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DETERMINE THE ANTI-BACTERIAL PROPERTIES OF MIMOSA PUDICA PLANT AGAINST SELECTED MICRO-ORGANISMS BY PLATING

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ABSTRACT

Plants have been the traditional sources of raw materials for medicine. The benefits of using the natural products has increased and the active plant extracts are frequently used for new drug discoveries and for the presence of antimicrobials.*Mimosa pudica* also called sensitive plant which folds itself when touched and spreads its leaves once again after a while. The plant extracts from various parts of *Mimosa pudica* may be used by those who practice Ayurveda. *M.pudica* is also used to avoid or cure several disorders like cancer, diabetes, hepatitis, obesity and urinary infections. This plant is famous for its anticancer alkaloid, mimosine with another valuable secondary metabolites like tannins, steroids, flavonoids, triterpenes and glycosylflavones.

The plant was identified using NCBI primer blast and to identify the antibacterial effects of aqueous extraction of root and flowers of *Mimosa pudica* plant, well diffusion method was done against *E.coli* JM 101 strain, *Bacillus spp.*, *Pseudomonas spp.*bacterial cultures. The result of this study confirmed that the in-vitro antibacterial activity indicatesno significant activity against this selected bacteria in aqueous extract and there were no zone of inhibition and showed that the extracted DNA could be used directly for Polymerase Chain Reaction.

This research presented information about *Mimosa pudica* plant and that claims there is a vast potential in this herb in view of therapeutics and furthermore, commercialization of this herb would be in line with the World Health Organization (WHO) guidelines is highly desirable for the benefits of humanity. The general data have provided the basis for its wide use as therapeutic both in traditional and folk medicine. In recently, there are emerging many multidrug resistant human pathogenic bacteria.

KEYWORDS : Antibacterial, Antimicrobial, Medicinal plants, *Mimosa pudica*

INTRODUCTION

Historically, nature has been a source of medicinal agents which used to treat chronic as well as acute infectious diseases that based on the premises contain natural substances (Reed-guy *et al.*, 2017). In developing countries, the recent emergence of strains by reducing susceptibility to antibiotics raises and to search for new infection strategies (Ahmad, Mishra and Gupta, 2012). To develop alternative antimicrobial drugs to treat infectious diseases from medicinal plants because they have enormous therapeutic potential to heal many infectious diseases and that increasing prevalence of multidrug resistant strains of bacteria (Wong *et al.*, 2018). This strategies forced scientists to search for new antimicrobial substances because medicinal plants are not associated with many side effects (Samy, Ignacimuthu and Sen, 1998).

1.1 Biological source of the *Mimosa pudica* plant

Mimosa pudica is the herb first formally described by the Carl Linnaeus in 1753 which belongs to the taxonomic group Magnoliopsida and family *Mimosaseae* that herb showed sensation on touch (Muhammad *et al.*, 2016). This is a small or middle sized tree which contain pale brown seeds and the rods break into 2-5 segments and usually this is a short prickly plant with its branches growing close to ground which is well known for its rapid plant movement (Patro, Bhattamisra and Mohanty, 2016). Figure 1 illustrated the *Mimosa pudica* plant.

Figure 1: *Mimosa pudica*plant

All parts of the*Mimosa pudica* plant is considered to possess medicinal properties including the various biological and pharmacological activities (Linn, 2012). According to the Ayurveda, the root is bitter, acrid, vulnerary and used in the treatment of dysentery, biliousness, vagina and fatigue. Its leaves are useful in hydrocele, scrofula, cuts and bleeds and the whole plant is a rich sources of anti-diabetic, antitoxin, antioxidant, antipyretic, anti-inflammatory compounds (Chen *et al.*, 2004).

1.2 Principal constituents of Mimosa plant

Mimosa pudica plant is used to cure various alimentary due to the presence of secondary metabolites such as alkaloid, glycoside, flavonoid and tannins and *M. pudica* is also a valuable source of jasmonic acid and abscisic acid (Azmi, Singh and Akhtar, 2011). Several research works have been carried out to determine whether these phytochemical components of *Mimosa pudica* and this secondary metabolites of the plant extracts could be responsible for the antimicrobial activity (Patro, Bhattamisra and Mohanty, 2016). Table 1 showed the importance of the phytochemicals extracted from the plant.

Table 1: Importance of the phytochemicals extracted from the plant

Part of the plant	Compound	Known function/ Usage	References
Seeds	Hydrogelable materials and glucuronoxylan polysaccharide	Used for the delayed, sustained/ targeted release of different drugs.	(Akter *et al.*, 2010)
Leaves	Flavonoids	This is the phenolic compound that provide pigmentation for fruits, flowers, and seeds.	(Linn, 2012)

		flavonoids can resist to microbial because they inhibit the synthesis of nucleic acid, function of cytoplasmic membrane and energy metabolism	
Root and stem	Alkaloid	It has antispasmodic, antimalarial, analgesic and diuretic activities.	(Vijayalakshmi and Udayakumar, 2018)
Root	Mimosine	Mimosinehave potent antipolifertative and apoptotic effects.	(Muhammad *et al.*, 2016)
	Mucilage	This composed of d-xylose and d-glucuronic acid.	(Kaur *et al.*, 2011)
	Glycosides	It has antifungal, and antibacterial properties.	(Tomar, Shrivastava and Kaushik, 2014)
	Saponins	It possess anti-inflammatory, antiviral and plant defence activities.	(Joseph, George and Mohan, 2017)

1.3 Antimicrobial activity of *Mimosa pudica* plant

Mimosa pudica plant extracts of this plant has some properties under the laboratory study (Lakshmibai, 2018). In recently, antimicrobial properties would result in developing eco-friendly management of human infectious diseases by reducing side effects and the non-nutritive phytochemicals obtained from the *Mimosa pudica* plants have some characteristics including their difference mode of action (Medicine, 2017). Contemporary several research works are carrying out to study about the antibacterial, antifungal and insecticidal properties of the *Mimosa pudica* plants extracts has ability to disruption of bacterial membrane potential permeabilization and leakage of the cellular contents (Tomar, Shrivastava and Kaushik, 2014).

1.4 CetylTrimethylammonium Bromide (CTAB) method for plant extraction

DNA extraction from plant tissue can vary depending on the material used. In the CTAB extraction protocol, initially grinding stage is doing to break down cell wall materials. Once tissue has been sufficiently ground, it can then be resuspended in a CTAB buffer. In order to purify DNA, insoluble particulates are removed through centrifugation mixing with chloroform. DNA must be precipitated to remove contaminating salts (Alex and Aitken, 2012).

1.5 Polymerase Chain Reaction (PCR)

PCR is a molecular biology technique used to amplify a minute amount of DNA and the key ingredients of PCR reaction contain Taq polymerase, template DNA, primers, $MgCl_2$ and nucleoids (Patel *et al.*, 2015). There are three basic steps of the PCR including denaturation, annealing and the extension.

- Denaturation happens at 94^0C which breakdown the hydrogen bonds and denatures that into single stranded form.
- At the annealing stage the temperature is decreased to 50-65^0C and under this low temperature the DNA primers are attach to complementary sequence (Atawodi, 2016).
- Extension happens at 72^0C and at this step the reaction temperature is raise to make new strand of DNA by the Taq polymerase enzyme.

The steps of the polymerase chain reaction is summarized in the figure 2.

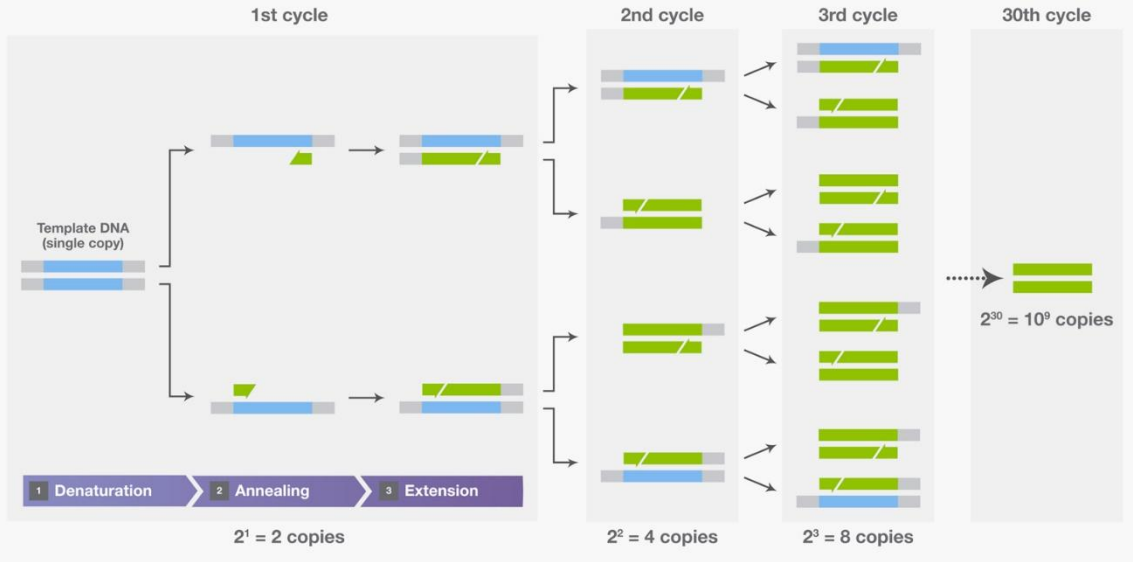


Figure 2: Steps of PCR (Journal et al., 2010).

1.5.1 Variants of conventional PCR

Polymerase chain reaction is a highly versatile technique and has been modified in different way to achieve specific outcomes suit specific applications.

1.5.1.1 Reverse Transcription PCR (RT-PCR)

In RT-PCR, RNA strand is reverse transcribed into its complementary DNA (cDNA) using reverse transcriptase enzyme and then the cDNA fragment is amplified using PCR. This is widely used in genetic disorder diagnostics and to obtain eukaryotic exon sequences from mature mRNAs(Atawodi, 2016).

1.5.1.2 Quantitative PCR (qPCR)

qPCR is used to detect and quantify nucleic acids and the data collection can be done by using either fluorescently labelled primers or probes as well as a using DNA binding dye such as SYBR green dye (Brasileira *et al.*, 2013).

1.5.1.3 Gradient PCR

This is the technique that allows to determine the optimal annealing temperature of a particular primer in one experiment. Gradient PCR provides one single run evaluates up to 12 different annealing, elongation, or denaturation steps and during this cycles, numbers of possible concentration parameter can be tested (Brasileira *et al.*, 2013).

1.5.2 Use of PCR for plant identification

DNA barcoding technique is used to sequence and analysis of sequenced products in PCR (Brasileira *et al.*, 2013). Fabaceae is one of the variety and complex family, because of this reason some of the major universal DNA barcode primers are used for PCR. Primers are short, synthetic, single stranded DNA sequence that binds to a complementary DNA sequence and enables addition of new deoxyribnucleotides by DNA polymerase at the 3' end (Wang, Qi and Cutler, 1993). The primers used for plant identifictions are trnH-psbA, trnL and atpF-atpH exhibited amplicon length polymorphism can simultaneously discriminate and the rbcL and matK produced monomorphic bands (Wallinger *et al.*, 2012). The basis of amplicon length polymorphism is important to visualize on agarose gel to identify the adulteration of herbal drug formulation. For plants, rbcL and matK are the standard DNA barcode markers which was recommended by Consortium for the Barcode of Life (CBOL)

Plant Working group, based on their high level of taxonomic resolution (Wallinger *et al.*, 2012).

Table 2: Characteristics of different primers(Sikdar et al., 2018), (Haider, 2015).

Primer name	Genomic source	Type
atpF-atpH	Plastid	Inter-genic spacer
matK	Plastid	Protein coding
rbcL	Plastid	Protein coding
trnH-PsbA	Plastid	Inter-genic spacer
trnL	Plastid	Intron

Table 2 represented the characteristics of different primers used for plant identification.

1.6 Agarose gel electrophoresis

This method is used in biochemistry, molecular biology, genetics and clinical chemistry to separate a mixed population of DNA in a matrix of Agarose(Li *et al.*, 2016). The Agarose gel apparatus is showed in figure 3. There are some factors which affect migration of nucleic acid in gel. They are,

- Concentration of the gel- The larger molecules are resolved better using a low concentration gel while small molecules separate better at high concentration gel.
- Size of the DNA fragment being electrophoresed- Smaller molecules travel faster than larger molecules in gel and double stranded DNA moves at a rate that is inversely proportional to the number of base pairs (Westermeier, Biosciences and Gmbh, 2005).
- Conformation of the DNA molecule- The movement of the DNA may be affected by the conformation of the DNA molecule (Anderson, Wright and Meksem, 2013).

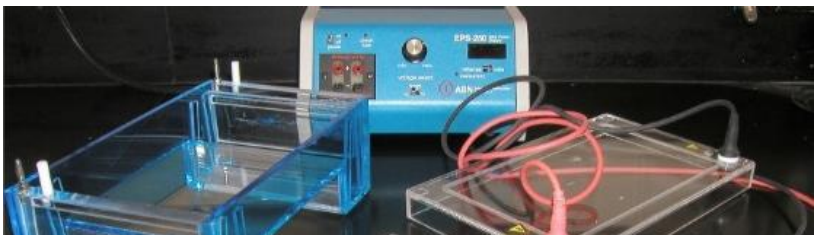
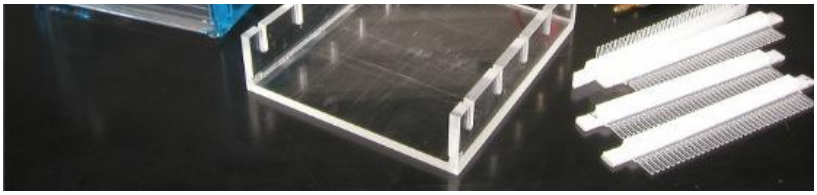


Figure 3: Gel electrophoresis Apparatus (Reddy and Raju, 2014)



1.7 Susceptibility testing for bacteria

The susceptibility test was performed to identify significant bacterial isolates and to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infection (Westermeier, Biosciences and Gmbh, 2005).

1.7.1 Susceptibility testing methods

1.7.1.1 Broth dilution tests

This is the oldest antimicrobial susceptibility testing method which can performed by 2 ways, using macro dilution or micro dilution method. This method involves the preparation of

twofold dilutions of antibiotics in a liquid growth medium dispensed in test tubes (Buller and Court, 2013).

1.7.1.2 Antimicrobial gradient method

This is the commercial method that provide microdilution panels, instrumentation and automated reading of plates which intended to reduce technical errors and time wastage(Buller and Court, 2013).

1.7.1.3 Disk diffusion method

This is the routinely used method used for determining antimicrobial resistance. Muller Hinton agar is considered to be the best media for this method. Because it shows the acceptable batch to batch reproducibility for susceptibility testing and gives satisfactory growth of nonfastidiuos pathogens (Hudzicki, 2016).

1.7.1.4 Agar dilution method

Agar dilution method involves the incorporation of varying concentrations of antimicrobial agent into an agar medium by using serial twofold dilutions, followed by the application of a defined bacterial inoculum to the agar plates. This method has the ability to test multiple bacteria and the potential to improve the identification of minimum inhibitory concentration (Buller and Court, 2013).

MATERIAL AND METHODS

2.1 Equipments and reagents

The autoclaving process was performed at 121^0C and 15Pa for 20 minutes unless otherwise specified. All glassware used for the experiment and distilled water used were sterilized by autoclaving and sterile plasticware was used to weigh Mueller Hinton agar powder (Eqitron media Pvt, Ltd).

PCR tubes, Eppendorf tubes, pipette tips were UV sterilized. The PCR cycling was carried out on ABI Thermal cycler (life technologies hold in Pvt, Ltd made in Singapore).Molecular grade reagents were used Dream Taq DNA polymerase 5U/µl, 500M, 10X Dream Taq Green buffer, 50-bp DNA ladder (Tracklt TM).

Centrifugation was done (TRASONS, SPINWIN MC 03, made in Brussels Belgium). TECHNO FAB dry bath was used and the analytical balance, Avery Berkel was used.

Bacterial culturing was done under the UV sterilized laminar flow and incubation of bacterial cultures were performed using incubator (Memmert GmbH Co. KG, Schwabach, FR of Germany) which require aseptic conditions to perform the experiment. Water bath Sshutzart, (DIN 40050/Sshutzart, Germany) was used to perform of bacterial tests at variants temperatures.

Dry bath was performed at 95˚C or 5 minutes (TECNO FAB).

2.2 Collection and preparation of plant material

The fresh plants of *Mimosa pudica* were collected from Welivita village, Colombo District, Sri Lanka. Then the roots, flowers and leaves were separated and washed thoroughly 10-15 minutes with running tap water to remove the soil particles and adhered debris and then with sterile water. They were air dried for 5 days at room temperature and then the plant materials were pulverized into powder and used for extraction.

2.3 Extraction of DNA from *Mimosa pudica* plant using CTAB method

100mg *Mimosa pudica*root sample was measured and transferred into labeled sterile 1.5ml micro-centrifuge tubes. Then 300µl of sterile deionized water was added into the tube and mixed followed by the addition of 500µl of CTAB buffer. After mixing the contents, 10µl of Proteinase K was added and incubate at 65^0C for 30-90 minutes. After the incubation period, 2µl of RNase A was added and shake. Then incubated at 65^0C for 5-10 minutes and centrifuged for 5 minutes at 13,000rpm. Supernatant was transferred into new tube containing

500µl chloroform and shake for 30 seconds. Then the sample was centrifuge for 5 minutes at 13,000rpm. 400µl upper layer was transferred to a new tubes containing 500µl chloroform and shake. Sample was centrifuge for 5 minutes at 13,000rpm and 400µl of supernatant was transferred to a new tube. The 2 volumes of CTAB precipitation solution was added and mixed by pipetting. Then the sample was incubate for 60 minutes at room temperature and centrifuge for 5minutes at 13,000rpm. After centrifugation, supernatant was discarded and precipitates were dissolved in 350µl of 1.2M NaCl. 350µl chloroform was added to tube and shake for 30 seconds. Then centrifuged for 5 minutes at 13,000rpm and 250µl of upper layer was transferred to the new tube followed by the addition of 0.6 volumes of isopropanol and shaked. Resulting solution was centrifuged for 10 minutes at 13,000rpm and discarded the supernatant. 500µl of 70% ethanol solution was added and shake carefully and centrifuged sample for 5 minutes at about 13,000rpm and discard the supernatant. Finally, pellet was air dried and dissolved DNA in 100µl sterile distilled water.
The same procedure was done for the flowers of *Mimosa pudica* plant and experiments were carried out in duplicates.

2.4 Polymerase Chain Reaction

Tubes were labelled accordingly and sterilized under the UV light for 15minutes, followed by preparation of master mix as in table 3.
Table 3: Reagents used for the master mix preparation

Reagents	**N=1(**µl)	**N=6(**µl)
Distilled water	13.5	81
5X PCR buffer	5	30
dNTP	0.75	4.5
PsbA3 forward primer	1	6
PsbA3 reverse primer	1	6
$MgCl_2$	2.5	15
Taq DNA polymerase	0.25	1.5

After preparing the master mix, 24µl was transferred into each tube and 1µl of DNA was added into each tube and PCR cycling was carried out as given in figure 4.

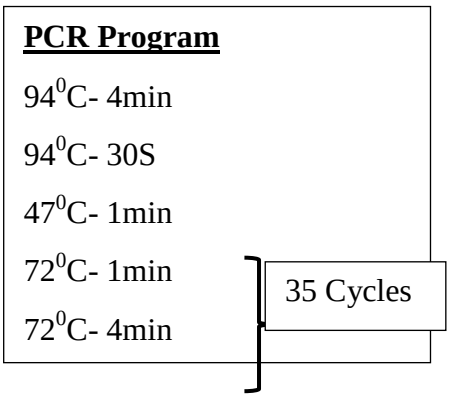


Figure 4: PCR program

2.5 Preparation of 2% Agarose gel

2 grams of Agarose powder was measured and transferred into a conical flask containing 100 ml of 0.5X TBE buffer and heated under a microwave oven until the powder completely dissolved. Once the Agarose was dissolved, conical flask was taken out and left the solution to cool down to the room temperature. Meanwhile, gel casting tray was taken and each ends were sealed with the aid of masking tapes and the comb was placed in the gel tray. Once the Agarose gel solution gets cooled, poured it into the tray and the

gel was kept for about 1hour to be set. After gel gets solidified, the comb was removed carefully and the gel was take out and kept for further use.

2.5.1 Gel electrophoresis

Initially, the gel was placed inside the gel electrophoresis apparatus and 0.5x TBE buffer was poured until the gel was fully submerged in the buffer.Then 50µl of Ethidium bromide was added into the buffer, mixed and samples were loaded. After that the lid was closed and electrodes were connected followed by providinga constant voltage of 50V for 5min.Finally the voltage was raised up to 100V and electrophoresis was carried out for another 1 hour duration and the gel was observed under UV light.

2.6 Antibacterial susceptibility test

2.6.1 Preparation of aqueous extracts

15 grams of powdered roots and flowers of *Mimosa pudica* were measured and 225ml of water was added into the conical flask. 15:1 solvent to sample ratio was used and the extractions were conducted at 40^0C for 4 hours in a thermostatic water bath. After that, the extracts were filtered using Whatman No. 1 filter paper and then the extracts obtained were labelled and stored at 4^0C for further use.

2.6.2 Preparation of bacteria culture

Escherichia coli, *Pseudomonas stutzeri*and *Bacillus cereus* isolates were provided by GenetechMolecular Diagnostics & School of Gene Technology, Colombo, Sri Lanka and those were cultured on LB broth for 24 hours at 37˚C.

2.6.3 Preparation of Muller Hinton agar plates

First, 19 grams of Muller Hinton agar powder was measured and transferred into a volumetric flask and top up to 500ml by adding distilled water. Then the solution was transferred into a media bottle and sterilized by autoclaving for 121^0C at 15psi for 30 minutes. Then plates were poured into sterilized petri dishes.

2.6.4 Bacterial inoculation

For prepare bacterial inoculation, serial dilution method was used. Initially 900µl of autoclaved distilled water was added to the tube and 100µl bacterial suspension was added to the same tube and mixed well. The second dilution sample was prepared using 100µl from the previous sample and added 900µl autoclaved water. By using same procedure 10^{-2}, 10^{-3} dilutions were prepared.

2.6.5 Treatment of plant extract

Antibacterial activity was determined using agar well diffusion method. First, 10µl of bacterial suspension was spread on the Muller Hinton agar plates using spreader. Then wells were made on the medium and 250µl and 500µl of the extract were dropped into the well separately. The plates were left for 24 hour time period to incubate at 37^0C.

RESULTS

3.1 Plant identification

The root extraction sample of *Mimosa pudica* plant was conducted in gradient PCR using the matK forward and reverse primers for identify the annealing temperature. The results obtained from the gel electrophoresis showed in figure 5.

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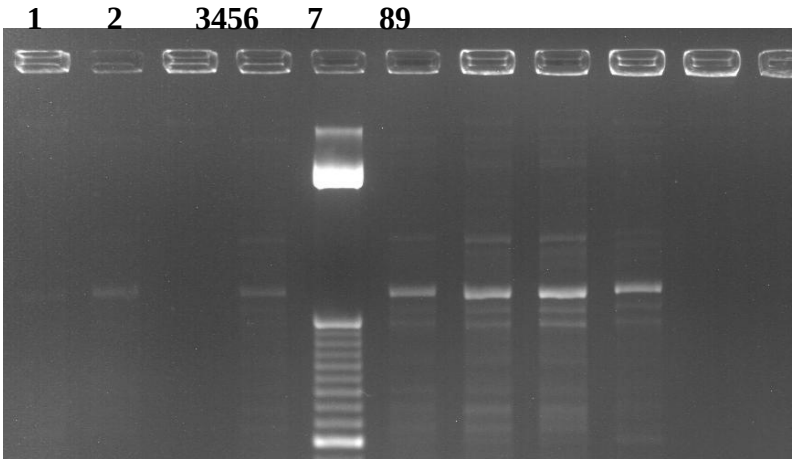
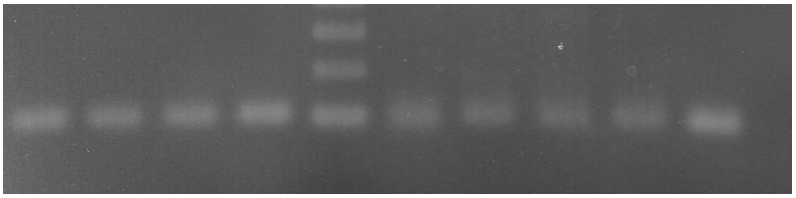


Figure 5: Gradient PCR gel electrophoresis of the *Mimosa pudica* root extraction.



The temperatures obtained from the gradient PCR is represented in Table 4.

Table 4: Annealing temperatures obtained from gradient PCR

Well Number	Annealing temperature
1	55.0
2	54.4
3	53.6
4	52.2
5 (50bp ladder)	-
6	50.5
7	49.3
8	48.4
9	48.0

The 48.4^{0}C annealing temperature was selected as the optimum temperature.

Using the gradient PCR results, the conventional PCR was conducted using PsbA3 primer because with the matk primer, the unclear sequence results were obtained.

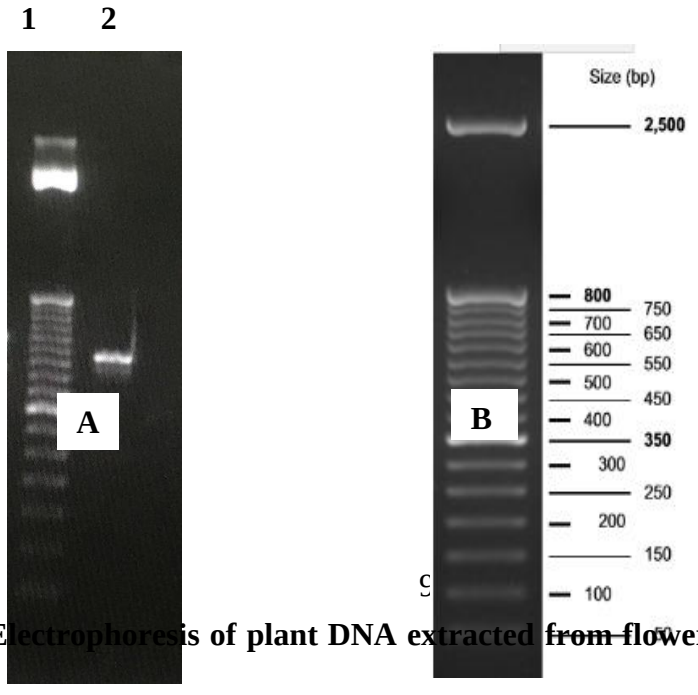


Figure 6: (A) Electrophoresis of plant DNA extracted from flowers of *Mimosa pudica* plant (B) 50bp ladder.

When compairing with 50bp ladder, the prodcut size can be 500-550bp in size and figure 6 represented the gel image obtanied from the PCR.
Then NCBI primer blasting was done to identify the plant that showed in figure 7.

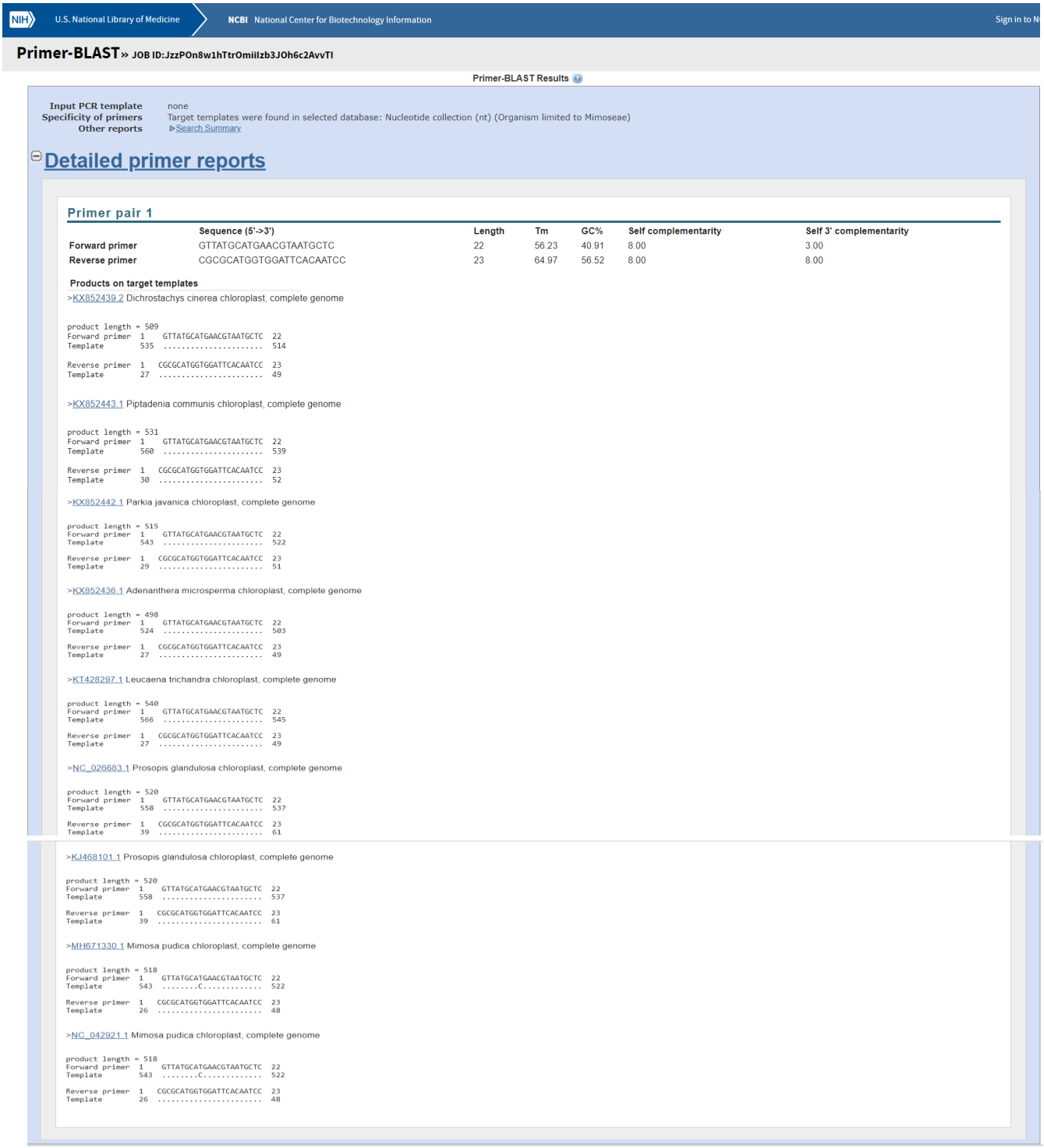


Figure 7: NCBI primer blasting report

According to the above results, the identified plant product length was 518 which can known as *Mimosa pudica* chloroplast, complete genome showed in figure 8.

>MH671330.1 Mimosa pudica chloroplast, complete genome

product length = 518
Forward primer 1 GTTATGCATGAACGTAATGCTC 22
Template 543 .....C..... 522

Reverse primer 1 CGCGCATGGTGGATTCACAATCC 23
Template 26 48

Figure 8: *Mimosa pudica* chloroplast complete genome details

3.2 Antibacterial activity

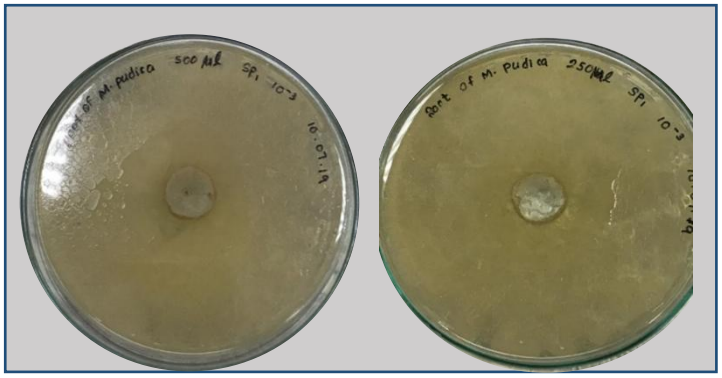


Figure 9: (A) Root aqueous extraction against *Bacillus cereus*in 10^{-3} concentration with 500µl extracts (B) Root aqueous extraction against *Bacillus cereus*in10^{-3} concentration with 250µl extracts. There were no inhibition.

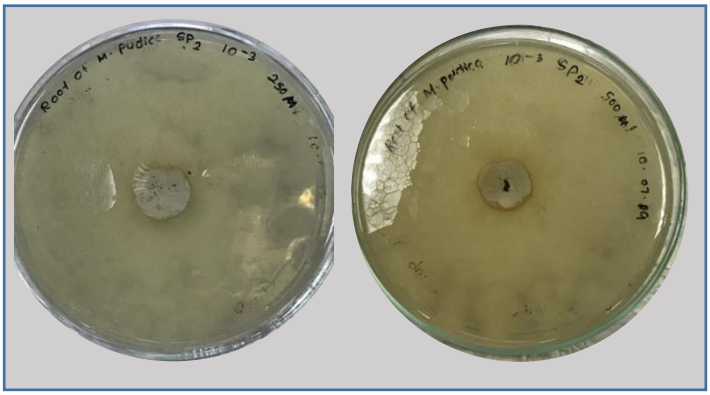


Figure 10:(A)Root aqueous extraction against *Pseudomonas stutzeri*in10^{-3} concentration with 250µl extracts (B) Root aqueous extraction against*Pseudomonas stutzeri*in 10^{-3} concentration with 500µl extracts. The zone of inhibition were not seen in the results.

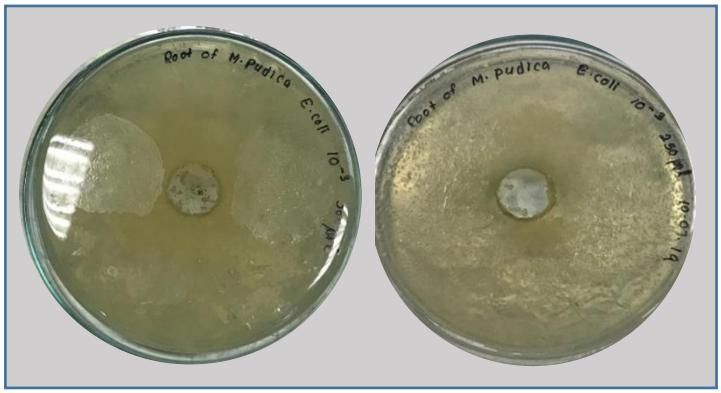


Figure 11:Root aqueous extraction against Escherichia coliin 10^{-3} concentration with 500µl extracts (B) Root aqueous extraction against Escherichia coliin 10^{-3} concentration with 250µl extracts. There were no inhibition zone.

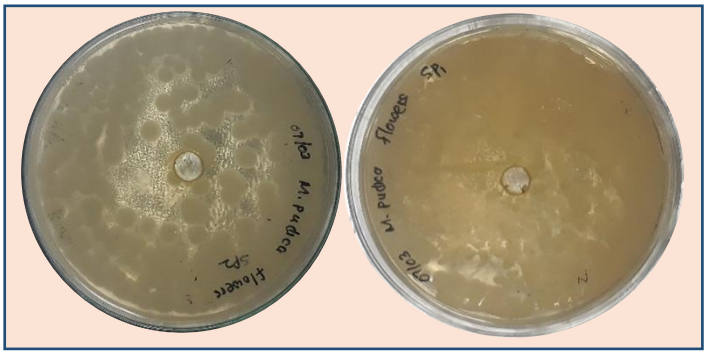


Figure 12: (A) Flower aqueous extraction against *Pseudomonasstutzeri* (B) Flower aqueous extraction against *Bacilluscereus.* In the results there were no inhibition against these bacteria cultures.

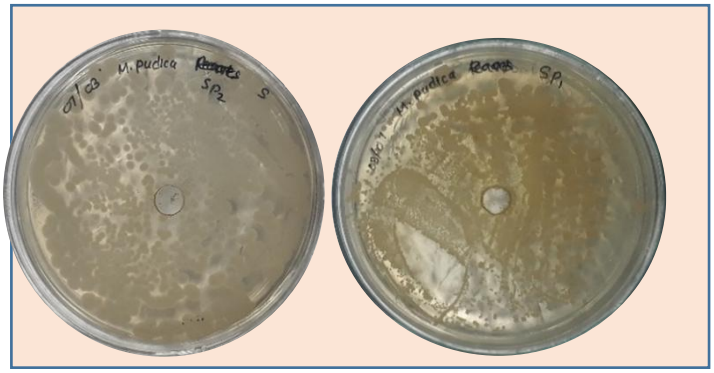
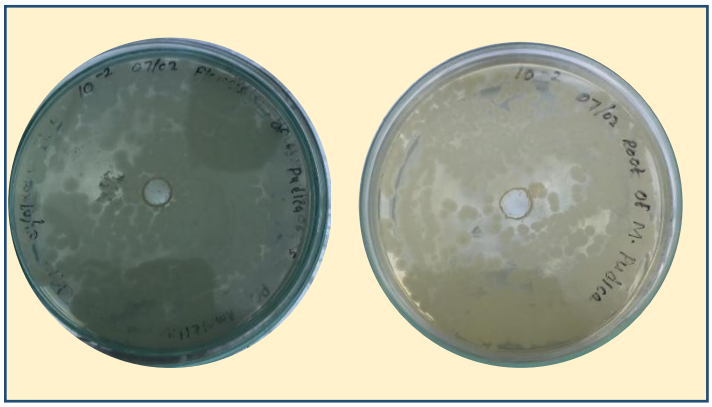


Figure 13:(A) Root aqueous extraction against *Pseudomonasstutzeri*(B) Root aqueous extraction against *Bacilluscereus.* There were no inhibition zone.



A **B**

Figure 14: (A) Flower aqueous extraction against *Escherichia coli*(B) root aqueous extraction against *Escherichia coli***.** There were no inhibition zone.

The antibacterial effects of the *Mimosa pudica* plant was performed using ampicillin as the positive control against these bacterial species.

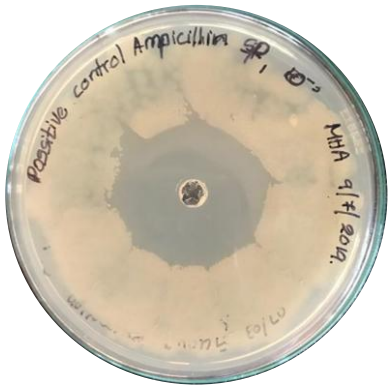


Figure 15: positive control against *Bacilluscereus.* Zone of inhibition was resulted.

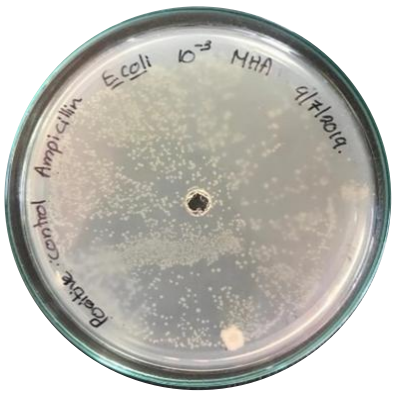


Figure 16: Positive control against*Escherichia coli*. There was no inhibition.

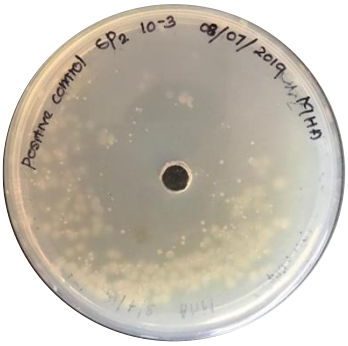


Figure 17: Positive control against *Pseudomonasstutzeri.*Zone of inhibition was resulted.

Escherichia coli	No inhibition zone
Bacilluscereu	3.5
Pseudomonasstutzeri.	1.8

Table 5: Measurements of zone of inhibition

DISCUSSION

In the present era, medicinal plants possess a variety of compounds of known to have therapeutic properties and have the traditional sources of raw materials for medicine. Hence, these medicinal plants derived compounds have their own defense system to use frequently for new drug discoveries and the therapeutic use of such plant products is an alternative strategy to prevent the spread of disease (Tomar, Shrivastava and Kaushik, 2014). In the present investigation the antimicrobial activity of the plant extract was also tested against potentially pathogenic microorganisms *Escherichia coli*, *Pseudomonas stutzeri* and *Bacillus cereus* at different concentrations of the extract to understand the most effective activity (Reed-guy *et al.*, 2017).

The antibacterial activity of aqueous extracts of flowers and root of *Mimosa pudica* at different concentrations 10^{-2} and 10^{-3}µl were analyzed against above mentioned bacterial cultures by well disc diffusion method. The observed results of the aqueous extracts at different concentrations was compared with the bacterial activity of positive control and both extracts solvent haven't inhibition against the selected bacteria.

The antibacterial activity evaluation of aqueous extract of *Mimosa pudica*root against *Bacillus cereus, Pseudomonas stutzeri*and *Escherichia coli*were carried out and the results were presented in figure 9 to figure 11. There were no inhibition zone observed against bacterial cultures. In the figure 12, that showed the flowers aqueous extraction against *Pseudomonas stutzeri* and *Bacillus cereus.* There also no inhibition against that bacterial cultures.

The organic extracts including ethanolic, benzene and diethyl ether extracts of *Mimosa pudica* has antibacterial actions than the aqueous extract because these organic extract against

these microorganisms which have used in many studies required to evaluate the efficacy of *Mimosa pudica*. The other organisms which have particularly exhibited better antibacterial activity and may be due to the antiracial principals which are either polar or non-polar and effectively extracted only the organic solvent medium (Lodangi *et al.*, 2016),(Molina, 2015). The variation in antibacterial activity of flowers and root of *M.pudica* against selected bacterial species based on different concentration and solvents of extracts haven't an inhibition, but the zone of inhibition can increased with the increased level of concentrations(Tomar, Shrivastava and Kaushik, 2014). The important function of plant extracts and their components is hydrophobicity and the aqueous extracts is not hydrophobicity which inhibit the growth of bacteria in the solvent extracts. According to the results of the present study, the growth of the microorganisms in this extracts of flowers and root of *Mimosa pudica* were controlled because it enables to partition the lipids of bacterial cell membrane and mitochondria and disturbing the cell structures and rendering them more permeable (Patro, Bhattamisra and Mohanty, 2016). This mechanism leads to extensive leakage of intracellular compounds from the bacterial cells and also the bacterial cell death is happen because it exit of molecules and ions from the cell (Akter *et al.*, 2010).

The bioactive ingredients of *Mimosa pudica* including the phytochemicals alkaloids, glycosides, flavonoids, saponins, steroids and tannins are responsible for the antibacterial activity (R *et al.*, 2014). In the present study, the antibacterial activity of flowers and root of *M.pudica* may also be control by the above mentioned phytochemical compounds. The *Escherichia coli*and the *Pseudomonasstutzeri*are gram negative bacteria and the *Bacilluscereu* is gram positive bacteria. The gram negative bacteria possess an outer membrane which is not present in the gram positive bacteria (Muhammad *et al.*, 2016). This gram negative bacterial outer membrane acts as a permeability barrier which limits access of the antibacterial agents to their targets in the bacterial cells. This can also involve for the growth of bacteria (Azmi, Singh and Akhtar, 2011).

The present results of the study showed that there were no inhibition zone against these human pathogenic microorganisms and according to the flavonoids compounds this bacterial growth can control because of some mechanisms of flavonoids (Sgt, 2015). They are,

- Normally flavonoids can inhibit the synthesis of nucleic acid since the B ring of the flavonoids relates to the intercalation or hydrogen bonding with the stacking f nucleic acid bases.
- Although, the flavonoids also influence the synthesis of protein and lipid but to a lesser degree.
- The other antimicrobial mechanism of flavonoids is the inhibition of cytoplasmic membrane function by reducing membrane fluidity of bacterial cells by changing the permeability of the cellular membrane function.
- Moreover, flavonoids has the inhibitory effect on bacteria by the inhibition of energy metabolism which is necessary for active uptake of various metabolites and for biosynthesis. But this mechanism decreases the inhibition growth of the bacteria.

The abilities of the flavonoids inhibited bacteria to resume their growth in the bacterial culture media because it had bacterial or bacteriostatic mode of action. Bacteriostatic is the capable of inhibiting the growth of bacteria and the use of antimicrobial substances with the bacteriostatic mode of action may have less side effects than those with bactericidal mode of action (Doss, 2011).

In plants, phytochemicals and polysaccharides involve with genomic extraction procedures and downstream reactions. In this study, a simple, rapid and efficient method for root and

flower DNA extraction was optimized. In this method the small amount of root and flowers materials to reduce inhibitory agents (Agne *et al.,* 2009).

The procedure involves homogenization of the *Mimosa pudica* root and flowers in extraction buffer, incubation at 60^0C, extraction by chloroform, iso-amyl alcohol and finally DNA precipitation by iso-propanol. The results showed that the extracted DNA could be used directly for polymerase chain reaction (Patel *et al.,* 2015). But isolation and purification of genomic DNA from the *Mimosa pudica* plant derived compounds are faced with some problems that includes,

- Degradation of DNA due to endonucleases- the DNA degradation is widely observed in apoptotic cells because of endonucleases the irreversible damage can cause to tissue which leads to the one or more cell death.
- Co-isolation of highly viscous polysaccharides.
- Co-isolation of inhibitor compounds including polyphenols which can reduce purity of extracted DNA and other secondary metabolites which can interfere with enzymatic reactions.

The CTAB method was used for this present study to extract the DNA but these method was time consuming (Linn, 2012). There are several methods to extract plant materials which can remove some contaminants, but required large amounts of plant tissue and the other hand, these methods require long periods for plant growth (Wallinger *et al.,* 2012). Using the liquid nitrogen was the other method which is not safe. In present era, there are DNA isolation kits, but the main problem with these commercially available kits is their cost. When analysis of these method, the CTAB method is selected because it require small amount of plan tissue and could extract optimal amount of DNA for the present study.

Using the root DNA extraction sample was done gradient PCR . This was done to determine the optimum annealing temperature for the polymerase chain reaction. Gradient PCR was done to solved the problems with the amplification of a specific DNA fragment using normal PCR are occur in the laboratories. Using the gradient PCR function of the universal block, a gradient of 45-55^0C was set. In well 1 and 3 showed faint bands and nonspecific bands can see in well 2 and 4. Although the DNA band was observed at 48.4^0C is very dense and thick. Because of these reasons 48.4^0C annealing temperature was selected as the optimum temperature.

For the gradient PCR, primer matK was used and according to the above temperature conventional PCR was done. But the plant was not identified because of unclear sequencing. Then using the PsbA3 primer again conducted the PCR and in that gel image visible bands were appeared which showed in the figure 6 and the 1st well contains the 50bp ladder and 2nd well contains the flower extraction of the *Mimosa pudica* plant. When comparing this results with 50bp ladder, the product size will be 500-550 in length. For plant identifiction NCBI primer blast was done and finally the identified plant was *Mimosa pudica* chloroplast, complete genome which is in 518bp length.

CONCLUSION

This study showed that the selected *Mimosa pudica* aqueous extract does not have antibacterial activity against *Escherichia coli JM 101 strain, Pseudomonas stutzeri*and *Bacillus cereus.*The benefits of using the natural products has increased and the active plant extracts are frequently used for new drug discoveries and for the presence of antimicrobials (Nanoparticles, 2017). The standardized plant extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Reed-guy *et al.,*

2017). In recently, there are emerging many multidrug resistant human pathogenic bacteria and the whole plant of the *Mimosa pudica* is very useful for various pharmacological and biological activities (Tomar, Shrivastava and Kaushik, 2014). Some studies concluded that the antivenomic potential of the plant is due to tannins. *M.pudica* has been extensively studied for its antimicrobial activities (Vijayalakshmi and Udayakumar, 2018). Further investigation should be done such as toxicity of the active compounds, their side effects, antibacterial activity of purified active compounds in order to deeply understand about bioactive components in *M.pudica*(Linn, 2012), (Thi *et al.*, 2016).

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