A Study On Endophytic Bacteria From *Aegle Marmelos* (L.) Correa (Leaves)

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Abstract

This study was to isolate and characterization of endophytic bacteria from the leaves of *Aegle marmelos*(L.) Correa and evaluate their antimicrobial activities. The plant samples were collected from Hlegu Township, Yangon Region. The plant *Aegle marmelos*(L.) Correa stone apple or Bael fruit (Ok-Shit) belongs to the family Rutaceae. Eleven endophytic bacteria were isolated from this plant. These eleven strains were identified by morphological and biochemical tests to get the genus level. These eleven strains were identified as Genus *Aeromonas, Streptococcus, Bacilllus, Corynebacterium, Pseudomonas* and *Azomonas.*

Keywords : endophytic bacteria, antimicrobial activities, strains

Introduction

Man cannot survive on earth for long time without plants. A number of traditional herbal medicinal plants are used for management of various diseases. Therapeutic use of medicinal plants has become popular because of its inability to cause side effects and combat antibiotic resistance in microorganisms. Endophytes are microorganisms (bacteria) which inhabit in healthy living plant tissue for all or part of their life cycle without causing apparent harmful symptoms to the host. Many of the endophytes are known to produce bioactive compounds that can be used by the host plant for their defense against different phytopathogens (Berde *et al*., 2015). *Aegle marmelos* (L.) Correa belongs to the family Rutaceae, which is commonly known as the bael trees (or) Bengal Quince in English. This plant is Ok-Shit known as in Myanmar (The Wealth of India, 1948; Indigenous drugs of India, 1982). The family Rutaceae consists of 120 genera and 1000 species. The Morphology of *Aegle marmelos* (L.) Correa. A deciduous tree, stems with spines. Leaves are alternate, palmately compound, trifoliate, exstipulate; petiolate, aromatic. Inflorescences are terminal and axillary scorpioid cymes. Flowers are ebracteolate, pedicllate, bisexual, actinomorphic, pentamerous, hypogynous aromatic. Calyx is synsepalous, 5-lobed, deciduous. Corolla is apopetalous, 5-petals, much longer than calyx, greenish yellow. Androecium is polyandrous, stamens numerous, the filaments short, the anthers are dithecous, basifixed, introse, longitudinal dehiscence. Ovary is ovoid, 5-lobed, carpels 5, syncarpous, 5-loculed, axile placentation, the ovules numerous in each locule, the style is short, the stigma is capitate (KyawSoe, Tin Myo Ngwe, 2004). An infusion of wood apple leaves is an effective remedy for peptic ulcers. Wood apple leaves are rich in tannin, which reduces inflammation and help in the healing of ulcers. It is also useful in preventing cancer of the breast and uterus and helps treat infertility caused by in sufficiency of the progesterone hormone. The leaves of the wood apple are used to prevent and treat cold and other respiratory disorders (Madki *et al*., 2010). In Myanmar, fresh leaves are used in cough and diarrhea as traditional medicine. It is also used as salad with fish-source. The bark is used in intermittent fever and the fruit is used in astringent, diarrhoea and dysentery as traditional medicine.

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Materials and Methods

Collection of Plant Samples

The endophytic microorganisms were isolated from the leaves of *Aegle marmelos* (L.) Correa. The fresh samples were then taken and experiments were carried out at the Microbiology Laboratory, Botany Department, Dagon University.

Methods

Aseptic Techniques

Sterilized Pyrex glass wares were used throughout the experiment. Glass wares were first acid washed and then rinsed in distilled water and sterilized by using autoclave for 15 minutes at 15 pounds per inch square at 121°C.

Selective Isolation of Endophytic Bacteria

Firstly, the leaf samples were washed in running tap water to remove thoroughly soil particles and were surface sterilized sequential immersion in 70% ethanol for five minutes, a solution of sodium hypochloride for in five minutes and distilled water for in five minutes. Then the samples were washed three times in sterile distilled water to remove surface sterilizing agent. Each of the samples was divided into small fragments (1cm) under aseptic conditions and was plated on three different culture media 1% Glucose Nutrient agar, 1% Sucrose Nutrient agar and 1% Lactose Nutrient agar.

Preparation of Culture Media

1% Glucose medium (Atlas, 1993)

Nutrient Agar	28.0 g
Glucose	10.0 g
Distilled Water	1000 ml
pH	7.0 $\pm$ 0.2

1% Sucrose medium (Atlas, 1993)

Nutrient Agar	28.0 g
Sucrose	10.0 g
Distilled Water	1000 ml
pH	7.0 $\pm$ 0.2

1% Lactose medium (Atlas, 1993)

Nutrient Agar	28.0 g
Lactose	10.0 g
Distilled Water	1000 ml
pH	7.0 $\pm$ 0.2

King's B medium (Atlas, 1993)

Agar	2.0 g
KH_2PO_4	1.0 g
$MgCl_2$	1.0 g
Peptone	5.0 g
Glycerol	5.0 g
Distilled Water	1000 ml
pH	7.0 $\pm$ 0.2

Isolation of Pure Culture from Plates (Atlas, 1993)

About 100 ml of four different media were separately distributed into test tubes. The test tubes were plugged with cotton wool and sterilized by autoclaving them at 15 pounds pressure per square inch for 15 minutes at 121°C. The sterilized media were cooled down. The separate colonies appear and different types of bacterial colonies were cultured in test tubes. The slants of media were repeatedly sub-cultured to obtain pure cultures.

Subculture Techniques (Atlas, 1993)

The isolated of bacteria species were sub cultured on four different media to check its purity and incubated at 28°C for 48 hours. Purified cultures were routinely maintained on 1% glucose medium, 1% sucrose medium, 1% lactose medium and King's B medium. The isolates were subjected to various physiological and biochemical tests, including sugar fermentation, assimilation of carbon and nitrogen compounds and other physiological tests.

Identification

Identification of each isolated of the bacteria up to genus level was carried on the basis of standard morphological and biochemical tests presented by Bergey's Manual of Determinative Bacteriology by Buchnan, 1957.

Morphological Characterization

Gram Staining (Aneja, 2005)

To identify the bacteria as either gram positive or gram negative, gram staining was used with counter stain, which contains crystal violet solution, iodine solution with 95% ethyl alcohol and safranin. A drop of sterile distilled water was placed on a free glass slide and a loopful of bacterium was smeared and dried by heating. Then, slide was covered with crystal violet solution and dried for kept 30 seconds. Iodine solution was added as drop by drop for 60 seconds. Next steps, 95% ethyl alcohol, about 20 seconds were used for decolourization and washed the slide with distilled water. Then, safranin was add kept 30 seconds and washed with distilled water. Finally, the slide was air dried and examined under the oil immersion objective of microscope. Those bacteria that appeared purple were referred to as gram positive, those appearing pink were described gram negative.

Endospore Staining (Santra *et al*., 1998)

The bacterial smear was prepared in the usual way and fix by passing the slide 20 times over a flame. The smear was covered with malachite green and allowed it to react for 10 minutes on water bath. It was necessary to the smear was covered with malachite green solution to avoid dryness to smear. Rinse with tap water for 10 seconds and then stain with safranin for 1 minute. Rinse with water, blot dry and examine under oil immersion.

Catalase Test (Salle, 1948)

A few drops of 3% hydrogen peroxide (H_2O_2) solution was added onto each slide culture and watched for immediate signs of bubbling, which represented positive test; absence of bubbles indicated a negative test.

Cultural Characteristics of Isolated Microorganisms

The distinct culture characteristics of isolated microorganisms on slant were studied according to the procedures described by Salle (1948), Harrigan and Mc Cane (1966) and Cruickshank (1968).

Biochemical Characteristics of Isolated Bacteria Strains

Carbohydrate Test

The digestion of carbohydrate by isolated microbes was evident as either acid production or gas production, carbohydrate was digested either by oxidation or fermentation process were carried out to identify the isolated microbes, digestions of glucose, sucrose, lactose and dextrose.

Acid Production from Various Sugars

Acid production from various sugars was tested according to the procedure described by Harrigan and Mc Cane (1996). The require amount of peptone water, provided with one percent each of the testing materials and 0.08 percent of phenol red indicator, was separately taken into test tubes and steamed in a tube for 30 minutes perday on 3 successive days. The sterilized medium was cooled down to room temperature and inoculated with the bacterial isolates under investigation. The inoculated tube was incubated at 30^{o}C for one to seven days. Acid production, as indicated by a change of in a red color of indicator to yellow, was daily observed in the course of 7 days incubation at 30^{o}C.

Citrate Utilization (Atlas, 1993)

Utilization of sodium citrate as a sole carbon source was tested using Simmon's citrate medium. Broth medium was inoculated with a straight wire and incubated at room temperature for two days. Greenish blue to blue color turn as positive reaction.

Nitrate Reduction

This test was carried out according to the technique Harrigan and Mc Cane (1996). A proper amount of nitrate broth was introduced into test tube and sterilized as usual. The sterilized medium was cooled down to room temperature and inoculated with the bacterial isolates under investigation. The inoculated tube was incubated at 30^{o}C for 3 days. Nitrite produced from nitrate was detected with Griess - II reagent, which is a mixture of sulphonilic acid and alpha - naphthylamine in acetic acid.

Urea Hydrolysis

Urea hydrolysis by the nitrogen fixing isolates was tested in Christensen's Urea medium (Christenson, 1946), and in a urea supplemented nitrogen free basal medium.

Christensen's medium included 0.1 percent peptone, 0.5 percent sodium chloride, 0.2 percent potassium dehydrogenate phosphate, 0.1 percent glucose and two percent agar with phenol red indicator. Sterilized medium was supplemented with sterile urea solution to make a final concentration of two percent.

All urease test media were prepared as broth and were inoculated heavily on the surface of the broth. They were kept a room temperature and checked daily for 7 days for the development of red color which indicated the hydrolysis of urea.

Hydrogen Sulphide (H_2S) Production (Cowan, 1975)

The basal nitrogen free agar medium was supplemented with 12 percent of gelatin and sterilized by autoclaving at 121^{o}C for 15 minutes. Before agar become solidified, freshly prepared 10 percent aqueous ferrous chloride solution was added to make five percent final concentration, and distributed into sterile test tubes. The tubes were inoculated with each test organism and incubated at room temperature and checked daily up to seven days for blackening which indicated the production of hydrogen sulphide gas.

VogesProskauer Reaction (VP test)

This test was carried out according to Barritts modified method described by Bisen and Verman, 1998. The requisite quality of glucose phosphate broth was taken into test tube and sterilized by autoclaving it at 121^{o}C (15 psi) for 15 minutes. The sterilized medium was cooled down to room temperature and inoculated with the bacterial isolate under investigation. The inoculated test tube was inoculated at room temperature for five days and acetyl-methyl-carbinol produced by the organisms was detected with 40% potassium hydroxide solution and 3ml of 5% solution of alpha-naphthol in absolute alcohol.

Methyl Red Test (Grag, 2003)

Each of the isolated strains was inoculated in glucose phosphate broth and incubates for five days at 30°C. Then, methyl red solution was prepared by dissolving (0.1 g methyl red in 300 ml or 95 percent ethanol, made up to 500 ml with distilled water). Add about five drops of the indicator was added to 5 ml of culture. A red color denoted that the pH had been reduced to 4.5 or less. It was recorded as positive reaction and a yellow color indicated a negative reaction.

NaCL Tolerance (Salt Tolerance Test) (Atlas, 1993)

Nutrient medium was prepared with a range of NaCL concentration is 1%, 3% and 6%. The isolated strains were inoculated into the NaCL nutrient medium by drop

method. Two tubes for NaCL concentration was prepared and incubated for three days at 25°C and growth of isolated bacterial strain was seen in the broth for positive result.

Oxygen Requirement (aerobic/ anaerobic) (Prescott, 2002)

Different test tube of slant cultures and broth cultures were prepared and then one was inoculated at room temperature. Another culture was kept in the anaerobic tin can. Different culture at agar test tubes was prepared as usual and the isolated strains were made as stab-culture and incubated at room temperature. The patterns of growth were checked daily and the oxygen requirement of the isolated strains were recorded.

Enzymatic Activity Tests

Starch Hydrolysis (Pelezar and Chan, 1972)

Soluble starch and wheat powder were utilized for source of starch powder. Starch medium (peptone 0.5g, yeast 0.5g, agar 2.5g, above each starch powder 1%, Distilled water 100 ml, pH 7.0 $\pm$ 0.2) was incubated with isolated strains and incubated for 24 hours. Then, dilute iodine solution was flooded on the surface of starch agar checked visually. Hydrolysis of starch was indicated by clear zones around the growth of bacterial strain and recorded as positive reaction. Unchanged starch gave a blue-back colour.

Antimicrobial Activity Test

Cultivation of Test Organisms

The study of antimicrobial activity was performed by agar well diffusion method. *Vibrio cholerae, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Proteus mirabilis, Candida albicans, Escherichia coli, Bacillus subtilis* and *Bacillus pumalis* were used for the used for the antimicrobial activities. They were inoculated into the nutrient broth and transferred into nutrient agar medium.

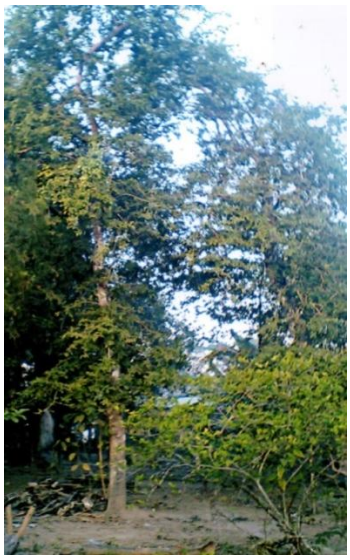

Figure (1) Habit of *Aeglemarmelos*(L.) Correa

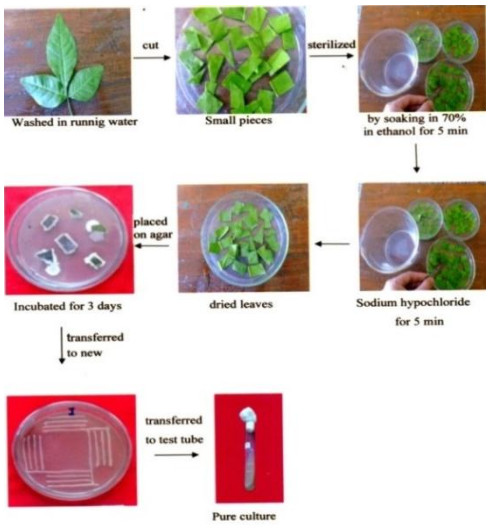

Figure (2) Isolation of Endophytic Bacteria from *Aeglemarmelos*(L.) Correa (Leaves)

Results

Isolation of the Endophytic Bacteria

Eleven bacterial strains (A1-A11) were isolated from *Aegle marmelos* (L.) Correa (Leaves). The morphological, cultural and biochemical characteristics of isolated bacteria were studied and the results were shown in Table (1-7) and Figure

(3-9). Antimicrobial activities of isolated strains were carried out by agar well diffusion with nine test organisms. Among them, all strains showed respectively antimicrobial activity on test organisms in Table (8-9) and Figure (11).

Identification of the Isolated Bacteria

The present investigation was undertaken in order to isolate and identify endophytic bacteria from leaves of *Aegle marmelos* (L.) Correa (Leaves). Eleven strains were isolated from this plant and these strains were assumed to be *Bacillus, Streptococcus, Azomonas, Coryne bacterium, Pseudomonas* and *Aeromonas*. In detail, characterization of the strains A4 was short rod, gram-positive non-motile, facultative anaerobic, hydrolysed starch and wheat, grown in NaCl 1% and not NaCl 3.0% and NaCl 6.0%, acid and gas production from glucose, sucrose, dextrose, maltose and L-arabinose. Catalase, citrate, urease and methyl red tests were positive results and nitrate, hydrogen sulphide, Voges Proskauer and phenylalanie test were negative results. This strain was recorded as the genus *Bacillus*. And strain A2 and A10 were gram-positive, facultative-anaerobic, short-rod, non-motile, spores were not forming, starch hydrolysed, acid and gas production from glucose, sucrose, dextrose, maltose and L-arabinose, not production from lactose and ribose. Catalase test, nitrate reduction and urease were positive. NaCl 1% was positive and NaCl 3.0% and NaCl 6.0% were negative results. Citrate, hydrogen sulphide, Voges Proskauer and phenylalanine were negative results. These strains were identified as *Streptococcus.* The strain A5 was gram-positive, facultative-anaerobic, short rod, non-motile, no spore forming hydrolysed starch and gas production from glucose, sucrose, dextrose, mannose, maltose and L-arabinose, catalase test, citrate, nitrate, urease and methyl red test were positive results and hydrogen sulphide (H_2S) and Voges Proskauer (VP) and phenylalanine test were negative results, grow in NaCl 1.0% was positive results, NaCl 3.0% and NaCl 6.0% were negative results. This strain was identified as *Coryne bacterium*. The strain A3 was gram-negative, aerobic, rod, non-motile, no spore forming hydrolysed starch, gas production from glucose, dextrose, maltose, mannose and L-arabinose, catalase test, nitrate, citrate, urease, methyl red, catalase and citrate tests give the positive results, sucrose, lactose, ribose, hydrogen sulphide, Voges Proskauer (VP) and Phenylalanine (PPA) test were negative results, NaCl 1.0%, NaCl 3.0% and NaCl 6.0% were negative results. This strain was identified as *Pseudomonas*. The strain A11 was gram-negative, rod, non-motile, spores forming, starch was not hydrolysed catalase test, lactose, dextrose, maltose and L-arabinose, nitrate, urea test, citrate were positive results. Methyl red, hydrogen sulphide, Voges Proskauer (VP) tests and Phenylalanine (PPA) were negative results. NaCl 1.0% was positive and NaCl 3.0% and NaCl 6.0% were negative results. This strain A11 was identified genus *Azomonas*.

The remaining strains A1, A6, A9 were gram-negative, short rod, the strains A7, A8 were gram negative, rod, non-motile, spores are not formed, starch hydrolysed, glucose, maltose and L-arabinose were production acid and gas. Strain A1 was glucose, sucrose, dextrose, maltose, L-arabinose, citrate, nitrate, urea test, methyl red, catalase were positive results and hydrogen sulphide and Voges Proskauer (VP) and Phenylalanine (PPA) were negative results. Strains A6. A7, A9 were positive for nitrate, urea and methyl red. These strains were identified as *Aeromonas.* In detail, characterization of the strain A4 was the genus *Bacillus,* A10 was the genus *Micrococcus*, A2 was the genus *Streptococcus*, A5 was the genus *Coryne bacterium*, A11 was the genus *Azomonas* and the remaining isolates A1, A6, A7, A8 and A9 were *Aeromonas* and A3 was the genus *Pseudomonas*. In the biological screening for antimicrobial activity tests, all the isolated strains against all test organisms were

exhibited and the zones of inhibition were respectively recorded in Table (9) and Figure (11).

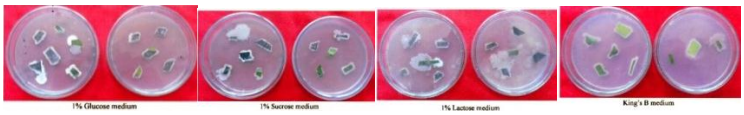

Figure (3) Colony characters of isolated bacteria from *Aegle marmelos*(L.) Correa (Leaves)

Characterization of the isolated Endophytic Bacteria

Table (1) Colony Characterization of Isolated Bacteria on 1% Glucose Medium

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
A 1	Medium	Entire	Red color	Flat, circular	Red pigment
A 2	Large	Undulate	Pale red color	Raise, irregular	Red pigment
A 3	Medium	Entire	Cream	Raise	Cream glistening

Table (2) Colony Characterization of Isolated Bacteria on 1% Sucrose Medium

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
A 4	Medium	Entire	Cream	Raise	Cream glistening
A 5	Large	Undulate	Cream	Flat, irregular	Red pigment
A 6	Medium	Entire	Cream	Flat, irregular	Cream glistening
A7	Medium	Entire	Cream	Raise, irregular	Brown glistening

Table (3) Colony Characterization of Isolated Bacteria on 1% Lactose Medium

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
A 8	Large	Lobate	Cream	Flat, filaments	Cream glistening
A 9	Large	Undulate	White	Flat, irregular	White glistening
A 10	Medium	Entire	Cream	Raise	Cream glistening

Table (4) Colony Characterization of Isolated Bacteria on King's B Medium

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
A 11	Medium	Entire	Cream	Convex, circular	Yellow pigment

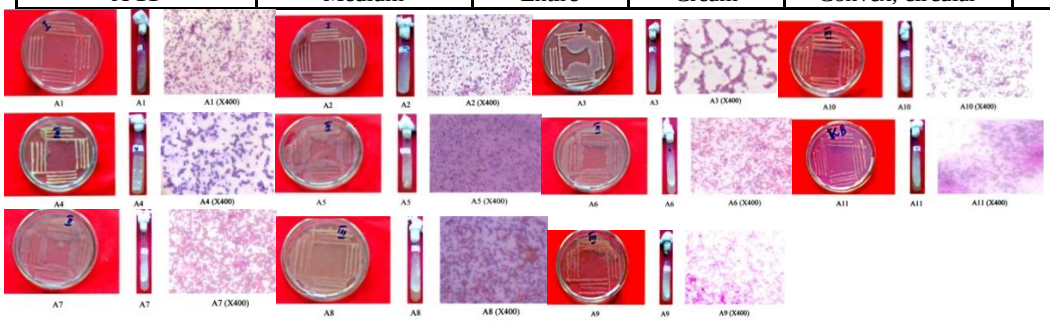

Figure (4) Cultural characteristics and cell morphology of isolated bacteria (A1 – A11)

Table (5) Morphological characteristics of isolated bacteria from *Aegle marmelos* (L.) Correa (Leaves)

Test \ Strains	A 1	A 2	A 3	A 4	A 5	A 6	A 7	A 8	A 9	A 10	A 11
Cell Morphology	Short rod	Short rod	Rod	Short rod	Short rod	Short rod	Rod	Rod	Short rod	Short rod	Rod
Endospore staining	-	-	-	+	-	-	-	-	-	-	+
Aerobic and Anaerobic	F-anaerobic	F-anaerobic	aerobic	F-anaerobic	F-anaerobic	F-anaerobic	F-anaerobic	F-anaerobic	F-anaerobic	F-anaerobic	F-anaerobic
Motility	-	-	-	-	-	-	-	-	-	-	-
Gram Staining	-	+	-	+	+	-	-	-	-	+	-

(+) = positive reaction (-) = negative reaction (F-) = facultative

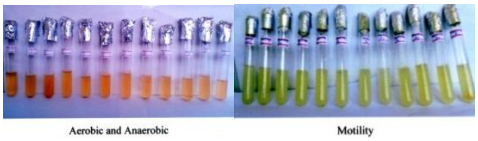

Table (6) Biochemical characteristics of isolated bacteria from *Aegle marmelos* (L.) Correa (L

No	Tests	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
1	Catalase	++	+	++	+++	+	+	-	+++	-	+++	++
2	Glucose(acid, gas)	++	++	+	++	++	++	++	++	++	+++	-
3	Sucrose (acid, gas)	+	+	-	+	+	++	+	-	+	+	-
4	Lactose (acid, gas)	-	-	-	-	-	+	+	++	+	-	+
5	Dextrose (acid, gas)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
6	Mannose (acids,gas)	-	-	+++	-	++	+	+	+	++	+	-
7	Maltose (acid, gas)	+	+	+	+	+	+	+	+	+	+	+
8	Ribose (acid, gas)	-	-	-	-	-	-	-	-	-	-	-
9	L.arabinose (acid, gas)	++	+	+	+	++	-	+	++	++	+	+
10	Citrate	+	-	+++	+++	++	+	+	+++	+	++	++

No	Tests	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
11	Nitrate	+++	+++	+++	-	+++	+++	+++	-	+++	+++	+
12	Urea test	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
13	Hydrogen sulphide	-	-	-	-	-	-	+	-	-	-	-
14	Methyl Red	+	++	++	+	+	++	+	+	+	-	-
15	Voges Proskauer	-	-	-	-	-	-	-	-	-	-	-
16	NaCl 1.0%	+	+	-	+	+	++	-	++	++	++	++
17	NaCl 3.0%	-	-	-	-	-	-	-	-	-	-	-
18	NaCl 6.0%	-	-	-	-	-	-	-	-	-	-	-
19	Phenylalanine (PPA)	-	-	-	-	-	-	-	-	-	-	-

(+) = positive (++) = high positive (+++) = betterpositive (-) = negative

Table (7) Enzymatic Activity Test of Isolated Bacteria from *Aegle marmelos* (L.) Correa (Leaves)

No.	Tests	A 1	A 2	A 3	A 4	A 5	A 6	A 7	A 8	A 9	A 10	A 11
1.	Strach hydrolysis	++	++	+++	+	+++	+++	+++	++	+++	+++	-
2.	Wheat hydrolysis	++	+	+++	+	-	++	++	++	+	-	++

+ = hydrolysis +++ = better hydrolysis ++ = high hydrolysis– = non hydrolysis

Figure (6) Catalase reaction of isolated bacteria from *Aeglemarmelos*(L.) Correa (Leaves)

Figure (7) Biochemical test of isolated bacteria from *Aegle marmelos*(L.) Correa (Leaves)

Starch powder

Wheat powder

Figure (8) Enzymatic activity for Starch hydrolysis of isolated bacteria from *Aegle marmelos* (L.) Correa (Leaves)

Identification of the isolated Endophytic Bacteria

According to the results, the isolated bacteria strains were assumed to be *Bacillus, Streptococcus, Corynebacterium, Pseudomonas, Azomonas* and *Aeromonas.*

Table (8) Identification of the isolated Endophytic Bacteria

No.	Strains No.	Genus
1	A1	*Aeromonas*
2	A2	*Sterptococcus*
3	A3	*Pseudomonas*
4	A4	*Bacillus*
5	A5	*Corynebacterium*
6	A6	*Aeromonas*
7	A7	*Aeromonas*
8	A8	*Aeromonas*
9	A9	*Aeromonas*
10	A10	*Sterptococcus*
11	A11	*Azomonas*

Table (9) Test for Antimicrobial Activity of Isolated Bacteria Strains

No.	Strains no. / Test organisms	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
1.	*Vibrio cholerae*	-	-	-	-	19.3 mm	3.3 mm	-	-	-	24.6 mm	-
2.	*Pseudomonas aeruginosa*	10.7 mm	-	-	-	-	-	-	-	-	-	-
3.	*Staphylococcus aureus*	3.5 mm	2.3 mm	-	-	3.9 mm	-	-	-	-	2.9 mm	2.2 mm
4.	*Klebsiella pheumoniae*	2.4 mm	-	-	-	-	-	-	-	-	-	-
5.	*Proteus mirabilis*	1.8 mm	-	7.7 mm	-	-	-	-	-	-	12.2 mm	-
6.	*Candida albicans*	-	-	-	-	-	-	-	-	-	4 mm	-
7.	*Esherichia coli*	3.8 mm	-	-	-	-	-	-	-	-	3.2 mm	-
8.	*Bacillus subtilis*	4.4 mm	4.4 mm	-	-	-	-	-	-	-	-	-
9.	*Bacillus pumalis*	-	-	-	-	-	-	-	-	-	-	-

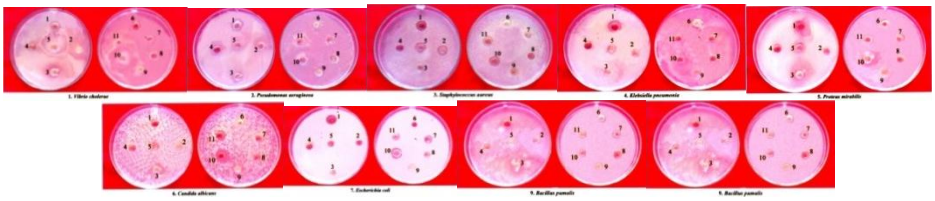

Figure (9) Antimicrobial activity of isolated bacteria from *Aegle marmelos* (L.) Correa (Leaves) (A1-A11)

Discussion and Conclusion

This research was undertaken to isolate and characterize the endophytic bacteria from *Aegle marmelos* (L.) Correa(Leaves).Eleven strains were isolated from these samples and all strains were characterized as *Aeromonas, Streptococcus, Bacillus, Coryne bacterium, Pseudomonas* and *Azomonaas.* These results were matched with description in Bergey's Manual of Determinative Baccteriology by Buchnan *et al.,* 1957. In the biochemical characterization strain A1, A3, A4, A8, A10 and A11 were positive result and other strains were negative results for catalase test. Strains A1-A10 were positive results and A11 was negative results for starch hydrolysis test. Strains of A1-A6 and A8-A11 were negative results and A7 was positive results for hydrogen sulphide production test. All strains showed negative results for motility test and Voges Proskaure test (VP).Strains A1, A3-A11 were positive results and A2 was negative result for citrate reduction test. Strains A1-A9 were positive results A10 and A11 were negative results for methyl red test. Strains A1, A2, A3, A5, A6, A7 and A9-A11 obtained positive results and A4 and A8 obtained negative results for nitrate reduction test. The strains, A1, A3-A10 showed positive results and strains A2 showed negative results for citrate test. For sugar fermentation tests, all strains were positive results for dextrose, maltose, gas produced from strains A1-A10 were positive and A11 were negative results for glucose. All strains showed negative results for ribose. Strains A1, A2, A4-A7, A9 and A10 showed the positive results and strains, A3, A8, A11 was negative results for sucrose. Strains A6-A9, A11 were positive results and A1 to A5, A10 showed negative results for lactose sugar. Strains A1-A5, A7-A11 exhibited positive results and A6 exhibited the negative results for L-arabinose. Antimicrobial activities of isolated strains were carried by nine test organisms. Among all these isolated strains, strain A1 showed the test clear zone against on *Pseudomonous aeruginosa.* Strain A2 showed the moderate clear zone against on *Staphylococcus aureus* and *Bacilllus subtilis*. The isolated strain A3 showed the best clear zone against on *Proteus mirabilis.* Strains A4, A7, A8, A9 were not showed clear zone on nine test organisms. The isolated strain A5 showed the best clear zone against on *Vibrio cholerae*. Strain A6 showed the best clear zone against on *Klebsiella pneumonia.* Strain A11 showed less clear zone against on *Staphyolococcus aureus* among all these results A1 and A10 showed the best antimicrobial activities. According to results, all the isolated strains were not against to the *Candida albicans.*This data was not agreed with Berde *et al*., 2015.In India, the biological roles of endophytic fungi that the ability of endophytic fungi is to produce new and interesting bioactive secondary metabolites, which are of pharamaceutical, and agricultural importance. The various natural products produced by endophytic fungi possess unique structures and bioactivities against various diseases (Mishra *et al.,* 2014).In conclusion, this research aimed to isolated the useful endophytic bacteria from *Aegle marmelos* (L.) Correa(Leaves).Eleven strains were obtained and they were identified as seven kinds of possible genus. This paper not only provides the

knowledge of isolation and identification methods but also contribute the knowledge that endophytic bacteria of the plant which possess the wide range of antimicrobial activity.

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