African Journal of Biotechnology Vol. 10(44), pp. 8709-8715, 15 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.528
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

DNA fingerprinting for the authentication of *Ruta graveolens*

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Accepted 5 August, 2010

Ruta graveolens **is a small aromatic shrub and has been used medicinally and magically, since ancient times. In this study, random amplified polymorphic DNA (RAPD) was employed to develop reproducible markers for authentication of this species from its adulterant *Euphorbia dracunculoides*. The random decamer oligonucleotide primers (42) were screened for identification of genuine and adulterant samples using the DNA isolated from the dried leaf, seed and stem of both samples. Out of 42 primers, 10 gave faint band, 12 gave species-specific reproducible unique band and the remaining did not amplify the DNA. RAPD could thus, serve as a complementary tool for quality control.**

Key words: Adulterant, *Euphorbia dracunculoides*, herbal drugs, random amplified polymorphic DNA.

INTRODUCTION

Medicinal plants are well known for their reputed medicinal properties; however, most of them are empirically used in Indian system of medicine to cure several human ailments. According to an estimate of the World Health Organization (WHO; http://www.who.int/research/en), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. Correct identification and quality assurance is indispensable to ensure medicinal quality of herbal drugs. Authentication is especially useful in cases of those medicinal herbs that are frequently substituted or adulterated with other species or varieties which are morphologically and phytochemically indistinguishable. Several herbal drugs on the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of a wrong herb may be ineffective or it may worsen the condition. Traditional Chinese medicine (TCM) contaminated with plants *Aristolochia* species in Belgium has resulted to an epidemic of subacute intestinal nephropathy in patients and many of them required kidney transplantation when examined histologically (Cosyns et al., 1999).

Several plants of *Rutaceae* family are used in traditional medicine world-wide. The most common medicinal plant of this family is *Ruta graveolens* (L.), known as rue and native to Europe. It is an ornamental evergreen shrub of one meter high (approximately), aromatic with sharp unpleasant odor and culinary. The leaves are small, oblong, deeply divided, pinnate and glandular dotted. The ramified stem has small yellow flowers in clusters and each has four petals except for the central flower which has five petals during spring and summer season. The fruits are small, rounded, lobulated and brown in color. The taste of the fruit is slightly stinging but is masked by a strong bitter taste (Zargari, 1990).

The roots and aerial parts of this plant contain more than 120 compounds of different classes of natural products such as acridone alkaloids, coumarines, essential oils, flavonoids and furoquinolines (Kostova et al., 1999; Kuzovkinaa et al., 2004) and is the main source of furanocoumarins such as psoralen, xanthotoxin (8-meth-

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Abbreviations: RAPD, Random amplified polymorphic DNA; **TCM,** traditional Chinese medicine; **PCR,** polymerase chain reaction; **CTAB,** cetyltrimethylammonium bromide; **EDTA,** ethylenediaminetetraacetic acid; **PVP,** polyvinylpyrrolidone.

oxypsoralen; 8-MOP) and bergapten (5-metho-xypsoralen; 5-MOP) (Milesi et al., 2001). *R. graveolens* is its being widely used for medicinal purpose in various clinical conditions from very ancient time but rationality of use is still controversial. In homeopathy, rue is an important remedy for deep aching pain and rheumatism besides being used for eyestrain-induced headache (Miguel, 2003). It has also been used as a remedy for gastric disorders, stiff neck, dizziness, headache and so on (Conway and Slocumb, 1979). Besides, rue is widely used as sedative, antihelmintic (Skidmore-Roth, 2001), hypotensive (Chiu and Fung, 1997; Trovato et al., 2000), anti-inflammatory, antiviral, antiplasmodial (Yamamoto et al., 1989; Raghav et al., 2006; Queener et al., 1991), antimicrobial, cytotoxic (Ivanova et al., 2005), antifungal (Oliva et al., 2003; Meepagala et al., 2005), as herbicide (Hale et al., 2004) and contraceptive (Maurya et al., 2004; Harat et al., 2008). The polyphenolic and alkanoid fractions of *R. graveolens* showed protective effects on acute and chronic inflammation in rat (Ratheesh et al., 2009) and total extract (70% ethanol) showed *in vitro* cytotoxicity against tumor cell lines of different origin (Varamini et al., 2009). This medicinal shrub also has some adverse effects such as anti-fertility and abortion in female which have been reported from countries, viz., Brazil (de Freitas et al., 2005), India (Gandhi et al., 1991), Peru (Gutierrez-Pajares et al., 2003) and Mexico (Conway and Slocumb, 1979). Another specie of different genus with similar morphology named '*Euphorbia dracunculoides* (L.)' is being sold in local markets or used clinically as a replacement of *R. graveolens* at a relatively reduced cost under the local name 'suddaba' (Rahman et al., 2003). This may results in reducing the efficacy and quality of *R. graveolens* and may have adverse effects such as epistaxis, nausea/vomiting and haematuria (Rahman et al., 2003). Limitations of chemical and morphological markers for authentication have generated a need for newer methods in quality control of *R. graveolens.* However, DNA-based molecular markers are important tools in quality assurance and preservation of germplasm of medicinal plant species. Genuine sample specific markers (*R. graveolens*) are needed to maintain the quality and efficacy for herbal formulations. Our major objective therefore, is to develop DNA-based molecular tool for accurate identification of *R. graveolens* from its adulterant *E. dracunculoides.*

MATERIALS AND METHODS

The genuine sample of *R. graveolens* was provided by the Central Council for Research in Unani Medicine (CCRUM), Hyderabad. The samples for authentication were purchased from local markets of Khari Baoli, Delhi, India. The material was identified at the National Institute of Science Communication and Information (NISCAIR) by Dr. H. B. Singh and voucher (NISCAIR/RHMD/consult/-2007-08/937/121) was kept in the herbarium. Leaves powder of 100 mg each was used for DNA extraction using modified cetyltrimethy-lammonium bromide (CTAB) method (Khan et al., 2007). The total genomic DNA was used in random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR) to develop fingerprints for the authentication of genuine as well as adulterant samples.

Reagents and chemicals

The stock solution concentration were: CTAB 3% (w/v), 1 M Tris-Cl (pH 8), 0.5 M ethylenediaminetetraacetic acid (EDTA) (pH 8), 5 M NaCl, absolute ethanol (AR grade), chloroform-IAA (24:1 (v/v)), polyvinylpyrrolidone (PVP) (40,000 mol wt) (Sigma) and β-mercaptoethanol. All the chemicals used in the experiments were of analytical grade. The extraction buffer consisted of CTAB 3% (w/v), 100 mM Tris-Cl (pH 8), 25 mM EDTA (pH 8) and 2 M NaCl, respectively. The PVP and β-mercaptoethanol were prepared freshly and added.

RAPD analysis

RAPD reaction was performed according to the method developed by McClelland et al. (1995). The total volume of reaction was performed in 25 µl. PCR reaction for RAPD analysis consisted of 15 mM $MgCl_2$, 2.5 µl 10x buffer, 2 µl 2 mM dNTPs (mix), 1.5 U Taq polymerase in buffer, 25 ng/µl of each primer, 30 ng/µl plant DNA and sterile water of 18 µl. All reagents were supplied by Bengalore Genei Pvt. Ltd (Bangalore, India) except for primers, which were supplied by Genetix Biotech Asia Limited (Bangalore, India). For DNA amplification, a Techne thermal cycler was programmed for 1 cycle of 3 min at 94°C, 30 s at 35.5°C and 1 min at 72°C followed by 45 cycles of 1 min at 94°C, 30 s at 35.5 and 72°C for 1 min, then final extension for 5 min at 72°C. The RAPD fragments were separated on 1.2% agarose gel by electrophoresis in 1xTAE buffer and stained with ethidium bromide.

RESULTS

R. graveolens was chosen to test the reliability of quality control using RAPD technique. *E. dracunculoides* was found as an adulterant in the local market samples, when identified later at NISCAIR, New Delhi (voucher no, NISCAIR/RHMD/consult/-2007-08/937/121). The dried leaves, fruits and stem of *E. dracunculoides* is more or less similar in morphology with those of *R. graveolens*, there-by difficult to differentiate from each other (Figure 1). It leads to confusion among customers and *E. dracunculoides* is bought mistakenly instead of *R. graveolens* due to low cost. The RAPD technique was carried out in replicate (two) on both plants for reproducibility using 42 decamer primers. Out of 42 primers, 12 generated unique, monomorphic and polymorphic fragments, whereas remaining primer did not give amplification using genomic DNA extracted from leaves, seeds and stem, respectively. The unique fragments generated in RAPD profile have shown differentiation between genuine and adulterant samples. RAPD fingerprints generated from genomic DNA isolated from dried leaves, fruits and stem was same with all decamer primers. The total number of unique fragments specific to genuine as well as adulterant samples with different primers is summarized in Figures 2 - 4 and Table 1.

Figure 1. Morphological slides of genuine and adulterated samples. **A.** Genuine samples of *R. graveolens* provided by Central Council for Research in Unani Medicine (CCRUM) Hyderabad; **B.** adulterated samples of *R. graveolens* with *E. dracunculoides* purchased from Khari Baoli, New Delhi, India.

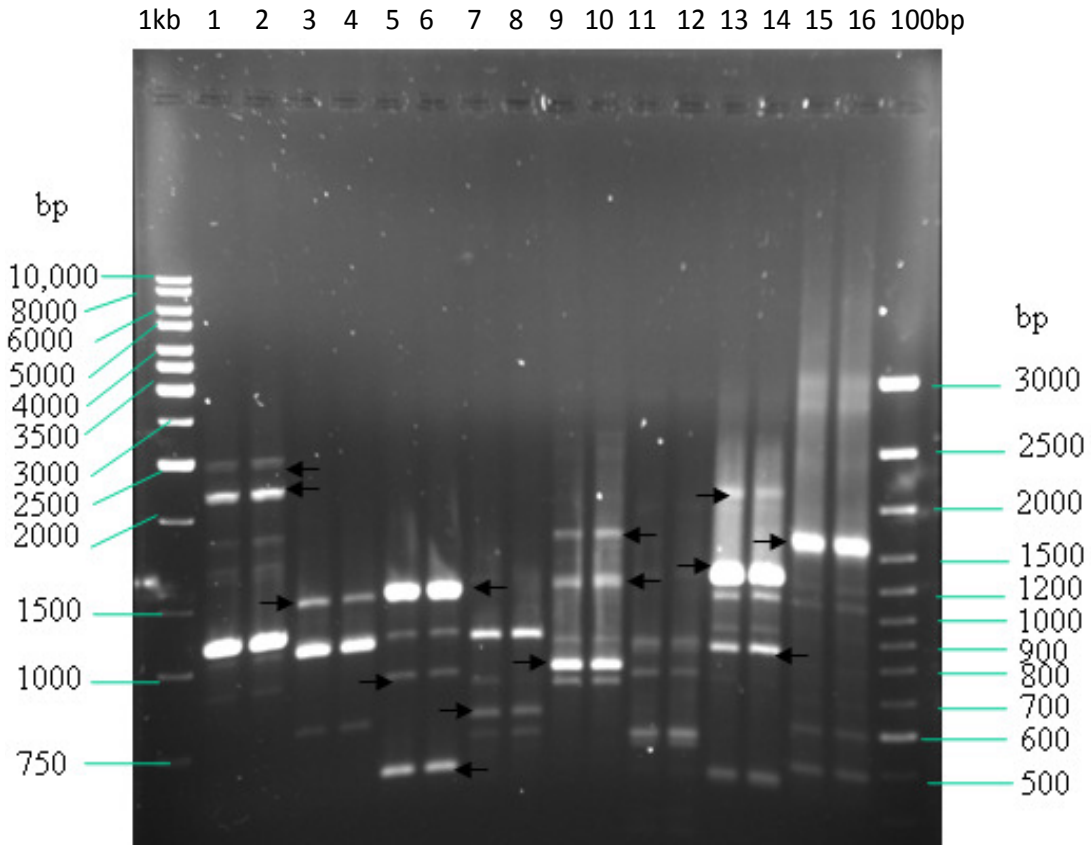

Figure 2. RAPD analysis carried out with primers. P-11 (lanes: 1 - 4), P-12 (lanes: 5 - 8), P-13 (lanes: 9 - 12), P-14 (lanes: 13 - 16) on genomic DNA extracted from leaf of *R. graveolens* (lanes: 1, 2, 5, 6, 9, 10, 13 and 14) and *E. dracunculoids* (lanes: 3, 4, 7, 8, 11, 12, 15 and 16). 1 kb and 100 bp are DNA ladder.

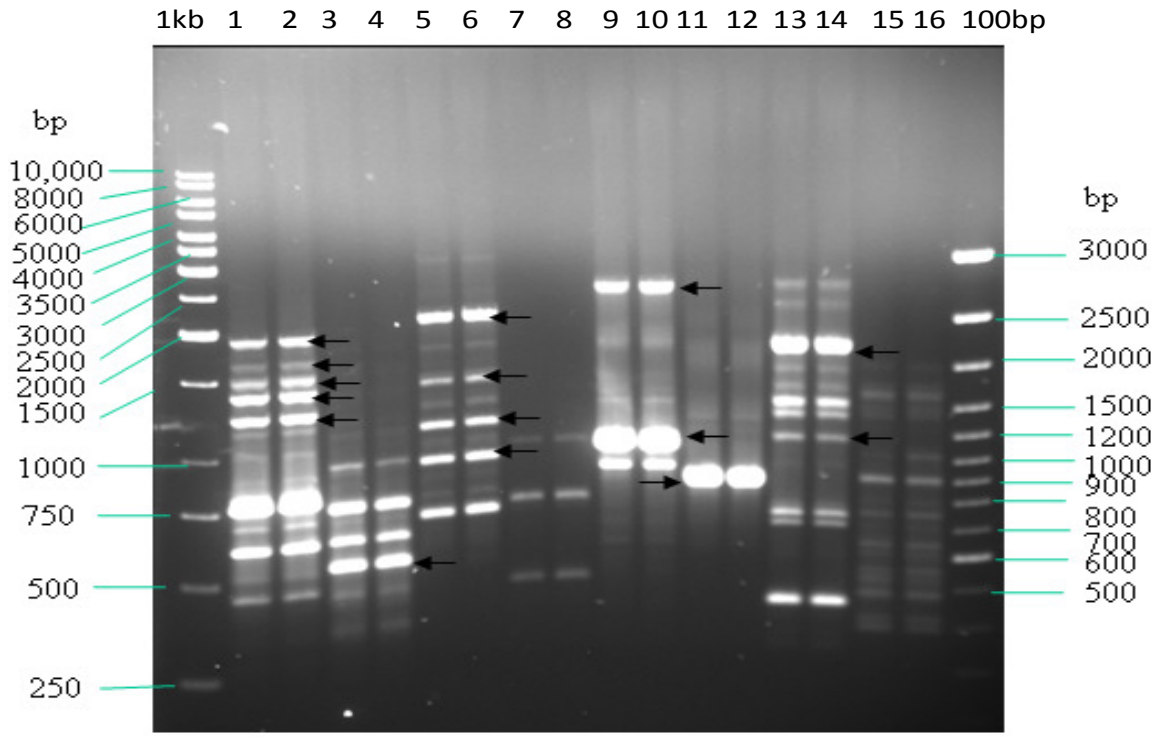

Figure 3. RAPD analysis carried out with primers. OPC-1 (lanes: 1 - 4), OPC-2 (lanes: 5 - 8), OPC-3 (lanes: 9 - 12), OPC-4 (lanes: 13 - 16) on genomic DNA extracted from leaf of *R. graveolens* (lanes: 1, 2, 5, 6, 9, 10, 13 and 14) and *E. dracunculoids* (lanes: 3, 4, 7, 8, 11, 12, 15 and 16). 1 kb and 100 bp are DNA ladder.

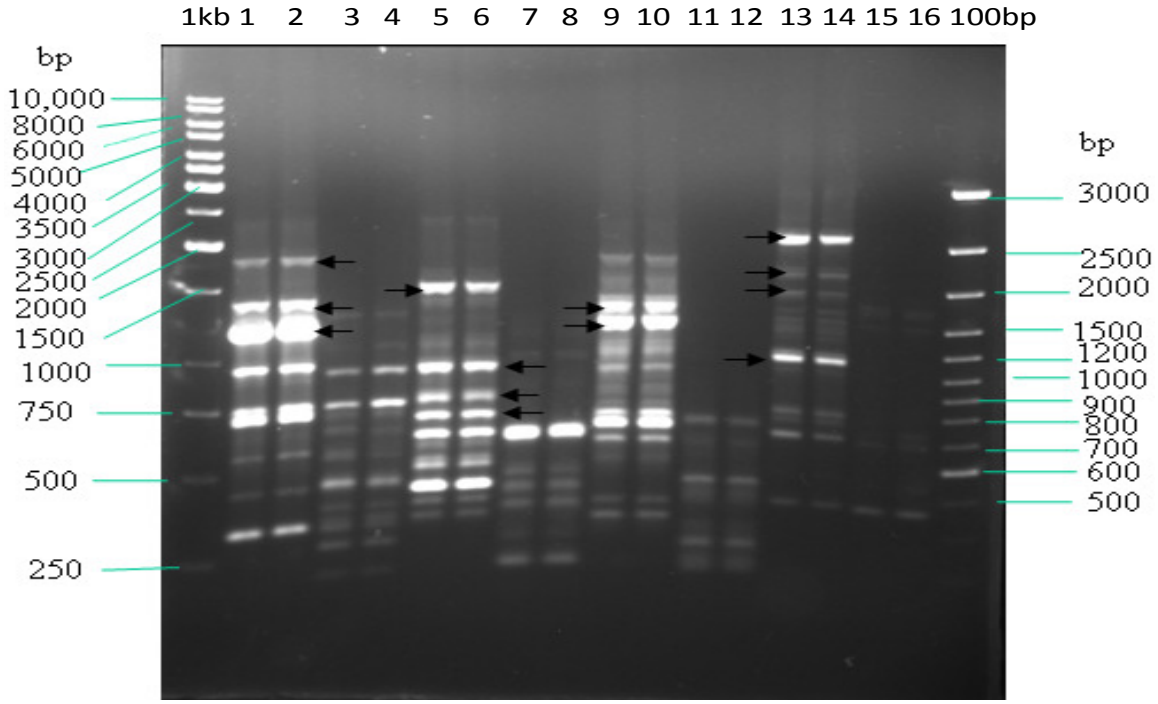

Figure 4. RAPD analysis carried out with primers. OPC-11 (lanes: 1 - 4), OPC-12 (lanes: 5 - 8), OPC-13 (lanes: 9 - 12), OPC-14 (lanes: 13 - 16) on genomic DNA extracted from leaf of *R. graveolens* (lanes: 1, 2, 5, 6, 9, 10, 13 and 14) and *E. dracunculoids* (lanes: 3, 4, 7, 8, 11, 12, 15 and 16). 1 kb and 100 bp are DNA ladder.

Table 1. The unique amplicons specific to *R. graveolens* and *E. dracunculoides* samples with 12 decamer oligonucleotides primers obtained in PCR amplification.

Plant species	Size of unique fragments (bp)				Size of unique fragments (bp)				Size of unique fragments (bp)			
	P-11	P-12	P-13	P-14	OPC-1	OPC-2	OPC-3	OPC-4	OPC-5	OPC-6	OPC-7	OPC-8
R. graveolens	2400, 2200	1200, 800, 550	1800, 1300, 800	2300, 1300, 900	2400, 2000, 1800, 1500, 1400	2500, 1900, 1400, 1000	2800, 1200	2400, 1200, 450	2500, 1900, 1500	2000, 1100, 900, 850	1900, 1600	2600, 2200, 2000, 1200
E. dracunculoides	1200	650	-	1600	600	-	950	-	-	-	-	-
Total no of unique fragments/ primer	3	4	3	4	6	4	3	3	3	4	2	4

(Genders, 1994). Since, dried parts of this scented plant such as leaves, fruits and stem are used in herbal formulations, morphological characteristics of dried parts of genuine and adulterants are more or less similar, thereby presenting difficulty in discriminating by conventional methods (Figure 1). However, another species, named 'suddaba' (*E. dracunculoides*), is easily adulterated and/or substituted with *R. graveolens* in local herbal markets (Rahaman et al., 2003). The leaves of *R. graveolens* are bipinnate or tripinnate and obviate-oblong, whereas *E. dracunculoides* are lanceolate or linear oblong, subacute, base rarely rounded or subcordate. The odour and taste are not distinct in *R. graveolens* and *E. dracunculoides*. The fruit of *R. graveolens* is capsule, 4 - 5 lobed globose, numerous tiny blackish seeds triangular in shape, while fruit of *E. dracunculoides* is capsule with subglobose shape, 3-celled and seeds are ovoid-terete with gray or dark gray color. However, these morphological markers sometimes deviate for precise authentication of *R. graveolens* and leads to degradation of quality and efficacy of this medicinal shrub.

Our study showed clear discrimination of *R. graveolens* and *E. dracunculoides* based on unique fragments generated with 12 decamer primers. However, each fragment in RAPD is derived from a region of the genome that contains two short segments in inverted orientation on opposite strands and is complementary to the primer for reproducible amplification (Hon et al., 2003). The primer OPC-1 amplified more unique fragments such as 2400, 2000, 1800, 1500 and 1400 bp as compared to other primers which would be applicable at large scale for authentication of *R. graveolens* (Table 1). According to traditional usage and animal studies, both plants have different medicinal effects and adulteration of genuine sample may have undesirable effects. The developed RAPD fingerprints for genuine as well as adulterant samples would be helpful for correct preparation of herbal drug formulations. The RAPD analysis has been widely used for differentiation of a large number of medicinal species from their close relatives or adulterants, viz., *Panax* species (Shaw and But, 1995), *Coptis* species (Cheng et al., 1997), *Astragalus* species (Cheng et al., 2000), *Lycium barbarum* (Cheng et al., 2000), *Panax ginseng* (Um et al., 2001), *Echinacea* species (Nieri et al., 2003), turmeric (Sasikumar et al., 2004), *Astragali radix* (Na et al., 2004), *Dendrobium officinale* (Ding et al., 2005), *Typhonium* species (Acharya et al., 2005), *Dendrobium* species and its products (Zhang et al., 2005), *Tinospora cordifolia* (Rout, 2006), *Mimosae tenuiflorae* (Rivera-Arce et al., 2007), *Rahmannia glutinosa* cultivars and varieties (Qi et al., 2008), *Desmodium species* (Irshad et al., 2009), *Glycirrhiza glabra* (Khan et al., 2009), *Piper nigrum* (Khan et al., 2010) and *Cuscuta reflexa* (Khan et al., 2010). The components of *Rasayana churna* was identified by RAPD-PCR which have three Ayurvedic medicines such as dried stem of *Tinospora cordifolia*, dried fruit of *Emblica officinalis* and dried fruit of *Tribulus terestris* (Shinde et al., 2007). The marker of size 600 bp was specific to *T. cordifolia*, 500 bp specific to *E. officinalis* and the remaining >1000 bp was present in *T. terestris* (Shinde et al., 2007). The same species of *Selaginella* which has medicinal value was collected from different habitats and authenticated by RAPD marker (Li et al., 2007). The advantages of RAPD technique is their simplicity, rapidity, requirement of low amount of genomic DNA and avoidance of radioactive substances (Micheli et al., 1994). However, synthesis of decamer primers also avoids prior genetic information of studying plants. Our RAPD marker therefore, may prove to be reproducible under a wide variation of amplification conditions such as an annealing temperature, origin of the primer, *Taq* polymerase and thermal cycler. In this study, we could suggest that RAPD technique is convenient for identification of *R. graveolens* and its adulterant *E. dracunculoides* in the local herbal markets.

ACKNOWLEDGEMENTS

This work was supported by the Central Council for

Research in Unani Medicine (CCRUM) New Delhi, Gov. of India.

REFERENCES

Acharya L, Mukherjee AK, Panda PC, Das P (2005). Molecular characterization of five medicinally important species of *Typhonium* (Araceae) through random amplified polymorphic DNA (RAPD). *Zeitschrift fur Naturforschung. C* 60(7-8): 600-604.

Cheng K, Chang H, Huang H, Lin C (2000). RAPD analysis of *Lycium barbarum* medicine in Taiwan market. Bot. Bull. Acad. Sin. 41: 11-14.

Cheng KT, Chang HC, Su CH, Hsu FL (1997). Identification of dried rhizomes of *Coptis* species using random amplified polymorphic DNA. Bot. Bull. Acad. Sin. 38: 241-244.

Cheng KT, Su CH, Chen CT, Lin CC (2000). RAPD analysis of *Astragalus* medicines marketed in Taiwan. Am. Chinese. Med. 28: 273-278.

Chiej R (1984). Encyclopaedia of Medicinal Plants. *MacDonald* 0-356-10541.

Chiu KW, Fung AY (1997). The cardiovascular effects of green beans (*Phaseolus aureus*), common rue (*Ruta graveolens*), and kelp (*Laminaria japonica*) in rats. J. Pharmacol. 29: 859-862.

Conway GA, Slocumb JC (1979). Plants used as abortifacients and emmenagogues by Spanish New Mexicans. J. Ethnopharmacol. 1(3): 241-261.

Cosyns JP, Jadoul M, Squifflet JP, Wese FX, Van Ypersele DS (1999). Urothelial lesions in Chinese herb nephropathy. Am. Kidney Dis. 33: 1011-1017.

De Freitas TG, Augusto PM, Montanari T (2005). Effect of *Ruta graveolens* (L.) on pregnant mice. Contraception, 71(1): 74-77.

Ding G, Ding XY, Shen J, Tang F, Liu DY, He J, Li XX, Chu BH (2005). Genetic diversity and molecular authentication of wild populations of *Dendrobium officinale* by RAPD. eCAM, 40: 1028-1032.

Gandhi M, Lal R, Sankaranarayanan A, Sharma PL (1991). Post-coital antifertility action of *Ruta graveolens* in female rats and hamsters. J. Ethnopharmacol. 34: 49-59.

Genders R (1984). Scented Flora of the World. Robert Hale. London 0-7090-5440-8.

Gutierrez-Pajares JL, Zuniga L, Pino J (2003). *Ruta graveolens* aqueous extract retards mouse pre implantation embryo development. Reprod. Toxicol. 17: 667-672.

Hale AL, Meepagala KM, Oliva A, Aliotta G, Duke SO (2004). Phytotoxins from the leaves of *Ruta graveolens*. J. Agric. Food Chem. 52: 3345-3349.

Harat ZN, Sadegh MR, Sadeghipour HR, Kamalinejad M, Eshraghian MR (2008). Immobilization effect of *Ruta graveolens* (L.) on human sperm: A new hope for male contraception. J. Ethnopharmacol. 115: 36-41.

Hon CC, Chow YC, Zeng FY, Leung FCC (2003). Genetic authentication of ginseng and other traditional Chinese medicine. Acta. Pharmacol. Sin. 24: 841-846.

Irshad S, Singh J, Kakkar P, Mehrotra S (2009). Molecular characterization of *Desmodium* species-An important ingredient of 'Dashmoola' by RAPD analysis. Fitoterapia, 80: 115-118.

Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I (2005). Antimicrobial and cytotoxic activity of *Ruta graveolens*. Fitoterapia, 76: 344-347.

Khan S, Mirza KJ, Anwar F, Abdin MZ (2010). Development of RAPD markers for authentication of *Piper nigrum* (L.). Environ. We Int. J. Sci. Technol. 5: 553-562.

Khan S, Mirza KJ, Abdin MZ (2010). Development of RAPD Markers for Authentication of Medicinal Plant *Cuscuta Reflexa*. EurAsia. J. BioSci., 4(1): 1-7.

Khan S, Mirza KJ, Tayaab M, Abdin MZ (2009). RAPD profile for authentication of medicinal plant *Glycyrrhiza glabra* Linn. MAPSB. 3(1): 48-51.

Khan S, Qureshi MI, Kamaluddin, Tanweer A, Abdin MZ (2007). Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. Afr. J. Biotechnol. 6: 175-178.

Kostova I, Ivanova A, Mikhova B, Klaiber I (1999). Alkaloids and coumarins from *Ruta graveolens*. Monatsh. Chem. 130: 703-707.

Kuzovkina I, Al'terman I, Schneider B (2004). Specific accumulation and revised structures of acridone alkaloid glucosides in the tips of transformed roots of *Ruta graveolens*. Phytochem. 65: 1095-1100.

Li J, Wan DR, Chem KL (2007). RAPD analysis of 8 medicinal species of *Selaginella*. Zhong Yao Cai. 4: 403-406.

Maurya R, Srivastava S, Kulshreshta DK, Gupta CM (2004). Traditional remedies for fertility regulation. Curr. Med. Chem. 11: 1431-1450.

McClelland M, Mathieu-Daude F, Welsh J (1995). RNA fingerprinting and differential display using arbitrarily primed PCR. Trends Genet. 11: 242-246.

Meepagala KM, Schrader KK, Wedge DE, Duke SO (2005). Algicidal and antifungal compounds from the roots of *Ruta graveolens* and synthesis of their analogs. Phytochemistry, 66(22): 2689-2695.

Micheli MR, Bova R, Pascale E, Ambrosio ED (1994). Reproducible DNA fingerprint with the random amplified polymorphic DNA (RAPD) method. Nucleic Acids Res. 22: 1921-1922.

Miguel ES (2003). Rue (*Ruta* L., Rutaceae) in traditional Spain: frequency and distribution of its medicinal and symbolic applications. Econ. Bot. 57: 231-244.

Milesi S, Massot B, Gontier E, F Bourgaud, Guckert A (2001). *Ruta graveolens* L.: a promising species for the production of furanocoumarins. Plant Sci. 161: 189-199.

Na H, Um J, Kim S, Koh K, Hwang W, Lee K, Kim C, Kim H (2004). Molecular discrimination of medicinal *Astragali radix* by RAPD analysis. Immunopharmacol. Immunotoxicol. 26: 265-272.

Nieri P, Adinolfi B, Morelli I, Breschi MC, Simoni G, Martinotti E (2003). Genetic characterization of the three medicinal *Echinacea* species using RAPD analysis. Planta Med. 69: 685-686.

Oliva A, Meepagala KM, Wedge DE, Harries D, Hale AL, Aliotta G, Duke SO (2003). Natural fungicides from *Ruta graveolens* (L.) leaves, including a new quinolone alkaloid. J. Agric. Food. Chem. 51(4): 890-896.

Qi J, Li X, Song J, Eneji AE, Ma X (2008). Genetic relationships among *Rehmannia glutinosa* cultivars and varieties. Planta Med. 74: 1846-1852.

Queener SF, Fujioka H, Nishiyama Y, Furukawa H, Bartlett MS, Smith JW (1991). *In vitro* activities of acridone alkaloids against Pneumocystis carinii. Antimicrob. Agents Chemother., 35: 377-379.

Raghav SK, Gupta B, Agrawal C, Goswami K, Das HR (2006). Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. J. Ethnopharmacol. 104: 234-239.

Rahman SZ, Latif A, Singhal KC (2003). A case report of ADR due to misidentification of an indigenous drug. Indian Pharmacol. 35: 128-136.

Ratheesh M, Shyni GL, Sindhu G, Helen A (2009). Protective effects of isolated polyphenolic and alkaloid fractions of *Ruta graveolens* on acute and chronic models of inflammation. Inflammation, 33(1): 18-24.

Rivera-Arce E, Gattuso M, Alvarado R, Zarate E, Aguero J, Feria I, Lozoya X (2007). Pharmacognostical studies of the plant drug *Mimosae tenuiflorae cortex*. J. Ethnopharmacol. 113: 400-408.

Rout GR (2006). Identification of *Tinospora cordifolia* (Willd.) Miers ex Hook F & Thomas using RAPD markers. Zeitschrift fur Naturforschung C. 6: 118-122.

Sasikumar B, Syamkumar S, Remya R, Zachariah TJ (2004). PCR based detection of adulteration in the market samples of turmeric powder. Food Biotechnol. 18: 299-306.

Shaw PC, But PPH (1995). Authentication of *Panax* species and their adulterants by random-primed polymerase chain reaction. Planta Med. 61: 466-469.

Shinde M, Dhalwal K, Mahadik KR, Joshi KS, Patwardhan BK (2007). RAPD analysis for determination of components in herbal medicine. eCAM, 4(1): 21-23.

Skidmore-Roth L (2001). Mosby's Handbook of Herbs and Natural Supplements, 1st Ed., Mosby, New York, pp. 741-744.

Townsend CC (1968) Rutaceae. In: Tutin, T.G. (Ed.), Flora Europea, vol. 2, Rosaceae to Umbelliferae. Cambridge University Press, Cambridge, pp. 227-230.

Trovato A, Monforte MT, Forestieri AM, Pizzimenti F (2000). *In vitro* anti-mycotic activity of some medicinal plants containing flavonoids.

Bollettino chimico farmaceutico, 139(5): 225-227.
Um J, Chung H, Kim M, Na H, Kwon H, Kim J, Lee K, Lee S, Lim JP, Do K, Hwang W, Lyu Y, An N, Kim H (2001). Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. Biol. Pharm. Bull. 24: 872-875.
Varamini P, Soltani M, Ghaderi A (2009). Cell cycle analysis and cytotoxic potential of *Ruta graveolens* against human tumor cell lines. Neoplasma, 56(6): 490-493.
Yamamoto N, Furukawa H, Ito Y, Yoshida S, Maeno K, Nishiyama Y (1989). Anti-herpesvirus activity of citrsinine-I, a new acridone alkaloid, and related compounds. Antivir. Res. 12: 21-36.
Zargari A (1990). Medicinal Plants. Tehran University Press, Tehran, in Persian.
Zhang Y, But PP, Wang Z, Shaw P (2005). Current approaches for the authentication of medicinal *Dendrobium* species and its products. Plant Genet. Res. 3: 144-148.