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Isolation, Morphological Identification and *In vitro* Antibacterial Activity of Endophytic Bacteria Isolated From *Morus nigra* (Mulberry) Leaves

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ABSTRACT

Plant-associated bacteria that live inside plant tissues without causing any harm to plants are defined as endophytic bacteria. Different parts of mulberry plant (root, stem and leaves) are reported to possess different pharmacological activity.The present study was done to isolate endophytic bacteria from *Morus nigra* (mulberry) leaves, their identification and investigate their antibacterial activity against three gram positive bacteria, *Staphylococcus aureus*, *Streptococcus pyogens* and *Bacillus cereus* and gram negative bacteria *Escherichia coli*, *Salmonella* Typhimurium and *Klebsiella pneumoniae.* A total 25 leaves samples were taken, they were sterilized with 0.1 per cent sodium hypochlorite, 0.01 per cent bavistin, 0.05 per cent and 70 per cent ethanol. Sterilized leaves of the plants were embedded in kings B petri plates. for the isolation of endophytic bacteria. Maximum isolated sample on kings b media were irregular in shape, flat elevation, undulated margin, glistening growth surface, opaque and white in colour, the microscopic examination revealed that isolated endophytic bacteria were gram positive and rod shaped. The antibacterial effect was studied by the disc diffusion method with known antibiotic ciprofloxacin (Ci) as standard. The antibacterial activity of endophytic bacteria isolated from *Morus nigra* (mulberry) showed good antibacterial activity against *Streptococcus pyogens.*

Keywords: Endophytic bacteria, *morus nigra* (mulberry), leaves, antibacterial activity, ciprofloxacin

An increase in the number of people in the world having health problems leading to various types of cancers, drug-resistant bacteria, parasitic protozoans and fungal infection is a cause for alarm. An intensive search for newer and more effective agents to deal with these disease problems is now underway and endophytes are a novel source of potentially useful medicinal compounds.

Endophytes are microorganisms including bacteria that live in the intercellular spaces of plant without showing any disease symptoms to the host plant (Compant *et al*., 2005). Many studies have emphasized endophytes from medicinal plants and their application in different areas (Garcia *et al.*, 2012). Recently many known as well as new endophytic bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, anti-inflammatory, antioxidant etc., have been identified (Strobel and Daisy, 2003).

Mulberry belongs to the genus Morus of the family Moraceae, mulberry has three main species; white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*). Black mulberry (*Morus nigra*) is a small to medium tree that reaches nine meters in height. Different parts of mulberry plant (root, stem and leaves) possess different pharmacological activity. The mulberry plant is considered to be effective against HIV (Venkatesh and Chauhan, 2008), has the ability to reduce blood sugar in diabetic patients and also has some effect on the pancreas and glycogenylsis (Venkatesh and Chauhan, 2008) and can

control blood cancer (Ahmad *et al*., 1985), antimicrobial and anti-inflammatory properties as well (Butt *et al*., 2008).

The objective of the present study was to isolate endophytic bacteria from *Morus nigra* (mulberry) leaves, their identification and investigate their antibacterial activity against three gram positive bacteria, *Staphylococcus aureus*, *Streptococcus pyogens* and *Bacillus cereus* and gram negative bacteria *Escherichia coli*, *Salmonella* Typhimurium and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Plant material

Fresh leaves of *Morus nigra* (mulberry) was procured from the Department of Botany, J.N.K.V.V., Jabalpur. Fresh healthy plant leaves were collected by selected medicinal plants viz *Morus nigra* (mulberry). Five samples were taken and each sample was divided into 5 sub samples and separated for further isolation of endophytic bacteria. Samples were immediately brought to laboratory and were used within 24 hrs and finally processed for isolation of endophytic bacteria.

Sterilization of leaves

The sterlization of leaves and isolation of endophytic bacteria from the leaves was done according to Mahajan *et al.* (2014), with some modifications. Leaves were treated with double distilled water for 2-3 minutes, then surface sterilized with 0.1 per cent sodium hypochlorite for 5 minutes, washed with double distilled water for 2-3 minutes. Later, surface sterilization was done with 0.01 per cent bavistin and kept in distilled water for 5 minutes. For further sterilization the leaves were exposed to 0.05 per cent streptomycin followed by treatment with double distilled water for 5 minutes. Then the leaves were exposed to 70 per cent ethanol and again were kept in double distilled water for 5 minutes and were excised with autoclaved scalpel and forceps in laminar air flow chamber, then air dried in laminar flow.

Sterility check

To confirm that the surface of leaves were effectively sterilized 1 ml of the sterile distilled water that was used in final rinse of surface sterilization procedures were planted onto nutrient agar media and incubated at 37°C for 24 hrs. Bacterial growths were observed after 24 hrs. Also surface sterilized leaves were rolled on nutrient agar plates and incubated at 37°C for 24 hrs and checked for possible microbial growth.

Preparation and sterilization of media

King's B (KB) media, mueller hinton media, blood agar media and BHI broth were prepared by adding agar into the distilled water. Hot plate was used for the proper mixing of media and autoclaved at 121°C for 15-20 minutes at 15 lbs.

Inoculation of leaves

The media was poured into different autoclaved petri plates and leaves of the plants were embedded in petri plates. These plates were then incubated at 37°C for 24 hrs. Characterization of the bacteria was done according to its morphology and by grams staining. After that a single colony was transferred into BHI broth and incubated at 37°C for 24 hrs.

Purification of endophytic bacteria

For purification of endophytic bacteria subculturing was mainly done by streaking a loop full of BHI broth on the fresh pre solidified blood agar plates and then incubated at 37°C for 24 hrs. After incubation the colonies were transferred into BHI broth and then incubated at 37°C for 24 hrs and purity is checked by grams stain and stored at -20°C in deep freez for further work.

Antibacterial activity of endophytic bacteria

In vitro study

Procurement of known culture

Bactria were procured for the antibacterial activity from Hi media. The bacterial culture were as following *Escherichia coli* (ATCC No. 25922), *Klebsiella pneumoniae* (ATCC No. 700603), *Salmonella* Typhimurium (ATCC No. 13311), *Bacillus cereus* (ATCC No. 11778), *Staphylococcus aureus* (ATCC No. 6538), *Streptococcus pyogens* (ATCC No. 12384).

Preparation of inoculums

The above prepared bacterial inoculums were evenly spread on sterile mueller hinton agar plates as described by Bauer *et al.* (1969) and antibacterial effect was studied by the disc diffusion method in these plates. The known antibiotic ciprofloxacin (Ci) was simultaneously used and placed as control for antibiotic sensitivity. The dried discs were immediately used and incubated at 37°C for 24 hrs.

Preparation of Antibacterial disc

For determination of antibacterial activity of endophytic bacteria, broths were centrifuged at 4°C at 12000rpm for 30 minutes. Supernatant of each of these broths were taken, sterile discs were soaked in these broths in a sterile test tubes for 24 hrs and dried in laminar flow. After drying the discs were used immediately for disc impregnation in the inoculated plates as described by Kirubaharan *et al*. (1999) with slight modifications. Ciprofloxacin dics were used as control drug to compare the effect of treatment during *in vitro* study.

Antibacterial test

The prepared bacterial inoculums were evenly spread on a sterile Mueller Hinton agar plate as per method described by Bauer *et al.* (1969). The known antibiotic Ciprofloxacin (Ci) was simultaneously placed as a control for antibiotic sensitivity. The dried disc was incubated at 37°C for 24 hrs. Results were recorded as positive (growth) or negative (no growth) and zone of inhibition of growth exerted by these impregnated discs.

Table 1: Growth of endophytic bacteria isolated from Morus nigra (mulberry) on kings B media

Sl. No.	Isolate No.	Form	Elevation	Margin	Surface	Opacity	Chromogenesis
1	M1a	Irregular	Flat	Undulated	Glistening	Opaque	Absent
2	M1b	Irregular	Flat	Undulated	Glistening	Opaque	Absent
3	M1c	Irregular	Flat	Undulated	Glistening	Opaque	Absent
4	M1d	Irregular	Flat	Undulated	Glistening	Opaque	Absent
5	M1e	Irregular	Flat	Undulated	Glistening	Opaque	Absent
6	M2a	Circular	Raised	Entire	Dull	Opaque	Absent
7	M2b	Irregular	Flat	Undulated	Glistening	Opaque	Absent
8	M2c	Irregular	Flat	Undulated	Glistening	Opaque	Absent
9	M2d	Irregular	Flat	Undulated	Glistening	Opaque	Absent
10	M2e	Irregular	Raised	Undulated	Glistening	Opaque	Absent
11	M3a	Irregular	Flat	Undulated	Rough	Opaque	Absent
12	M3b	Irregular	Flat	Undulated	Glistening	Opaque	Absent
13	M3c	Irregular	Flat	Undulated	Glistening	Opaque	Absent
14	M3d	Irregular	Flat	Entire	Glistening	Opaque	Absent
15	M3e	Circular	Flat	Undulated	Rough	Opaque	Absent
16	M4a	Irregular	Flat	Undulated	Glistening	Opaque	Absent
17	M4b	Irregular	Flat	Undulated	Rough	Opaque	Absent
18	M4c	Irregular	Flat	Undulated	Glistening	Opaque	Absent
19	M4d	Irregular	Flat	Undulated	Dull	Opaque	Absent
20	M4e	Irregular	Flat	Undulated	Glistening	Opaque	Absent
21	M5a	Circular	Flat	Undulated	Glistening	Opaque	Absent
22	M5b	Irregular	Flat	Undulated	Glistening	Opaque	Absent
23	M5c	Irregular	Flat	Undulated	Glistening	Opaque	Absent
24	M5d	Irregular	Raised	Undulated	Glistening	Opaque	Absent
25	M5e	Irregular	Flat	Undulated	Glistening	Opaque	Absent

Table 2: Growth of endophytic bacteria isolated from Morus nigra (mulberry) on 5 per cent sheep blood agar

Sl. No.	Isolates No.	Form	Elevation	Margin	Surface	Opacity	Chromogenesis
1	M1a	Irregular	Flat	Undulated	Rough	Opaque	Absent
2	M1b	Circular	Flat	Undulated	Rough	Opaque	Absent
3	M1c	Irregular	Raised	Undulated	Dull	Opaque	Absent
4	M1d	Irregular	Flat	Undulated	Rough	Opaque	Absent
5	M1e	Irregular	Flat	Undulated	Rough	Opaque	Absent
6	M2a	Irregular	Raised	Undulated	Rough	Opaque	Absent
7	M2b	Irregular	Flat	Undulated	Smooth	Opaque	Absent
8	M2c	Irregular	Flat	Undulated	Rough	Opaque	Absent
9	M2d	Irregular	Flat	Undulated	Rough	Opaque	Absent
10	M2e	Irregular	Flat	Undulated	Rough	Opaque	Absent
11	M3a	Circular	Flat	Undulated	Rough	Opaque	Absent
12	M3b	Irregular	Flat	Undulated	Smooth	Opaque	Absent
13	M3c	Irregular	Flat	Undulated	Dull	Opaque	Absent
14	M3d	Irregular	Flat	Undulated	Rough	Opaque	Absent
15	M3e	Circular	Flat	Undulated	Rough	Opaque	Absent
16	M4a	Irregular	Raised	Undulated	Rough	Opaque	Absent
17	M4b	Irregular	Flat	Undulated	Rough	Opaque	Absent
18	M4c	Circular	Flat	Undulated	Rough	Opaque	Absent
19	M4d	Irregular	Flat	Undulated	Smooth	Opaque	Absent
20	M4e	Irregular	Flat	Undulated	Rough	Opaque	Absent
21	M5a	Circular	Raised	Undulated	Rough	Opaque	Absent
22	M5b	Irregular	Flat	Undulated	Rough	Opaque	Absent
23	M5c	Irregular	Flat	Undulated	Rough	Opaque	Absent
24	M5d	Irregular	Flat	Undulated	Rough	Opaque	Absent
25	M5e	Irregular	Flat	Undulated	Dull	Opaque	Absent

Table 3: Growth of endophytic bacteria isolated from Morus nigra (mulberry) on BHI broth

Sl. No.	Isolate No.	Turbidity	Flocullant	Pellicle	Sediment	Ring formation
1	M1a	Present	Absent	Present	Absent	Absent
2	M1b	Present	Absent	Present	Absent	Absent
3	M1c	Present	Absent	Present	Absent	Absent
4	M1d	Present	Absent	Present	Absent	Present
5	M1e	Present	Absent	Present	Absent	Absent
6	M2a	Present	Absent	Present	Absent	Absent
7	M2b	Present	Absent	Present	Present	Absent
8	M2c	Present	Absent	Present	Absent	Absent
9	M2d	Present	Absent	Present	Absent	Present
10	M2e	Present	Absent	Present	Absent	Absent
11	M3a	Present	Absent	Present	Absent	Absent

12	M3b	Present	Absent	Present	Absent	Absent
13	M3c	Present	Absent	Present	Absent	Absent
14	M3d	Present	Present	Present	Absent	Absent
15	M3e	Present	Absent	Present	Absent	Absent
16	M4a	Present	Absent	Present	Absent	Present
17	M4b	Present	Absent	Present	Absent	Absent
18	M4c	Present	Absent	Present	Absent	Absent
19	M4d	Present	Absent	Present	Absent	Absent
20	M4e	Present	Absent	Present	Absent	Absent
21	M5a	Present	Present	Present	Absent	Absent
22	M5b	Present	Absent	Present	Absent	Absent
23	M5c	Present	Absent	Present	Absent	Present
24	M5d	Present	Absent	Present	Absent	Absent
25	M5e	Present	Absent	Present	Absent	Absent

Table 4: Grams staining of endophytic bacteria isolated from *Morus nigra* (mulberry)

Sl. No.	Isolate No.	Grams staining	Shape	Types of bacteria
1	M1a	Negative	Bacillus	1
2	M1b	Negative	Bacillus	1
3	M1c	Negative	Bacillus	1
4	M1d	Negative	Bacillus	1
5	M1e	Negative	Bacillus	1
6	M2a	Negative	Bacillus	<1
7	M2b	Negative	Bacillus	1
8	M2c	Negative	Bacillus	1
9	M2d	Positive	Bacillus	1
10	M2e	Negative	Cocci	1
11	M3a	Negative	Bacillus	<1
12	M3b	Negative	Bacillus	1
13	M3c	Positive	Bacillus	1
14	M3d	Negative	Bacillus	1
15	M3e	Negative	Bacillus	1
16	M4a	Negative	Bacillus	1
17	M4b	Negative	Bacillus	1
18	M4c	Positive	Bacillus	<1
19	M4d	Negative	Bacillus	1
20	M4e	Negative	Bacillus	1
21	M5a	Positive	Bacillus	<1
22	M5b	Negative	Bacillus	1
23	M5c	Negative	Cocci	1
24	M5d	Negative	Bacillus	1
25	M5e	Negative	Bacillus	1

RESULTS

Preliminary characterisation of isolated endophytic bacteria

Growth of endophytic bacteria in kings B medium

Growth characteristics of endophytic bacteria isolated from mulberry leaves indicated that 88 per cent were irregular in shape while 12 per cent circular in shape, 88 per cent had flat elevation on petri plate while 12 per cent raised elevation, margin of the 92 per cent colonies were undulated while 8 per cent entire, the surface of the growth was glistening in 80 per cent while 8 per cent were dull and 12 per cent rough and the growth was opaque and white in colour in 100 per cent isolates. (Table 1)

Growth of endophytic bacteria on 5 per cent sheep blood agar medium

Colonies of endophytic bacteria grown on kings B agar were transferred to the 5 per cent sheep blood agar plates and incubated at 37°C for 24 hrs. The growth of endophytic bacteria from *Morus nigra* (mulberry) was studied. Growth characteristics of endophytic bacteria isolated from mulberry leaves presented that 80 per cent samples were irregular in shape while 20 per cent circular in shape, 84 per cent flat elevation on petri plate while 16 per cent raised elevation, margin of the 100 per cent colonies were undulated, the surface of the growth was rough in 76 per cent while 12 per cent dull, 12 per cent smooth. All the isolates were non haemolytic and non chromogenic (Table 2).

Growth of endophytic bacteria in BHI broth

Colonies of endophytic bacteria grown on blood agar were transferred to the sterile BHI broth tubes and incubated at 37°C for 24 hrs. The growth of endophytic bacteria from *Morus nigra* (mulberry) was studied. Endophytic bacteria from mulberry leaves shown characteristics as all isolates with turbidity, 92 per cent isolates without flocullant growth, 100 per cent isolates with pellicle formation, in 4 per cent isolates with sediment formation was present and in 84 per cent isolates ring formation was present. (Table 3)

Microscopic examination

The microscopic examination of endophytic bacteria was done by using grams stain. Endophytic bacteria isolated from mulberry showed that 84 per cent isolates shown gram positive reaction while 16 per cent were gram negative, 92 per cent endophytic bacteria were rod shape and 8 per cent were cocci, Microscopic examination showed that one type of endophytic bacteria were present in 84 per cent of isolate (Table 4).

In vitro antibacterial activity

Antibacterial sensitivity

The antibacterial activity of endophytic bacteria was evaluated against various gram positive and gram negative pathogenic bacteria namely *Bacillus cereus, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae and Salmonella* Typhimurium. Results were recorded for zone of inhibition around the disc. The inhibitory zone around the disc indicated absence of bacterial growth reported as sensitive and absence of zone reported as resistant.

For gram positive bacteria

The *in vitro* antibacterial activities of the endophytic bacteria against different gram positive bacteria are shown in table 5. The endophytic bacteria isolated from *Morus nigra* (mulberry) shown antibacterial activity as 12 per cent of isolates inhibited growth of *Staphylococcus aureus,* 80 per cent of isolates inhibited growth of *Streptococcus pyogenes* and 8 per cent isolates inhibited growth of *Bacillus cereus.*

For gram negative bacteria

The *in vitro* antibacterial activities of endophytic bacteria against different gram negative bacteria have been shown in table 6. The endophytic bacteria isolated from *Morus nigra* (mulberry) presented antibacterial activity as 12 per cent of isolates inhibited growth of *Escherichia coli,* 8 per cent of isolates inhibited growth of *Salmonella* Typhimurium and 12 per cent isolates inhibited growth of *Klebsiella pneumoniae.*

Table 5: In vitro antibacterial activity of endophytic bacteria isolated from Morus nigra (mulberry) against gram positive bacteria

Sl. No.	Isolate No.	Staphylococcus aureus	Streptococcus pyogens	Bacillus cereus
1	M1a	R	S	R
2	M1b	R	S	R
3	M1c	R	S	R
4	M1d	R	R	R
5	M1e	R	S	R
6	M2a	R	S	R
7	M2b	S	S	S
8	M2c	R	R	R
9	M2d	R	S	R
10	M2e	R	S	R
11	M3a	R	S	R
12	M3b	R	S	R
13	M3c	S	R	R
14	M3d	R	S	R
15	M3e	R	S	S
16	M4a	R	R	R
17	M4b	R	S	R
18	M4c	R	S	R
19	M4d	R	S	R
20	M4e	R	S	R
21	M5a	R	S	R
22	M5b	R	S	R
23	M5c	S	R	R
24	M5d	R	S	R
25	M5e	R	S	R

Table 6: In vitro antibacterial activity of endophytic bacteria isolated from Morus nigra (mulberry) against gram negative bacteria

Sl. No.	Isolate No.	*Escherichia coli*	*Salmonella* Typhimurium	*Klebsiella pneumonia*
1	M1a	R	R	R
2	M1b	R	R	R
3	M1c	R	R	R
4	M1d	R	S	R

5	M1e	R	R	R
6	M2a	S	R	R
7	M2b	R	R	R
8	M2c	R	R	R
9	M2d	R	R	S
10	M2e	R	R	R
11	M3a	R	R	R
12	M3b	R	R	R
13	M3c	R	R	R
14	M3d	S	R	R
15	M3e	R	R	S
16	M4a	R	R	R
17	M4b	R	R	S
18	M4c	R	R	R
19	M4d	R	R	R
20	M4e	R	R	R
21	M5a	S	R	R
22	M5b	R	R	R
23	M5c	R	S	R
24	M5d	R	R	R
25	M5e	R	R	R

Table 7: Over all in vitro antibacterial activity of endophytic bacterial isolates

Sl. No.	Samples	Activity against
1	M2b, M3c, M5c	Staphylococcus aureus
2	M1a, M1b, M1c, M1e, M2a, M2b, M2d, M2e, M3a, M3b, M3d, M3e, M4b, M4c, M4d, M4e, M5a, M5b, M5d, M5e	Streotococcus pyogens
3	M2b, M3e	Bacillus cereus
4	M2a, M3d, M5a	Escherichia coli
5	M1d, M5c	*Salmonella* Typhimurium
6	M2d, M3e, M4b	Klebsiella pneumoniae

Over all *in vitro* antibacterial activity of endophytic bacterial isolates

Out of 25 isolates from *Morus nigra* (mulberry) 3 isolates were effective against *Staphylococcus aureus*, 20 against *streotococcus pyogens*, 2 against *Bacillus cereus*, 3 against *Escherichia coli,* 2 against *Salmonella* Typhimurium, 3 against *Klebsiella pneumoniae*. (Table 8)

Discussion

Twenty five strains of endophytic bacteria were isolated from leaves of *Morus nigra* (mulberry). Endophytic bacteria are found in virtually every plant on earth (Ryan *et al*., 2008). Different plant parts such as root, stem and nodule (Hung and Annapurna, 2004), leaves, stems and root (Sobral *et al*., 2005) can also be used for isolation of endophytic bacteria. Costa *et al.,* (2012) had isolated culturable endophytic bacteria from common bean (*Phaseolus vulgaris*) leaves.

The preliminary identification of the bacterial isolates was done based on various morphological features of isolated endophytic bacteria. The colony characteristics of endophytic bacteria isolated from mulberry are having irregular shape, flat elevation on petri plate, undulated margin, glistening growth surface and the growth was opaque and white in colour. The microscopic examination of endophytic bacteria isolated from mulberry showed that 84 per cent isolates shown gram positive reaction while 16 per cent were gram negative, 92 per cent endophytic bacteria were rod shape and 8 per cent were cocci, Microscopic examination showed that one type of endophytic bacteria were present in 84 per cent of isolate.

The isolation of endophytic bacteria was in agreement with the findings of (Hung and Annapurna, 2004), had found equal percentages of gram positive 49 per cent and gram negative 51 per cent bacteria. Sobral *et. al*. (2005) and Ebrahimia *et al*. (2010) has also found equal percentage of gram positive and gram negative bacteria. However, Baghat *et al*. (2014) found the 90 per cent of gram positive bacteria.

As summarized in results antibacterial activity of endophytic bacteria was calculated by the presence of zone of inhibition produce by endophytic bacteria against pathogenic bacteria. All the isolates from endophytic bacteria were screened for the antibacterial activity against pathogenic bacteria *Bacillus cereus, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae and Salmonella* Typhimurium.

The overall *in vitro* antibacterial results shown that maximum sensitivity was observed against *Streotococcus pyogens*. Most of the isolates from mulberry had not showed antibacterial activity against both gram positive (*Staphylococcus aureus, Bacillus cereus*) and gram negative bacteria (*Escherichia coli, Salmonella* Typhimurium*, Klebsiella pneumoniae*).

Verma *et al.* (2009) observed antibacterial activity of endophytic actinomycetes from *Azadirachta indica* against *Escherichia coli*. Ebrahimia *et al*. (2010) observed antibacterial activity of endophytic bacteria isolated from leaves of *Hypericum scabrum* against *S. aureus*. Jalgaonwala *et al.* (2010) observed antibacterial activity of endophytic bacteria isolated from roots of *Aloe vera* possess strong antibacterial activity against *S. typhi* in dual culture assay. Roy and Banerjee (2010) isolated endophytic bacteria from a medicinal plant *Vinca rosea*. One of the isolated endophytes produced potential antimicrobial activity against *Bacillus cereus, Klebsiella pneumoniae, Escherichiae coli*. Pal *et al.* (2012) reported the antimicrobial activity of the bacterial endophytes of *P. foetida* indicating the inhibitory effect of majority of the isolates against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae*. The present study is also very near to all the above authors.

CONCLUSION

Endophytic bacteria were present in leaves of *Morus nigra* (mulberry), gram positive cocci shaped bacteria were present in leaves of *Morus nigra* (mulberry). Endophytic bacteria from mulberry possess maximum antibacterial activity against *Streptococcus pyogens*

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