ejpmr, 2016,3(1), 259-266

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

MOLECULAR IDENTIFICATION OF THE MEDICINAL PLANT JUSTICIA GENDARUSSA USING MATK GENE

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Article Received on 20/10/2015 **Article Revised on 13/11/2015** **Article Accepted on 05/12/2015**

ABSTRACT

Herbal plants play a major role in the development of modern civilization. *Justicia gendarussa* is an interesting example for traditional plant with medicinal values and have been proved by many research works. The present study which is focused on the Molecular characterization for the plant *Justicia Gendarussa* using Matk gene shows that the DNA isolated from was isolated from *Justicia gendarussa* has purity of purity 1.8. The purified DNA obtained was amplified using a matK primer. The amplified product of matK gene was found in the region of 750 – 1000 base pairs corresponding to the ladder. The PCR product using two sets of reverse primers were obtained and the result showed that reverse 1 primer was able to amplify the CO I gene better than reverse 2 primer. This plant is found to have a broad spectrum of activities due to the presence of active constituents like alkaloids, flavus compounds, phenolic compounds, steroids, carbohydrate, carotenoids and terpenoids. Various studies explored this plant has various pharmacological actions like antioxidant, analgesic, anti-inflammatory, anti-anxiety, antiangiogenic, antiarthritic, prevent damage to the liver, anticancer, active against bacteria, destroying fungi and anthelmintic. The study on the mechanism of action of this plant leaves could be useful for the development of commercial drugs.

KEY WORDS: Barcodes, COI gene, sequence alignment.

INTRODUCTION

Justicia gendarussa Burm f. Syn: Gendarussa vulgaris is an erect under shrub, 0.6 to 1.2 m in height with subterete leaves, simple, much longer or linear shape and it has 7.5 to 12.5 cm long, glabrous, short- petioled, pale green in bottom and dark violet green at top, contains 8 pairs of main nerves, mid rib and main nerves prominent on the under surface. Stems and branches are dark violet. Flowers are 5 – 12.5 cm long from the uppermost leaf axils, white colored, spotted with purple and clustered in the routes spikes. Shapes of the fruits are smooth. Calyx is 3.8 – 5 mm with nearly smooth linear segments. The leaves and roots are pungent, febrifuge, thermogenic, antiemetic, grievances, as an emmenagogue, diaphoretic, insecticidal and antipyretic[1].

Transverse section of leaf of *J. gendarussa* shows midrib and lamina portion. The midrib shows a cross section outline of the shape forming and biconvex in middle and apical regions. It has a single layered epidermis that are covered externally with flexible and presence of both covering and glandular hairs having unicellular stalk and multi cellular head. Collenchyma are well developed, which is present in upper epidermis of bottom part and the lower epidermis of top. There are three vascular bundles (VB) in middle region, which are collateral and open. The central one is large and arc shaped. Lamina is extending from dorsal and lateral, differentiated into palisade and spongy parenchyma. Upper epidermisis with mono layered, covered with a cuticle. It shows the presence of glandular hair with unicellular stalk and multi cellular of head and cystoliths. Lower epidermises are similar to upper, but mostly contains cross cells and few of the unequal cells. The defense cells are with bilayered in the lamina and becomes single layered in the midrib. Presence of fragments in upper and lower epidermis shows epidermal cells, pore, cystoliths, and glandular hairs. The epidermal cells are irregularly shaped having unequal and circumference of a plant organ. Pores are cross cell and unequal size and more in number, with numerous secreted hairs[2].

Lower epidermal cells are polygonal with glandular hairs in which few are unicellular or multi cellular stalk and multicellular head. Simple covering hairs are comparatively less than pores, cystoliths are oval to rectangular with unsmooth and starch grains are simple. The leaf was found to be rich in carotenoids, phenolics,

alkaloids, flavonoids and carbohydrates, triterpeninic acids[3].

Fig. 1: Justicia gendarussa

DNA Barcoding

DNA barcoding is a taxonomic method in which a short genetic marker DNA is used to identify to which an organisms or a particular species belongs. It helps to identify an unknown sample in terms of preexisting classification. Barcodes are used to identify whether unknown species in sample should be combined or separated. The most commonly used barcode region in animal is a segment of mitochondrial gene cytochrome oxidase I (COI) that contains 600 base pairs approximately. Applications include identifying plant leaves due to absences of flowers or fruit, helps to identifying larvae stages of insects, which may not have significant characters than adults and are less well known, identifying the nutrition level of an animals, and identifying products of herbal supplements, wood, or skin and other animal parts[4].

Specific locus should be standardized in DNA barcoding, that are present in most of the texa of interest and sequenceable without specific PCR primers , short time to be easily sequenced with current technology, and provide a large variation between species but relatively less variation within a species. A several loci set as standardized regions were selected by the respective committees[5]. The mitochondrial COI genes are used for animals and other eukaryotes as standard. For the plant, the concatenation of the rbcL and matK, chloroplast genes are used. These genes are providing poor resolution for land plants, and the regions to be assessed that could complement rbcL and matK. It can be applied in algae, animal and also fungi, perhaps to a lesser degree due to a lower incidence of hybridization compared to higher plants. Population genetic studies done by these techniques have large numbers of specimens at their disposal when the DNA quality is a lesser concern and high-quality DNA samples gives more accurate in barcoding techniques[6, 7].

To improve species concepts, it is important to develop a more sophisticated approach for barcoding[8]. It ideally include sequences from multiple independent markers, a multi-locus barcode, and specific inference tools that could be used to be limits and identify genetic 'gaps', and also improve the information base depend on cruder plastid and mitochondrial DNA barcodes[9]. Molecular analyse data says that several barcodes DNA regions are suitable in plants. It has four significant limitations in identification of species. First, both phenotypic plasticity and genetic variability in the characters employed for species recognition can leads to incorrect identifications. Secondly, the morphological cryptic taxa, which are common in many groups. Third, since morphological keys are often effective only for a particular life stage or gender, many individual species cannot be identified. Finally, the modern interactive versions represent a major advance; the use of keys often demands in high level of expertise that misdiagnoses are common[10].

Maturase K gene (matK)

The chloroplast maturase K gene (matK) is present within the introns of trnk gene, with the exception of some ferns. The gene contains approximately 1535 base pairs in monocotyledons that are called as encoded group of chloroplast intron maturase. Universal primers located in within the trnK gene are used to amplify the entire gene region for phylogenetic studies in orders or families, but are sometimes effectively used on genus or species level, i.e. in the genus Paeonia (Paeoniaceae). Only a 600-800 bp region of the matK gene are utilized for DNA-barcoding purposes. The matK gene evolves three times faster than rbcL and atpB. Some studies suggested that it can be effectively discriminate between species in the angiosperms.[11]

***Fig.2*: The matK chloroplast coding region based on the schematic drawing of Wakasugi *et al.* (1998), Matsumoto *et al.* (1998), Shaw *et al.* (2005) and Barthet & Hilu (2007)**

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Scrophulariales
Family	:	Acanthaceae
Genus	:	*Justicia*
Species	:	*gendarussa*

Local names of the plant

English	:	Black vasa, Black Malabar nut
Hindi	:	Nilinargandi, Udasanbhalu
Kannada	:	Karinekki
Marathi	:	Bakas, Kalaadulsa
Malayalam	:	Vatankolli, Vatankutti, Karinochil
Sanskrit	:	Krishnanirgundi, Nilanirgundi, Indrani
Tamil	:	Vadaikkutti, Karunochi.
Telugu	:	Addasarambu, Nallavavili,
Bengal	:	Jagatmadan, Jogmodon

Bombay : Kalaadulsa
Chinese : Ch'in ch'iu
Deccan : Kalishanbali
Burma : Bavanet, Bawanet

Medicinal Uses

From many studies it is evident that *Justicia gendarussa* have antioxidant, hepatoprotective, anthelmintic, anti inflammatory, antiarthritic, antiangiogenic, antimicrobial, analgesic and antianxiety activities. Phytochemical screening indicated that the leaf was rich in carotenoids, phenolic compounds, alkaloids, carbohydrates, flavonoids (flavanone), sterols and triterpenoides as other important constituents. The analysis of phytochemical are useful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also the source for pharmaceutical chemical compounds[12].

It can be used as a folk medicine[13]. The ethanolic extract form *Justicia gendarussa Linn* leaves shows significant in anti-arthritic activity similarly to aspirin which is against Freund's adjuvant-induced and collagen-induced arthritic rat models[14].

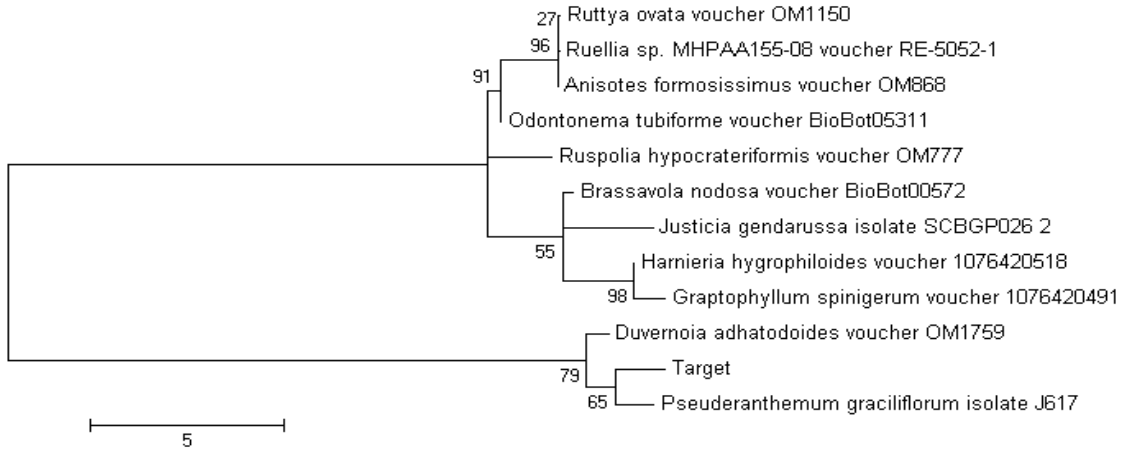
The methanolic extract from leaf of *Justicia gendarussa* possesses potent anti-inflammatory activity by inhibiting the release of prostaglandins or other endogenous compounds from cell membrane[15]. It is possible to formulate natural anti inflammatory, anti-bacterial drugs[16].

Chemical constituents

The quantitative estimation of leaves of *Justicia gendarussa* shows the presence of alkaloids, flavus compounds, tri terpenoids, carotenoids, phenolic compounds ,sugar and starch[17]. Some simple aromatic amine like benzyl alcohol and their respectively 0-methyl ethers, 2-amino benzyl alcohol stigmasterol, lupeol , 16-hydroxylupeol, 28 ß-sitosterol, aromadendrin, ß -Sitosterol- ß -D-glycoside 29 and can also be isolated male antifertility compound like gendarusin A and gendarusin B 30 from the plant. The leaves also contain betasitosterol, lupeol, an alkaloid, friedelin and aromatic amines[18]. The present study of us focused on the Molecular characterization for the plant *Justicia Gendarussa* using Matk gene.

Phylogenetic tree of *Justicia Gendarussa*

Original Tree



Bootstrap tree

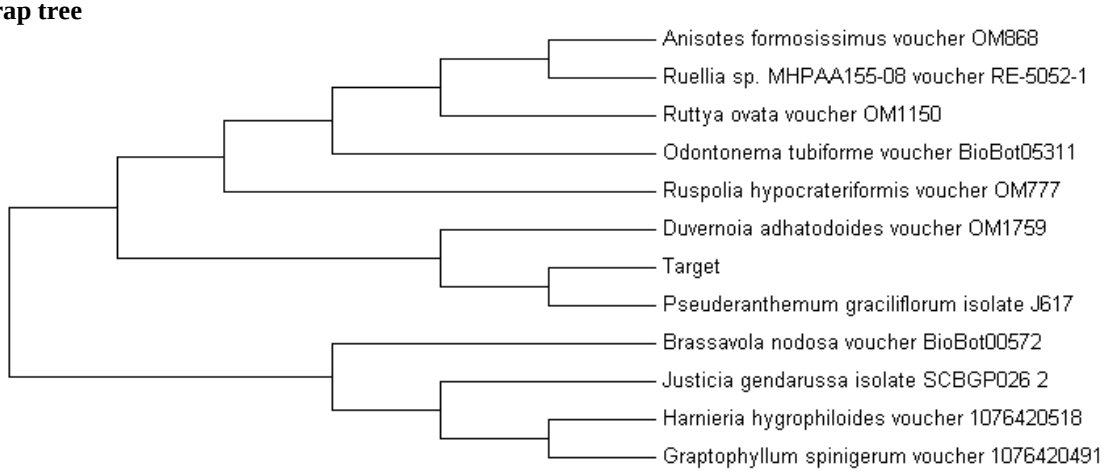


Fig.3: Phylogenetic tree of justicia *gendarussa*

MATERIALS AND METHODS

DNA isolation

The isolation of DNA from the plant was done by method followed by Doyle and Doyle, 1990: Fresh-leaf tissue (0.5 g) was ground in a 1.5-mL centrifuge tube with a mortar and pestle and 4mL of preheated, freshly prepared CTAB extraction buffer was immediately added to the tube. The tubes were allowed to incubate at 65°C for 60min, with inversion during incubation. Add an equal volume of chloroform: isoamyl alcohol (24:1, v/v) to the sample tubes and then inverted into 8-10 times. The tubes were centrifuged at 10,000 rpm for 15 min. The supernatant was relocated to a new centrifuge tube. An equal volume of absolute ice-cold isopropanol was added. The tubes were centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was washed with 70% (v/v) ethanol. The pellet was air-dried at room temperature and then dissolved in 50 µL TE buffer. The DNA samples were stored at -20°C until further use[19].

Qualitative Analysis - Agarose Gel Electrophoresis

Agarose gel was prepared with 1X TAE buffer and stained with 2µlof ethidium bromide. The % of agarose depends upon the molecule to be separated. Samples loaded with loading dye (2µl of loading dye is used). Electrophoresis of DNA fragments by electrical power is 50volts. Visualization of DNA fragments in the UV trans-illuminator.

Quantitative Analysis- Spectrophotometric Method

A solution of nucleic acids strongly absorbs UV with an absorbance maximum of 260nm and proteins at 280nm which is linearly related with the concentration of DNA, RNA and the amount of contamination of the solution in the solution. The intense absorption is primarily due to the presence of aromatic rings in the purine, pyrimidine. The concentration of nucleic acid in a solution can be calculated if one knows the value of A_{260} of the solution. Proteins are usually the major contaminants in nucleic acids extract and these have absorbsance maximum in 280nm. The ratio of absorbance between in 260 and 280nm hence provides a clear idea about the extent of contamination in the preparation. A ratio between 1.7 and 1.9 is indicative of fairly pure DNA preparation. But values less than 1.8 signify the presence of proteins as impurities. The values greater than 1.8 signify the presence of organic solvent in the DNA preparations. A ratio of 1.8 determines the pure DNA preparation.

The concentration of DNA sample was calculated using the given formula:

1. Concentration of dsDNA = A_{260} X 50µg X dilution factor
2. Purity of the DNA = A_{260} : A_{280} ratio = A_{260} / A_{280}
   = 1.8; pure DNA
   = 1.7 – 1.9; fairly pure DNA (acceptable ratio for PCR)
   = less than 1.8; presence of proteins.
   = greater than 1.8; presence of organic solvent

Polymerase Chain Reaction

PCR was carried out in Eppendorf Personnel Master Cycler (Germany). The PCR reaction constituents are the following

Optimized PCR condition: *Matk*

Milli Q water	-	7.8µl
10x Buffer with 20mM $Mgcl_2$	-	2.0µl (1x)
2mM DNTP's	-	2.0µl (0.2µM)
DNA	-	4.0 µl
3µM Forward Primer	-	2.0 (0.3µM)
3µM Reverse Primer	-	2.0µl (0.3µM)
Taq polymerase (5U/µl)	-	0.2µl (1unit/1 µl)

The Total Volume of the reaction is 20µl. The concentration of DNA was varied from 0.5 µl to 1 µl for optimization. The Whole reaction setup was carried out at 4°C.

PCR Programme

Step 1	Initial denaturation cycle	-95°C for 2minutes
Step 2	Denaturation	-94^{0}C for 45seconds
Step 3	Primer annealing	-47^{0}C for 1minute
Step 4	Extension	-72^{0}C for 1minute 20 seconds
Step 5	Go to step 2 repeat 35 times	
Step 6	final extensions	-72^{0}C 7 minutes
Step 7	Hold	- 4^{0}C

Gel Electrophoresis of PCR Products

DNA quality was assessed on a 1.5% Agarose Gel (in Tris Acetate EDTA buffer) electrophoresis at 50 Volts. DNA was stained with Ethidium Bromide visualized on a UV transilluminator[20].

RESULTS

Genomic DNA isolation

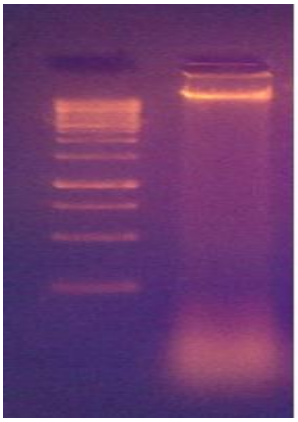


Fig.4: Genomic DNA of Justicia gendarussa

Lane 1: 1 Kb Ladder **Lane 2:** Genomic DNA of *Justicia gendarussa*

PCR matK

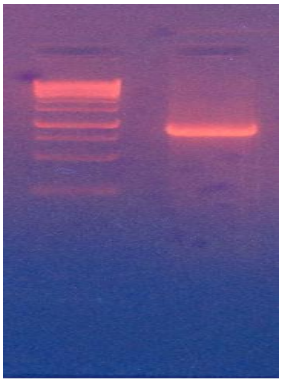


Fig. 5: PCR matK

Lane 1: 1 Kb Ladder **Lane 2:** *Justicia gendarussa* 780bp

Sequence

>TCAACGAAAAGGGCGTCTTCTTCTTTGCATTTA
GTACGGTTTTTTCTCAACGAGTATTGTAATTGGA
ATACT
CTTATTAGGCTAAATAAAGCCAGTTTCTCTTTTT
CAAAAAGAGATGAAAGGTTATTCGTATTCTTAT
ATAA
TTCTCATGTAGGGGAATATGAATCCGTTTTATTC
TTTTTACGTAACCAATCTTCTCATTTACGATCAA
CAT
CTTTTGGAGTTTTTATTGAACGAATCTCTTTCTAT
GGAAAAATAGAATATTTTGTGAACGTCTTTGTTA
AA
GCTAAGGATTTTCAGGGAAACCTGTGGTTGGTC
AAGGAACCTTGCATGCATTATATTAGGTATCAA
AGAAG
GTTCATCTTGGCTTCAAAAGGGACGTCACTTTTC
ATGAATAAATGGAAATGGTACCTTGTCACTTTTT
GGC
AAGGGCATTTTTCACTATGGTTTCATCCAAGAAG
GATTTATCGAAACGAATTATGCAATCATTTCCTT
GAA
TTTTTGGGTTATCTTTCAAGCGTGTGGATGAAAC
CTTCAGTGATACGGAGCAAAATTTTGGAAAATG
CATT
CCCAATCAATAATGCTATTAAGAAGTTCGATAC
CCTTATTCCAATTTTTTTAATAATTGCGTCATTG
GCTA
AAGCGAAATTTTGTAACGTATTAGGCCATCCTAT
TAGTAAGTCGGTTTGGGCTGATTTATCAGATTCT
AAT
ATTATTCAACGATTTGGGCGTATATGCAGAAAC
CTTTCTCATTATCATAGCGGATCT

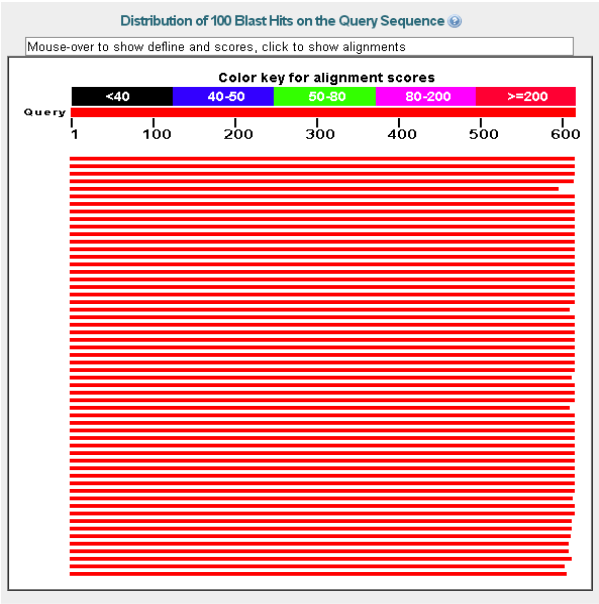


Fig. 6: Graphical Representation for sequence alignment

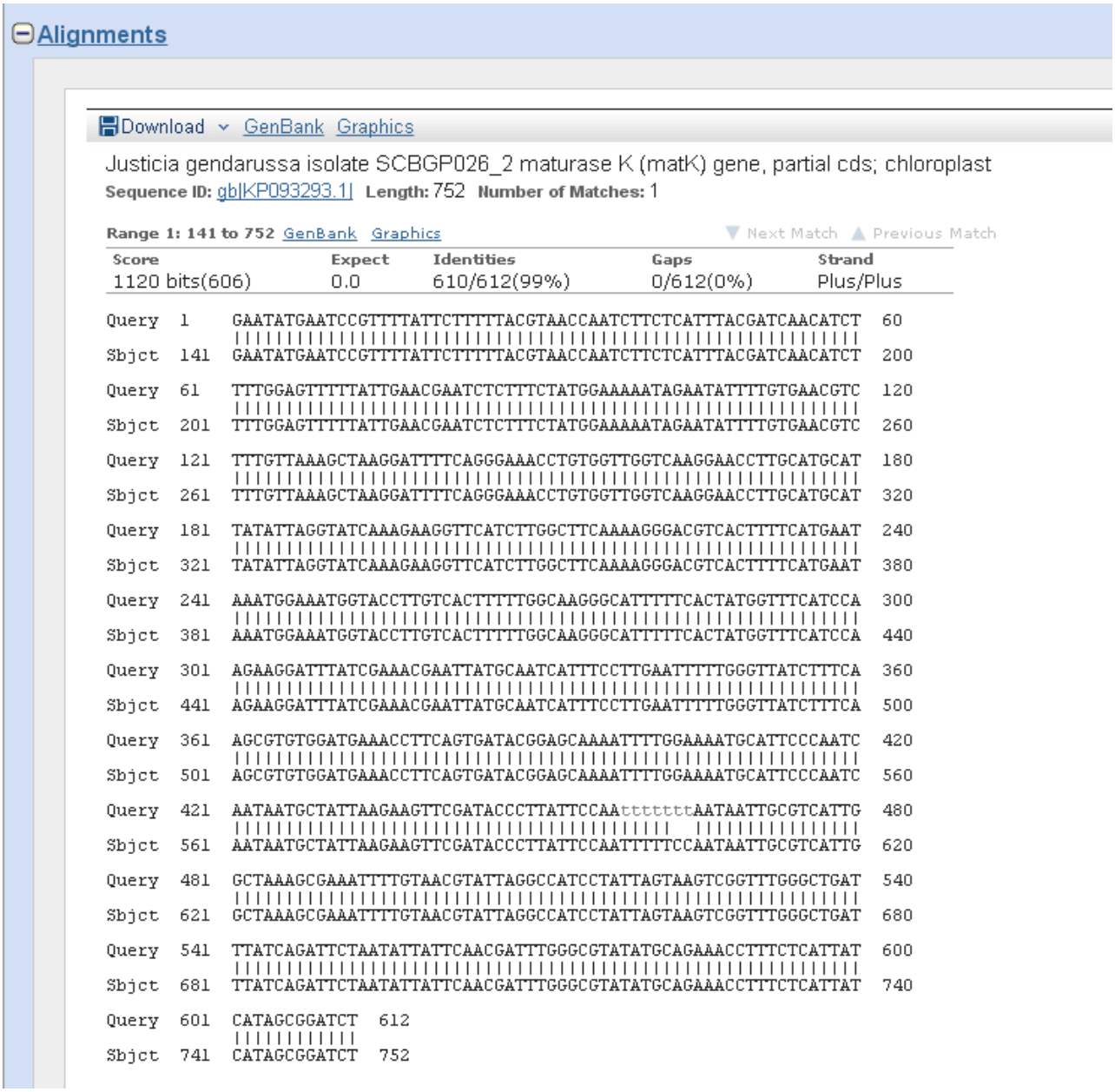
Sequence alignment representation

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Justicia gendarussa isolate SCBGP026_2 maturase K (matK) gene, partial cds; chloroplast	1120	1120	100%	0.0	99%	KP093293.1
Justicia gendarussa isolate SCBGP026_1 maturase K (matK) gene, partial cds; chloroplast	1120	1120	100%	0.0	99%	KP093292.1
Justicia ventricosa isolate SCBGP070_1 maturase K (matK) gene, partial cds; chloroplast	1103	1103	100%	0.0	99%	KP093371.1
Justicia ventricosa isolate SCBGP070_2 maturase K (matK) gene, partial cds; chloroplast	1101	1101	99%	0.0	99%	KP093372.1
Justicia gendarussa isolate OciSeq_J2 maturase K-like gene, partial sequence; chloroplast	1051	1051	96%	0.0	99%	JF499943.2
Justicia campylostemon voucher OM2299 maturase K (matK) gene, partial cds; chloroplast	1037	1037	100%	0.0	97%	JX518170.1
Duvernoia adhatodoides voucher OM1759 maturase K (matK) gene, partial cds	1037	1037	100%	0.0	97%	JF270753.1
Duvernoia aconitiflora voucher OM1816 maturase K (matK) gene, partial cds	1037	1037	100%	0.0	97%	JF270752.1
Metarungia longistrobus voucher CS15 maturase K (matK) gene, partial cds	1037	1037	100%	0.0	97%	JF270864.1
Justicia adhatoda isolate Ada11 maturase K (matK) gene, partial cds; chloroplast	1020	1020	100%	0.0	97%	KC616330.1
Justicia adhatoda isolate Ada8 maturase K (matK) gene, partial cds; chloroplast	1020	1020	100%	0.0	97%	KC616327.1
Justicia adhatoda isolate Ada7 maturase K (matK) gene, partial cds; chloroplast	1020	1020	100%	0.0	97%	KC616326.1
Justicia adhatoda isolate Ada4 maturase K (matK) gene, partial cds; chloroplast	1020	1020	100%	0.0	97%	KC616323.1
Justicia adhatoda isolate Ada1 maturase K (matK) gene, partial cds; chloroplast	1020	1020	100%	0.0	97%	KC616320.1
Justicia adhatoda isolate Ada3 maturase K (matK) gene, partial cds; chloroplast	1014	1014	100%	0.0	97%	KC616322.1
Justicia adhatoda isolate Ada2 maturase K (matK) gene, partial cds; chloroplast	1014	1014	100%	0.0	97%	KC616321.1
Justicia macrantha voucher BioBot10260 maturase K (matK) gene, partial cds; chloroplast	1014	1014	100%	0.0	97%	JQ586412.1
Justicia macrantha voucher BioBot10258 maturase K (matK) gene, partial cds; chloroplast	1014	1014	100%	0.0	97%	JQ586410.1
Ruellia sp. MHPAA155-08 voucher RE-5052-1 maturase K (matK) gene, partial cds; chloroplast	1009	1009	100%	0.0	96%	JQ589888.1
Ruellia sp. MHPAA156-08 voucher RE-5052-2 maturase K (matK) gene, partial cds; chloroplast	1009	1009	100%	0.0	96%	JQ589889.1
Justicia macrantha voucher BioBot10259 maturase K (matK) gene, partial cds; chloroplast	1009	1009	99%	0.0	97%	JQ586411.1
Justicia brenesii voucher BioBot05854 maturase K (matK) gene, partial cds; chloroplast	1009	1009	100%	0.0	96%	JQ586398.1
Justicia brenesii voucher BioBot05853 maturase K (matK) gene, partial cds; chloroplast	1009	1009	100%	0.0	96%	JQ586397.1



Alignments

Download GenBank Graphics

Justicia gendarussa isolate SCBGP026_2 maturase K (matK) gene, partial cds; chloroplast

Sequence ID: gb|KP093293.1| **Length:** 752 **Number of Matches:** 1

Range 1: 141 to 752 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1120 bits(606)	0.0	610/612(99%)	0/612(0%)	Plus/Plus

```
Query  1    GAATATGAATCCGTTTTATTCTTTTTACGTAACCAATCTTCTCATTTACGATCAACATCT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  141  GAATATGAATCCGTTTTATTCTTTTTACGTAACCAATCTTCTCATTTACGATCAACATCT  200

Query  61   TTTGGAGTTTTTATTGAACGAATCTCTTTCTATGGAAAAATAGAATATTTTGTGAACGTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  201  TTTGGAGTTTTTATTGAACGAATCTCTTTCTATGGAAAAATAGAATATTTTGTGAACGTC  260

Query  121  TTTGTTAAAGCTAAGGATTTTCAGGGAAACCTGTGGTTGGTCAAGGAACCTTGCATGCAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  261  TTTGTTAAAGCTAAGGATTTTCAGGGAAACCTGTGGTTGGTCAAGGAACCTTGCATGCAT  320

Query  181  TATATTAGGTATCAAAGAAGGTTCATCTTGGCTTCAAAAGGGACGTCACTTTTCATGAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321  TATATTAGGTATCAAAGAAGGTTCATCTTGGCTTCAAAAGGGACGTCACTTTTCATGAAT  380

Query  241  AAATGGAAATGGTACCTTGTCACTTTTTGGCAAGGGCATTTTTCACTATGGTTTCATCCA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381  AAATGGAAATGGTACCTTGTCACTTTTTGGCAAGGGCATTTTTCACTATGGTTTCATCCA  440

Query  301  AGAAGGATTTATCGAAACGAATTATGCAATCATTTCCTTGAATTTTTGGGTTATCTTTCA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  441  AGAAGGATTTATCGAAACGAATTATGCAATCATTTCCTTGAATTTTTGGGTTATCTTTCA  500

Query  361  AGCGTGTGGATGAAACCTTCAGTGATACGGAGCAAAATTTTGGAAAATGCATTCCCAATC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  501  AGCGTGTGGATGAAACCTTCAGTGATACGGAGCAAAATTTTGGAAAATGCATTCCCAATC  560

Query  421  AATAATGCTATTAAGAAGTTCGATACCCTTATTCCAAttttttAATAATTGCGTCATTG  480
            ||||||||||||||||||||||||||||||||||||||||||  ||||||||||||||||
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Query  481  GCTAAAGCGAAATTTTGTAACGTATTAGGCCATCCTATTAGTAAGTCGGTTTGGGCTGAT  540
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Query  541  TTATCAGATTCTAATATTATTCAACGATTTGGGCGTATATGCAGAAACCTTTCTCATTAT  600
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Sbjct  681  TTATCAGATTCTAATATTATTCAACGATTTGGGCGTATATGCAGAAACCTTTCTCATTAT  740

Query  601  CATAGCGGATCT  612
            ||||||||||||
Sbjct  741  CATAGCGGATCT  752
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DISCUSSION

Our work on *Justicia gendarussa* is mainly focused on identification, classification and gene characterization. The gene characterization is done by generating a DNA barcode using the mature K gene (matK) in the chloroplast of the leaf sample. The DNA was isolated by using Doyle and Doyle method and on quantification the purity of DNA is 1.8. The purified DNA thus obtained was amplified using a matK primer. The amplified product of matK gene was found in the region of 750 – 1000 base pairs corresponding to the ladder.

Justicia gendarussa of genus classified under the same family *Acanthaceae* were collected for comparison of analysis of the COI gene. Identification was based on species-specific diagnostic characters, frozen, surface sterilized prior to DNA extraction. The DNA was extracted using a DNA extraction kit with a standard protocol. The extracted DNA was visualized in gels under UV trans illuminator, and quantitative estimation of the sample was done using UV spectrophotometer at 260nm and 280nm to check the concentration of DNA extracted from the respective specimens. The results of purity ratio obtained show variations in readings. The PCR product using two sets of reverse primers were obtained and the result showed that reverse 1 primer was able to amplify the CO I gene better than reverse 2 primer.

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