mmol/L Na<sub>2</sub>EDTA) at 4 °C.

The contaminating RNA was eliminated by treating the sample with RNase A (20µg/µL) for 30 min at 37°C. DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 nm and 280 nm. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide.

#### **RAPD amplification:**

The RAPD amplification was performed according to the method developed by <sup>20</sup>. PCR reactions were carried out in 25µL reaction tubes using 25 random decanucleotide primers, OPAA-1, OPAA-2, OPAA-3, OPAA-4, OPAA-5, OPAA-6, OPAA-7, OPAA-8, OPAA-9, OPAA-10, OPAA-11, OPAA-12, OPAA-13, OPAA-14, OPAA-15, OPAA-16, OPAA-17, OPAA-18, OPAA-19, OPAA-20 (Operon Technologies Inc., USA), Bg26, Bg27, Bg28, Bg29 and Bg30 (Banglore Genei, India). Each reaction tube contained 50 ng template DNA, 1.5 mmol/L MgCl<sub>2</sub>, 300 µmol/L dNTPs, 1×*Taq* DNA polymerase buffer, 250 pmol decanucleotide primer and 2 units of *Taq* DNA polymerase (Promega, USA).

Amplification was performed in a DNA thermal cycler (Ependroff, USA), using the following conditions : 95°C for 3 min; 40 cycles at 94 °C for 30 sec, 45 °C for 1 min and 72 °C for 1 min; final extension at 72 °C for 5 min. PCR products were resolved on 1.2% agarose gel in 1xTAE buffer, agarose gel containing 0.5 µg/ml ethidium bromide visualised under UV light and photographed using gel documentation system (Image Master VDS, Pharmacia, USA).

APD markers suffer from a lack of reproducibility. Consequently, to confirm the electrophoretic patterns and the obtained polymorphic bands, every PCR was repeated twice under the same conditions of composition of reaction volume, amplification profile and thermalcycler.

#### **Cloning and sequencing of specific RAPD fragment**

A band of 429 bp, which is common in all the six accessions, was excised from gels and eluted using a Gel Extraction Kit (QIAGEN, Germany). The eluted DNA was cloned into pGEM<sup>®</sup>-T easy vector (Promega, USA) following the manufacturer's instruction. The ligated plasmid was introduced into *Escherichia coli* strain DH5<sub>α</sub>, following the protocols for preparing competent cells and transformation using the calcium chloride method <sup>21</sup>. White colonies were picked from LB-X-gal plates and grown overnight in LB medium containing ampicillin.

The plasmid DNA was isolated from the bacterial culture using plasmid isolation kit (QIAGEN, Germany). The inserted fragment was sequenced at The Center for Genomic Application, New Delhi, India with T7 primer. Nucleotide sequence of 429 bp was identical for all the six accessions. This sequence was used for designing primers for LAMP reaction.

#### **Primers design for LAMP:**

Four oligonucleotide primers, forward inner primer (FIP), back inner primer (BIP), and two outer primers (F3 and B3), were designed by using the sequence of DNA from 429 bp RAPD amplicon. All primer sequences were designed with the software program Primer Explorer V3 (<http://primerexplorer.jp/elamp3.0.0/index.html>).

The primers were selected based on the criteria described by <sup>16</sup>. Briefly, the design of the two outer primers, F3 and B3, is the same as that of regular PCR primers, while the design of the two inner primers, FIP and BIP, is different from that of PCR <sup>16</sup>. FIP consists of the sense sequence of F2 at the 3` end and the F1c region at the 5` end that is complementary to the F1 region. BIP consists of a B2 region at the 3` end that is complementary to the B2c region and the same sequence as the B1c region at the 5` end.

#### **LAMP Assay:**

LAMP reaction (25 µl) contained the one of outer primers (0.2 µM) and one pair of inner primers (1.6 µM), 2.5 µl of 10x *Bst* DNA polymerase reaction buffer [1µl containing 20mM Tris-HCl, 10mM

KCl, 10mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2mM MgSO<sub>4</sub>, 0.1% Triton X-100 (pH 8.8)], 400 µM each dNTP, 1 µL of an 8-U/µL concentration of *Bst* DNA polymerase (New England Biolabs, Inc., MA), 2 mM MgSO<sub>4</sub> (2 µl), 5 µl of betaine (Sigma-Aldrich, St. Louis, MO), and 5 µl of double-stranded target DNA. The LAMP reaction was performed in a heating block (Genei, India) at 65 °C for 1 h. For comparison, the reaction was also performed by using a conventional thermal cycler (Bio-Rad, USA).

### Visualization of LAMP product:

The inspection for amplification was performed through observation of a color change following addition of 1µl (1:1000) of SYBR Green I dye to the tube. This color was visualized by naked

without any UV source and produced fluorescence in under UV transilluminator.

### RESULTS:

RAPD method was performed in search for DNA polymorphisms, which can be used for generating informative LAMP-based molecular markers and defining the individual cultivars. Twenty five RAPD primers were used. Only the fragments confirmed by repeated amplifications were considered useful for generating LAMP-markers. Preliminary screening of 25 random decanucleotide primers showed that nineteen primers were able to prime genomic DNA of *P. hexandrum*, and resulted in amplified PCR products of a variable number of DNA bands (09-43 bands per primer) (**Table 2**). A total of 460 DNA bands were obtained (**Table 3**).

**TABLE 2: NUMBER OF AMPLIFICATION PRODUCTS, FRAGMENTS SIZE, NUMBER OF POLYMORPHIC BAND AND NUMBER OF MONOMORPHIC BAND WITH 25 ARBITRARY PRIMERS IN ALL THREE ACCESSION OF *P. HEXANDRUM***

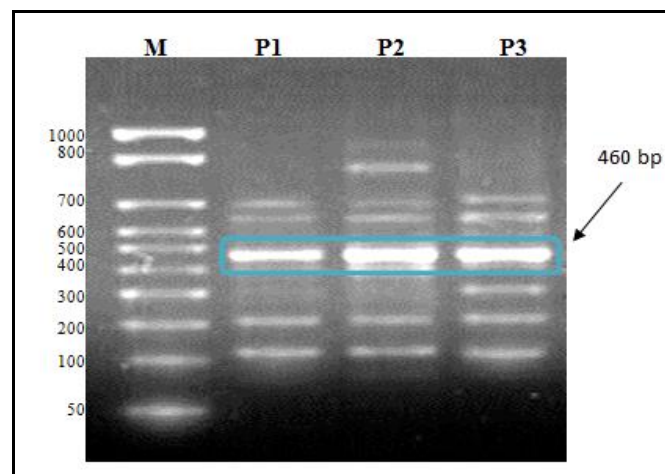
Primer code	Sequence 5' – 3'	No. of Amplification Product	Fragment Size (bp)	No. of Polymorphic Band	No. of Monomorphic Band
OPAA -01	AGACGGCTCC	19	331-1,400	2	9
OPA A-02	GAGACCAGAC	32	191-1,392	9	2
OPAA -04	AGGACTGCTC	38	195-1,093	10	3
OPAA -05	GGCTTTAGCC	35	79-1,194	12	1
OPAA -06	TCAAGCTAAC	37	65-1,194	10	0
OPAA -07	CTACGCTCAC	26	337-1,085	4	1
OPAA -08	TCCGCAGTAG	22	311-1,000	3	5
OPAA -09	AGATGGGCAG	29	272-10,96	5	3
OPAA -10	TGGTCGGGTG	32	222-1,100	3	6
OPAA -11	ACCCGACCTG	21	323-1,094	4	3
OPAA -12	GGACCTCTTG	26	290-918	5	3
OPAA -13	GAGCGTCGCT	27	197-1,492	5	2
OPAA -14	AACGGGCCAA	24	314-1,486	4	3
OPAA -15	ACGGAAGCCC	26	147-1,432	3	4
OPAA -16	GGAACCCACA	27	310-1,315	5	5
OPAA -17	GAGCCCGACT	0	00	0	0
OPAA -18	TGGTCCAGCC	20	209-1,055	5	3
OPAA -19	TGAGGCGTGT	0	00	0	0
OPAA -20	TTGCCTTCGG	Nil	00	0	0
BG-26	AAGCCTCGTC	15	109-890	1	5
BG-27	TGCGTGCTTG	09	150-915	0	5
BG-28	GACGGATCAG	0	00	0	0
BG-29	CACACTCCAG	Nil	00	0	0
BG-30	TGAGTGGGTG	0	00	0	0
<b>Total</b>		<b>472</b>			

Amplification of *P. hexandrum* species with OPAA-02, OPAA-03, OPAA-04, OPAA-05 and OPAA-06 produced good quality, reproducible fingerprint patterns and showed a high level of consistency of fingerprints among samples of the same species collected from different localities

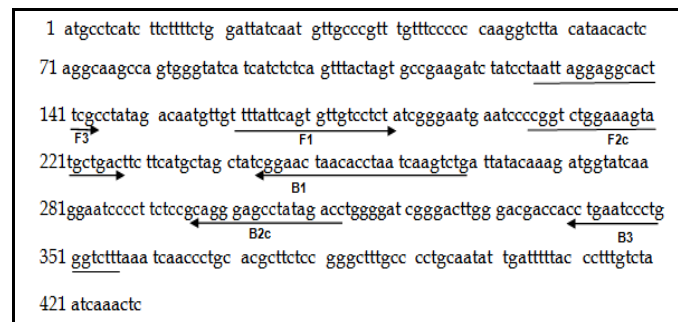
(**Fig. 1**). Several specific RAPD fragments of high intensity and reproducibility were eluted, cloned and sequenced. Nucleotide sequence of 429 bp amplicon (**Fig. 2A**), identical for all the three accessions of *P. hexandrum*, was used for designing primers for LAMP reaction (**Fig. 2B**).

**TABLE 3: NUMBER OF AMPLIFICATION PRODUCTS AND FRAGMENTS SIZE WITH 25 ARBITRARY PRIMERS IN ALL THREE ACCESSION OF P. HEXANDRUM**

Primer	P 1	P 2	P 3
OPAA -01	6	6	7
OPAA -02	10	10	12
OPAA -03	3	2	2
OPAA -04	12	13	13
OPAA -05	13	10	12
OPAA -06	12	13	12
OPAA -07	8	10	8
OPAA -08	7	5	10
OPAA -09	12	7	9
OPAA -10	12	11	9
OPAA -11	5	10	6
OPAA -12	9	9	9
OPAA -13	9	8	10
OPAA -14	7	7	10
OPAA -15	8	9	9
OPAA -16	12	7	8
OPAA -17	0	0	0
OPAA -18	6	6	8
OPAA -19	0	0	0
OPAA-20	0	0	0
BG-26	6	5	4
BG-27	1	4	4
BG-28	0	0	0
BG-29	0	0	0
BG-30	0	0	0
<b>Total</b>	<b>158</b>	<b>152</b>	<b>162</b>



**FIG.1: RAPD ELECTROPHORESIS PROFILE OF P. HEXANDRUM AMPLIFIED WITH OPAA-09 PRIMERS. LANES 1-3 CORRESPOND TO THE ALL THREE ACCESSIONS. LANE M, MOLECULAR MARKER 50-1000 bp (BIO BASIC INC, CANADA). THE NUMBERS ON THE LEFT SIDE OF THE FIGURE INDICATE THE DNA SIZE MARKERS IN BASE PAIR (bp).**



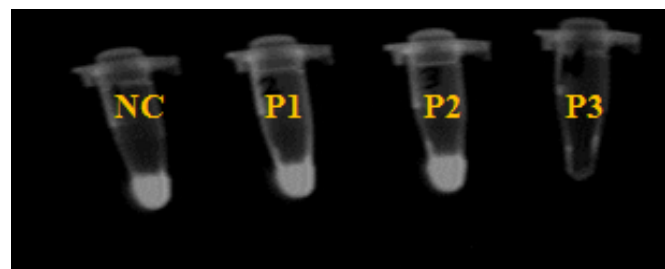
**FIG. A:**

LAMP primers of <i>Podophyllum hexandrum</i>		
Primers	Outer forward and backward Primers	Bases
pdF3	5'-aattaggaggcacttcgc-3'	18
pdB3	5'-aagaccaggaggattcagg-3'	18
Inner forward and backward Primers		
pdFIP (F1c+F2)	5'-gtcagcatactttccagaccgtgttttattcagtggtgtcct-3'	43
pdBIP (B1+B2c)	5'-cggaactaacacctaatacaagtctgtccccagggtctataggct-3'	43

**FIG.B:**

**FIG. 2: A. NUCLEOTIDE SEQUENCE OF RAPD AMPLICON (429 bp) OF P. HEXANDRUM AND UNDERLINED INDICATE THE DESIGNED LAMP PRIMERS, B LAMP PRIME FOR P. HEXANDRUM.**

The LAMP reaction relies mainly on autocycling strand displacement DNA synthesis that is similar to the cascade rolling-circle amplification reported by <sup>22</sup>. The minimum LAMP reaction unit consists of two outer (F3 and B3), two inner primers (FIP and BIP) and target DNA. Each inner primer contains two distinct sequences corresponding to the sense and antisense sequences of the target DNA and form stem-loop structures at both ends of the minimum LAMP reaction unit. These stem-loop structures initiate self-primed DNA synthesis and serve as the starting material for subsequent LAMP cycling reaction. The LAMP products were visualized on a UV transilluminator at 302 nm (Fig. 3) and naked eye without using any UV source (Fig. 4).



**FIG.3: ANALYSIS OF LAMP RESULT UNDER UV-TRANSILLUMINATOR. P1-P3 SHOWED ACCESSIONS OF P. HEXANDRUM, NC = NEGATIVE CONTROL**



**FIG. 4: ANALYSIS OF LAMP RESULT UNDER NAKED EYE WITHOUT USING ANY UV SOURCE. P1-P3 SHOWED ACCESSIONS OF P. HEXANDRUM, NC =NEGATIVE CONTROL.**

**DISCUSSION:** *P. hexandrum* has been studied extensively in India because of its wide-spread used in herbal medicine all over the world. We have developed a credible and convenient LAMP-based marker for the identification of *P. hexandrum*. Molecular marker technology proves to be valuable tools for genotyping medicinal plants and detecting adulterations and substitutions in herbal medicine. The LAMP-based markers obtained from this study are useful for identifying three morphologically similar herbaceous species of *P. hexandrum*. Using DNA markers developed in this study, *P. hexandrum*, the most widely used in traditional medicine can be efficiently differentiated from *P. hexandrum*, which is used to a much lesser extent and also from the least known *P. hexandrum*.

The LAMP operation is quite simple; it starts with the mixing of buffer, primers, DNA lysates, and DNA polymerase in a tube, and then the mixture is incubated at 65°C for a certain period. For the visualization of product, SYBR Green I was added. The tubes can also be inspected for white turbidity with the naked eye after a pulse spin to deposit the precipitate in the bottom of the tube<sup>18</sup>. However, detecting a small amount of the white precipitate by the naked eye is not always easy; therefore, the detection limit is apparently inferior to that of electrophoresis. To increase the rate of recognition by the naked eye, addition of SYBR Green I to the reaction solution is convenient<sup>23</sup>.

LAMP amplification is rapid (results can be obtained in less than 1 h), easy to perform and low in cost. Because of its easy operation without any sophisticated equipment, it will be simple enough for use in small-scale industries, hospitals, and testing laboratories in developing countries. The molecular based identification is especially useful when botanical identification based on morphology becomes difficult, such as in the case of incomplete or damaged samples, and in dried herbal products.

## REFERENCES:

1. Tyler VE, Brady LR and Robbers JE: Pharmacology. 9th edn. Lea & Febiger, Philadelphia 1988.
2. Broomhead AJ and Dewick PM: Tumor inhibitory aryltralin lignans in *Podophyllum versipelle*, *Diphyllicia cymosa* and *Diphyllicia grayi*. Phytochem. 1990; 29:3831-3837.
3. Kumar R, Singh PK, Arora R, Chawla R and Sharma RK: Radio protective Activities of *Podophyllum hexandrum*: Current Knowledge of the Molecular Mechanisms. Trees for life Journal 2009; 4:1-9.
4. Issell BF, Muggia FM, and Carter SK: Etoposide (VP-16)-Current Status and New Developments. Academic Press, Orlando, FL, USA 1984.
5. Stahelin HF and Wartburg AV: The chemical and biological route form podophyllotoxin glucoside to etoposide: Ninth Cain Memorial Award Lecture. Cancer Research 1991; 51:5-15.
6. Imbert TF: Discovery of podophyllotoxins. Biochemistry 1998; 80: 207-22.
7. Fay DA and Ziegler HW: Botanical source differentiation of Podophyllum resin by high performance liquid chromatography. Journal Liquid Chromatography 1985; 8:1501-1506.
8. Pandey H, Nandi SK, Kumar A, Palni UT and Palni LMS: Podophyllotoxin content in Podophyllum hexandrum Royle plants of known age of seed origin and grown at a lower altitude. Acta Physiol. Plant 2007; 29: 121-126.
9. Zhang YB, Shaw PC, Sze CW, Wang ZT and Tong YJ: Molecular authentication of chinese herbal materials. Food Drug Anal. 2007; 15:1-9.
10. Shcher NJ and Carles MC: Genome-Based Approaches to the Authentication of Medicinal Plants. Planta Medica 2008; 74:603-623.
11. Hosokawa K, Minami M, Kawahara K, Nakamura I and Shibata T: Discrimination among three species of medicinal Scutellaria plants using RAPD markers. Planta Medica 2000; 66:270-272.
12. Williams JG, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 1990; 18:6531-6535.
13. Penner GA, Bush A, Wise R, Kim W, Domier L, Kasha K, Laroche A, Scoles G, Molnar SJ and Fedak G.: Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Appl. 1993; 2:341-345.
14. Paran I and Michelmore RW: Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 1993; 85:985-993.
15. Nagamine K, Hase T and Notomi T: Accelerated reaction by loop-mediated isothermal amplification using loop primers. Molecular Cell Probes 2001; 16:223-229.
16. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanebe K, Amino N and Hase T: Loop-mediated isothermal amplification of DNA. Nucleic Acids Research 2000; 28: E63.
17. Chaudhary AA, Hemant, Mohsin M and Ahmad A: Application of loop-mediated isothermal amplification (LAMP)-based technology for authentication of *Catharanthus roseus* (L.) G. Don. Protoplasma 2012; 249:417-422.
18. Mori Y, Nagamine K, Tomita N and Notomi T: Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochem. Biophys. Research Communication 2001; 289: 150-154.
19. Doyle JJ and Doyle JL: Isolation of plant DNA from fresh tissue. Focus 1990; 12:13-15.
20. McClelland M, Mathieu-Daude F and Welsh J: RNA fingerprinting and differential display using arbitrarily primed PCR. Trends Gene 1995; 11:242-246.

21. Sambrook J and Russell DW: Molecular Cloning: a Laboratory Manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York 2001.
22. Hafner GJ, Yang IC, Wolter LC, Stafford MR and Giffard PM: Isothermal amplification and multimerization of DNA by *Bst* DNA polymerase. *BioTechnica* 2001; 30:852–860.
23. Hill J, Beriwal S, Chandra I, Paul VK, Kapil A, Singh T, Wadowsky MR, Singh V, Goyal A, Jahnukainen T, Johnson JR, Tarr IP and Vats A: Loop-mediated isothermal amplification assay for rapid detection of common strains of *Escherichia coli*. *Journal Clinical Microbiology* 2008; 46:2800–2804.

**How to cite this article:**

Al-Shaqha WM, Khan M, Azzi A and Chaudhary AA: Application and Cost-Effectiveness of LAMP (Loop-Mediated Isothermal Amplification) Technology for Authentication of Medicinal Plants *Podophyllum Hexandrum* Royle. *Int J Pharm Sci Res* 2015; 6(5): 2035-41. doi: 10.13040/IJPSR.0975-8232.6(5).2035-41.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)