# Antagonistic Activity of *Calotropis gigantea* and *Lactobacillus delbrueckii* Against Human Pathogens

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**Abstract:**
The antagonistic activity of *Lactobacillus delbrueckii* against target pathogens such as *Escherichia coli, Salmonella Sp. Shigella Sp, Aspergillus niger* and *Candida albicans* was tested. The antagonistic activity of *Lactobacillus delbrueckii* was compared with *Calotropis gigantea* extract. Antimicrobial activity of *Lactobacillus delbrueckii* and *Calotropis gigantea* was assessed by agar well assay technique. *Lactobacillus delbrueckii* was inoculated into broth and incubated at $37^0$C for 24 hrs. Cell free extracts were obtained by centrifugation. Wells of 6 mm diameter were made in each plate of Tryptone dextrose agar seeded with test pathogenic organisms. Cell free extract of *Lactobacillus delbrueckii* cultures were transferred at the rate of 50 µl into each well. In another well the plant extract was transferred at the rate of 50 µl. The plates were incubated at $37^0$C for 48 hours without inverting. Zones of inhibition formed around the well were measured.

**Key Words: Antagonistic activity, *Calotropis gigantean,* Human Pathogens**

**Introduction:**
*Calotropis gigantea* is a common shrub; it is about 3-6 feet height. Its use has been mentioned in ancient scripts of ayurveda. It belongs to the family *Asclepiadaceae*. It contains milky latex. The flowers are lilac, rosy or purple colored. It grows in dry land. From the leaves mudarine, calotropin, calotoxin, uscharin, and uscharidin have been isolated. Calotropin shows digitalis like action on the heart. In 1980, Pal and Sinha had isolated, crystallized and studied the properties of calotropins $D_1$ and $D_2$ from *C.*gigantea.[14] The new oxiopregnane-oligoglycosides named calotropin A and B have been isolated from the root of *C.gigantea* and their chemical structure have been elucidated by chemical and spectroscopy methods.[7] The cytotoxic principles of *'Akond mul'* (Root of *C.gigantea*) cardenoloids glycosides, calotropin frugoside and 4-O-Beta-D-glucopyranosyl frugoside were obtained as the cytotoxic principles.[8]

The human intestinal tract is a nutrient rich environment that is colonized by a vast and complex collection of microorganisms that play a major role in its function and development.[5,9]
The enteropathogens such as *Escherichia coli, Salmonella* sp *and Shigella* sp are found to cause intestinal disorders by colonization in the intestinal tract. The *Shigella sp* is found to cause shigellosis, a diarrhoeal illness.[3] The *Salmonella sp* causes typhoid fever; salmonellosis is most often caused by animal pathogens through contamination.[1] *Escherichia coli* inhabit in humans and animals intestine. It causes travelers diarrhoea. It produces heat labile and heat stable enterotoxins.[17] *Candida albicans* is found to cause candidiasis. It is a pathogenic fungus causing oral candidiasis, bronchocandidiasis, pulmonary candidiasis and meningeal candidiasis. *Aspergillus niger* is found to cause aspergillosis.[3]

This paper reports a study of antagonistic activity of *Calotropis gigantean* and *Lactobacillus delbrueckii* against human pathogens such as *Escherichia coli, Salmonella* sp*, Shigella* sp*, Candida albican, and Aspergillus niger.*

**Materials and Methods:-**
**Collection of plant:-**
The *Calotropis gigantea* was procured from in and around Kovilpatti.

**Preparation of plant extract:-**
From the plant latex was extracted aseptically onto the eppendorf tube, then ethanol was

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added (1:1) to the eppendorf tube containing the latex and it was shaken vigorously for proper mixing. It was then allowed to evaporate for 48 hours at $55^0$C. The dried extract was homogenized by adding ethanol. The homogenized extract was used for experimental purpose.[12]

**Lactic acid bacteria (LAB):-**

*Lactobacillus delbrueckii* (ATCC 9338) was procured from ATCC (American type culture Collection). *L. delbrueckii* was inocubated into de man Rogasa Sharpe (MRS) broth and was incubated at $37^0$C for 24 hours.

**Isolation and identification of test pathogens:-**

The test pathogens used in this study includes clinical isolates such as *Escherichia coli, Salmonella Sp, Shigella sp, Candida albicans and Aspergillus niger.*

The *E. coli, Salmonella* sp *and Shigella* sp were isolated from clinical specimens. These bacteria were serially diluted and plated onto selective media, Eosin methylene blue agar, *Salmonella Shigella* agar respectively. These plates were incubated at $37^0$C for 24 hours. The above test microorganisms were also confirmed by bio-chemical test. The *A. niger* was isolated from sputum and *C. albicans* was isolated from sore throat. The *A. niger* was plated onto Potato Dextrose agar and incubated at room temperature for 4 days, and then it was confirmed by slide culture technique using Lacto phenol cotton blue stain. *Candida albicans* was plated onto Corn meal agar and incubated at room temperature for 4 days, and then it was confirmed by germ tube test.[16]

**Antimicrobial testing:-**

The antagonistic activity of *C. gigantea* plant extract and *Lactobacillus delbrueckii* was assessed using agar well assay technique. The *L. delbrueckii* culture broth tubes were divided into two equal halves and were taken in two separate sterile tubes. In one tube the pH was increased to 9 and in another tube it was decreased to 3 in order to facilitate the breakage of cell wall. The cell free extracts were obtained by centrifugation of the samples at 4500 rpm for 10 minutes and were passed through 0.45 µm membrane filter. Three wells of 5 µm diameter were made in each plate of tryptone dextrose agar plate seeded with test pathogenic organism using sterile hollow borer. The plant extract and the cell free extract of *L. delbrueckii* culture were transferred at the rate of 50 µl into each corresponding well. Then the plates were kept in refrigerator for 3 hours for allowing the diffusion of sample into wells followed by incubation at $37^0$C for 48 hours without inverting.[16]

**Result and Discussion:-**

**Antimicrobial activity of *Calotropis gigantea* against human pathogens:**

The antimicrobial activity of *Calotropis gigantea* against target pathogens was tested under aerobic conditions. Table 1 shows that the strongest inhibitory activity was found against *Salmonella* sp (12-13 mm) and *Candida albicans* (10-12 mm) followed by *Shigella* sp (9-10 mm). The lowest inhibitory activity was found against *Escherichia coli* (8-10 mm) and *Aspergillus niger* (6-8 mm).

**Table 1: Antimicrobial activity of *Calotropis gigantea* against human pathogens:-**

| S. No | Test Organism | *Calotropis gigantean* Zone of inhibition (mm) |
|---|---|---|
| 1. | *Escherichia coli* | 8 – 10 |
| 2. | *Salmonella* sp | 12 - 13 |
| 3. | *Shigella* sp | 9 - 10 |
| 4. | *Candida albicans* | 10 – 12 |
| 5. | *Aspergillus niger* | 6 – 8 |

Based on our findings, table1 shows the maximum inhibitory activity of the plant extract which was against *Salmonella* sp and *Candida albicans*. This inhibition may be due to the presence of calotropins $D_1$ and $D_2$,[14] cardenoloids glycosides, calotropin frugoside and 4-O-Beta-D-glucopyranosyl frugoside. Kiuchi, F., *et.al.* also observed the same result.[8] The *Calotropis gigantea* is used as an antivenom against snake bite and scorpion bite. It is found effective against asthma, cough and cold. It is also useful in treating piles and dental aches.[8]

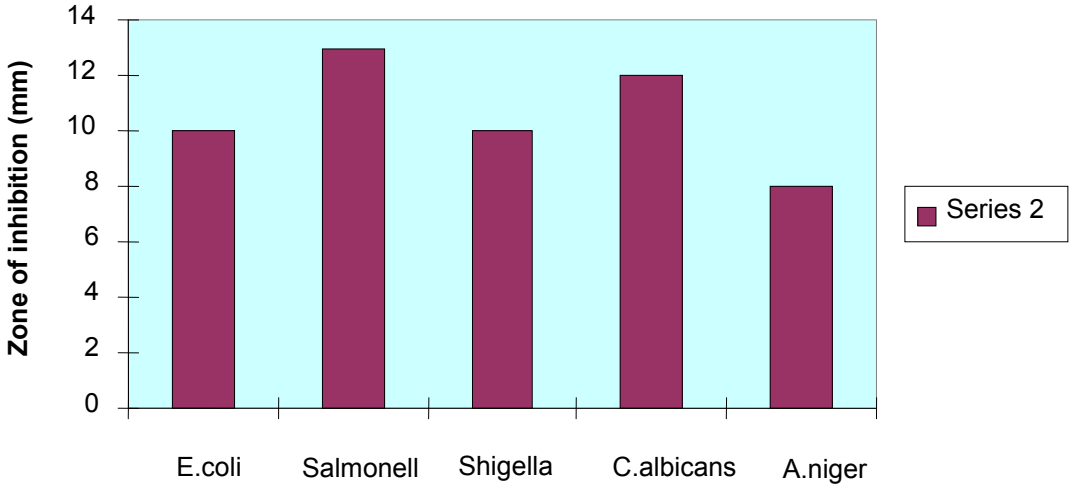



**Fig 1: Antimicrobial activity of *Calotropis gigantea* against clinical pathogens**

**Antagonistic activity of *Lactobacillus delbrueckii* with high pH and low pH against target pathogens:-**

The antagonistic activity of *L.delbrueckii* with high pH and low pH against target pathogens were tested under aerobic conditions.

**With high pH:-**

Table 2 shows that the maximum inhibitory activity was observed against *Aspergillus niger* (12-14 mm), *C.albicans* (9-11 mm) followed by *Shigella* sp (9-10 mm) and *Salmonella* sp (8-10 mm). The minimum inhibitory activity was observed against *E.coli.*

**With low pH:-**

Table 2 shows that the maximum inhibitory activity was found against *A.niger* (12-14 mm), *C. albicans* (9-11 mm) followed by *Shigella* sp (7-8 mm). The minimum inhilitory activity was found against *Salmonella* sp (5-7 mm) and *E. coli* (5-6 mm).

The ability of lactic acid bacteria (LAB) to inhibit the growth of various Gram positive, Gram negative bacteria and fungi is well known. This inhibition may be due to the production of organic acids such as lactic acid and acetic acid,[6] hydrogen peroxide, bacteriocin, bacteriocin-like substance and possibly bio-surfactants.[16] The lactic acid bacteria (LAB) have been shown to inhibit the *in vitro* growth of many enteric pathogens and have been used in both humans and animals to treat a broad range of gastrointestinal disorders.[15] Several researchers have reported on highly antagonistic *Lactobacilli* and associated this activity among others with production of organic acids, and the subsequent decrease in pH [11] (table 2). The inhibitory activities caused by the production of hydrogen peroxide by *Lactobacilli* have been documented previously.[4]

**Table: 2 Comparison of antimicrobial activity of *Lactobacillus delbrueckii* with high and low pH:-**

| S.No | Target bacteria | Zone of inhibition (mm) | |
|---|---|---|---|
| | | ***L. delbrueckii*** **high pH** | ***L. delbrueckii*** **low pH** |
| 1. | *Escherichia coli* | 6 - 8 | 5 - 6 |
| 2. | *Salmonella* sp | 8 - 10 | 5 - 7 |
| 3. | *Shigella* sp | 9 - 10 | 7 - 8 |
| 4. | *Candida albicans* | 9 - 11 | 9 - 11 |
| 5. | *Aspergillus niger* | 12 – 14 | 12 - 14 |

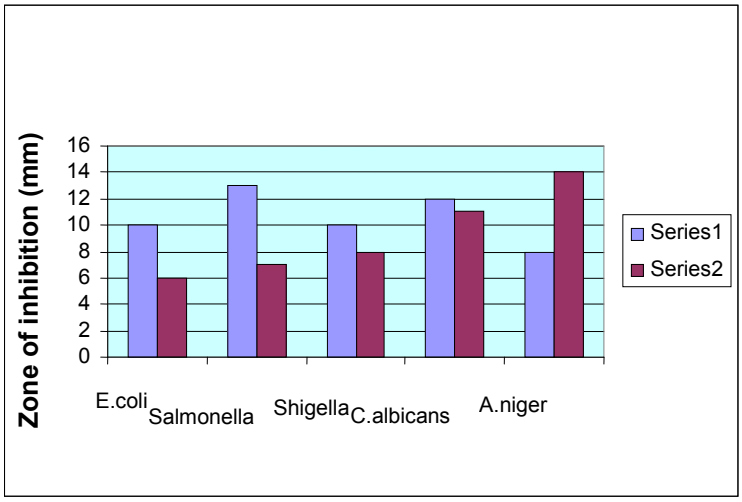



**Fig 2: Comparative antimicrobial activity of *Lactobacillus delbrueckii* with high and low pH.**

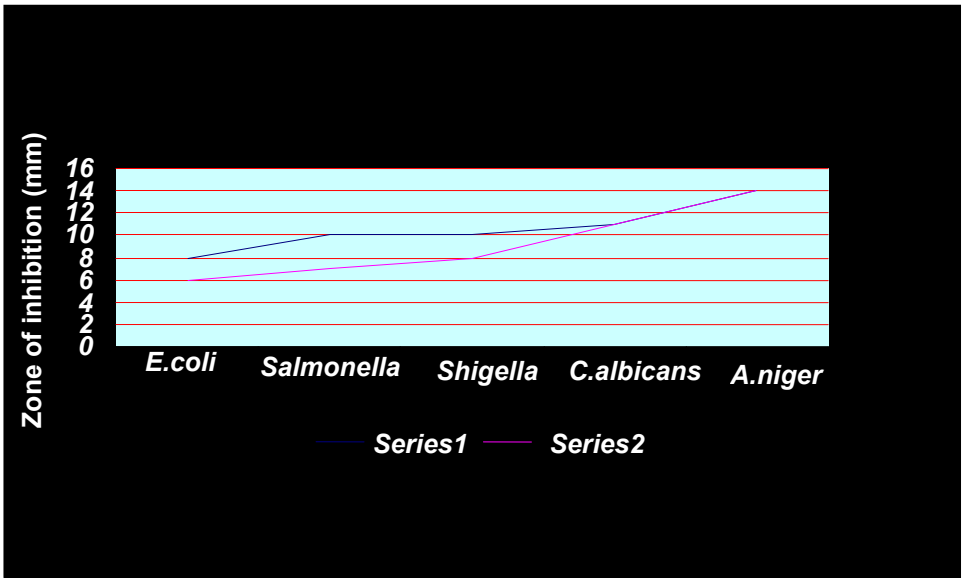



**Fig 3: Comparative antimicrobial activity of *Lactobacillus delbrueckii* with high and low pH.**

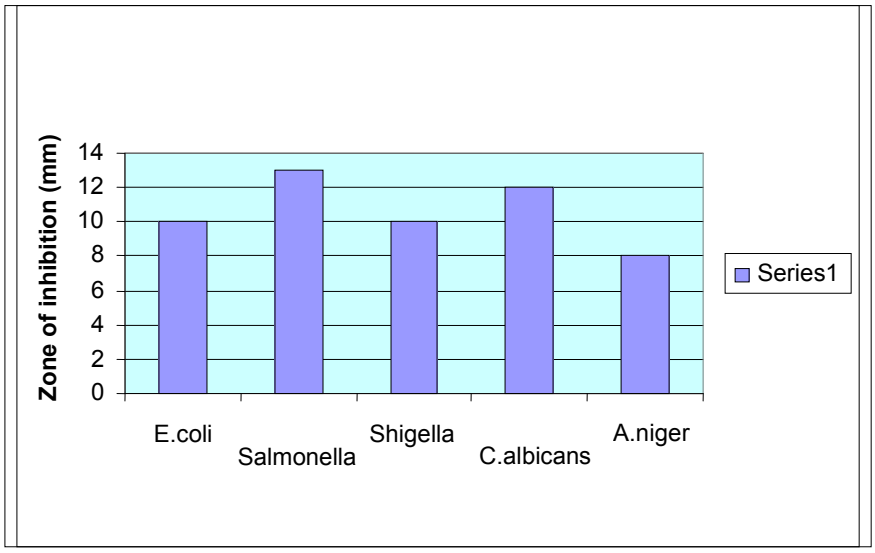



**Fig 4: Antimicrobial activity of *Lactobacillus delbrueckii* with high pH.**

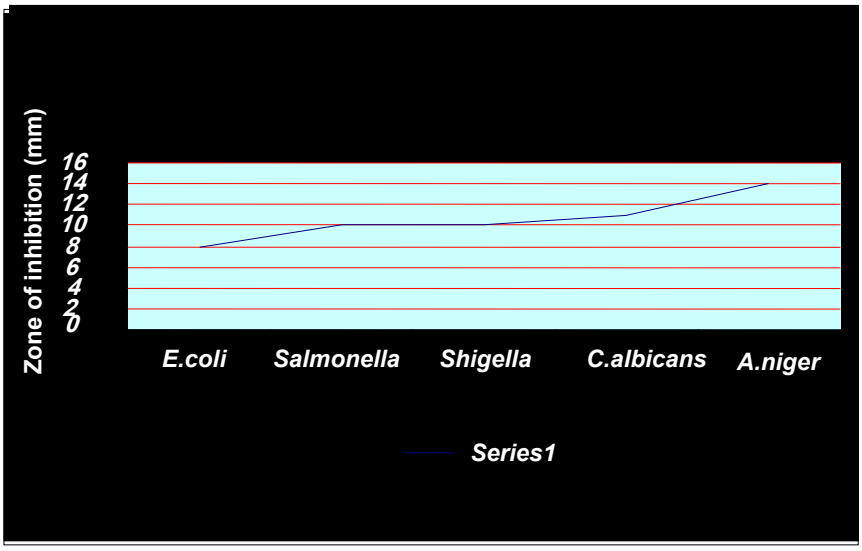



**Fig 5: Antimicrobial activity of *Lactobacillus delbrueckii* with high pH.**

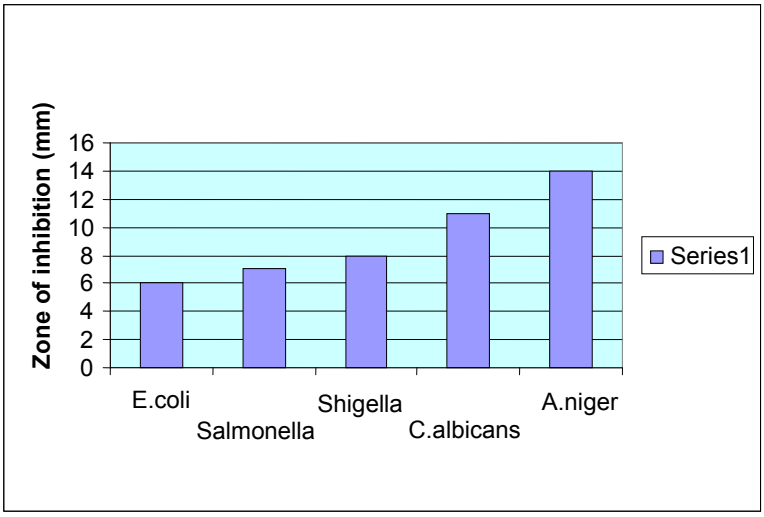



**Fig 6: Antimicrobial activity of *Lactobacillus delbrueckii* with low pH.**

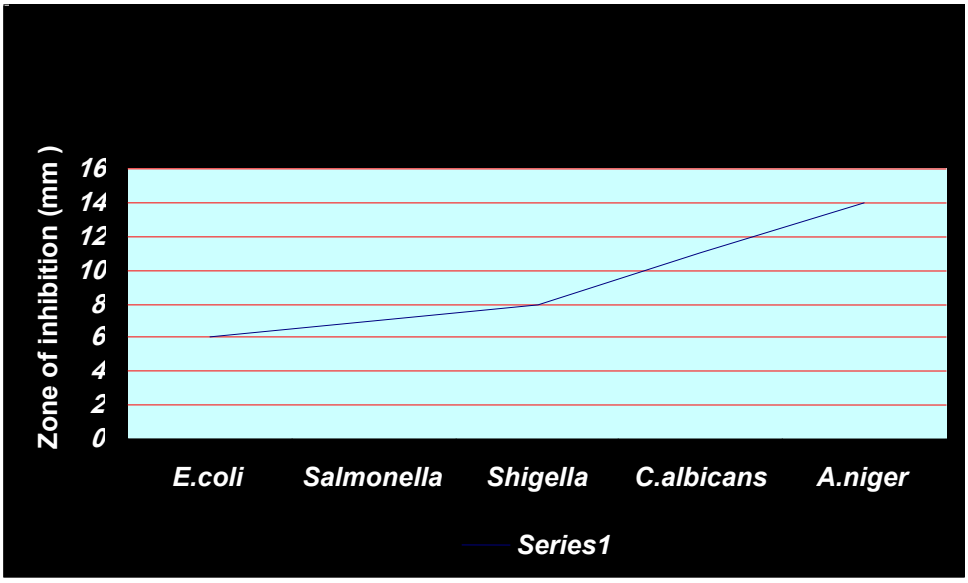



**Fig 7: Antimicrobial activity of *Lactobacillus delbrueckii* with low pH.**

*Lactobacilli* are nutritionally fastidious anaerobes. Table 2 and fig 2 and fig 3 show the antimicrobial activity of *L.delbrueckii* against the enteropathogens, *A.niger* and *C.albicans*. *L.delbrueckii* shows the maximum inhibitory activity against *A.niger* and *C.albicans*. Among the enteropathogens the maximum inhibition was found against *Shigella* sp and *Salmonella* sp. The adhesion of the probiotic microorganisms to the intestinal mucosa is a prerequisite for colonization and for antagonistic activity against enteropathogens [13].

**Comparison of antimicrobial activities of *Calotropis gigantea* and *Lactobacillus delbrueckii* against target pathogens:**

Table 1 shows that the extract showed its maximum inhibitory activity against *Salmonella* sp (12-13 mm) and its minimum inhibitory activity against *Aspergillus (A.) niger* (6-8 mm), whereas the table 2 shows that the *L. delbrueckii* showed its maximum inhibitory activity against *A. niger* (12-14) and its minimum inhibitory activity against *E.coli* (6-8). The lactic acid bacteria (LAB) have been shown to inhibit the *in vitro* growth of many enteric pathogens and have been used in both humans and animals to treat a broad range of gastrointestinal disorders like traveler's diarrhea, salmonellosis etc.[15]

**Conclusion**:

The antagonistic activity of *Lactobacillus delbrueckii* and *Calotropis gigantea* extract against target pathogens such as *Escherichia coli, Salmonella Sp. Shigella Sp, Aspergillus niger* and *Candida albicans* was tested. The antagonistic activity of *Lactobacillus delbrueckii* was compared with *Calotropis gigantea* extract. Cell free extract of *Lactobacillus delbrueckii* cultures were transferred at the rate of 50 µl into each well. In another well the plant extract was transferred at the rate of 50 µl. The plates were incubated at $37^0$C for 48 hours without inverting. Zone of inhibition formed around the well were measured. From our observation the *C.gigantea* extract showed its maximum inhibitory activity against *Salmonella* sp (12-13 mm) and minimum inhibitory activity against *A. niger* (6-8 mm), whereas the *L. delbrueckii* showed its maximum inhibitory activity against *A. niger* (12-14) and minimum inhibitory activity against *E. coli* (6-8).

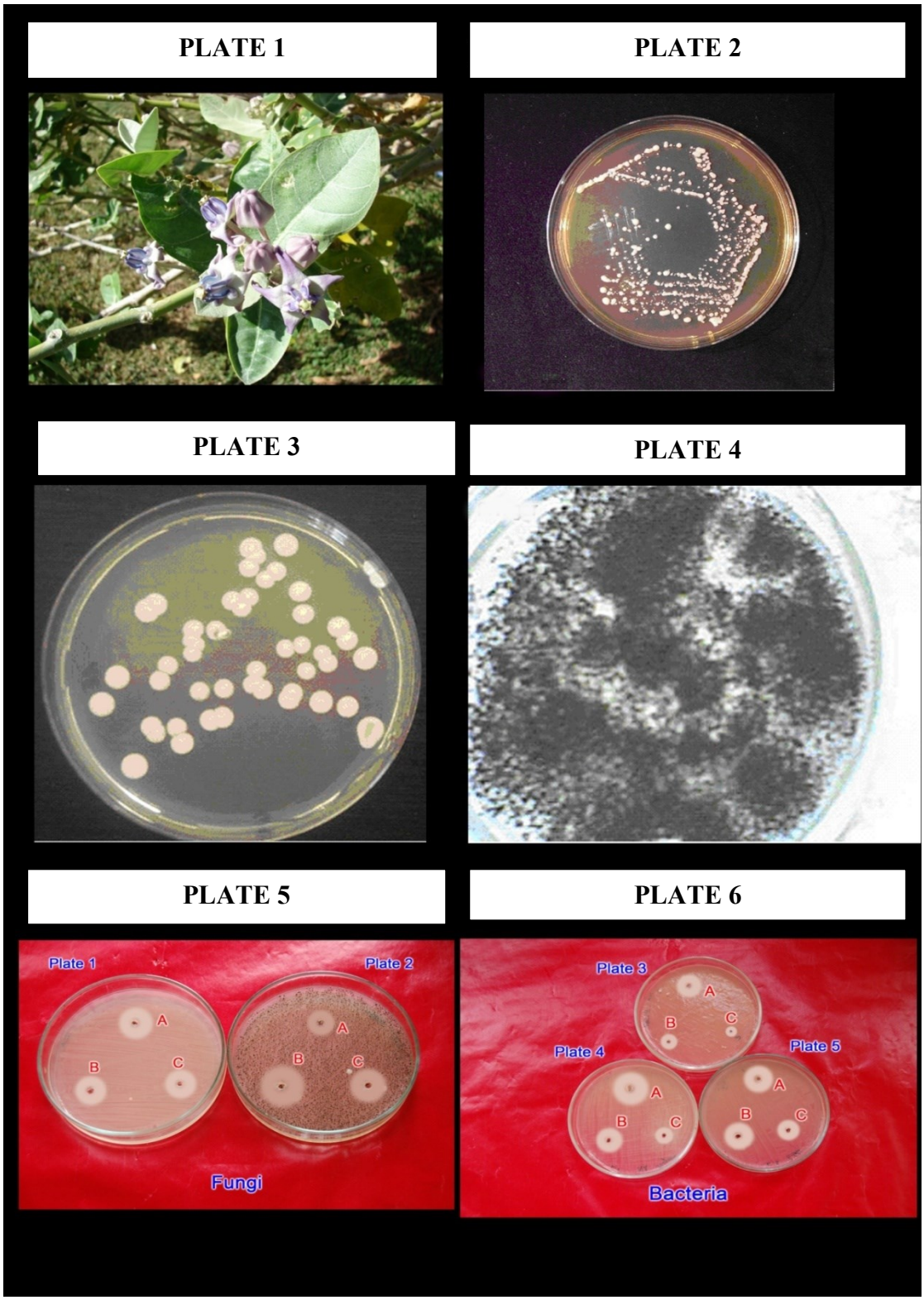



**Plate 1:** ***Calotropis gigantea*** **Plate 2:** ***Lactobacillus delbrueckii***
**Plate 3:** ***Candida albicans*** **Plate 4:** *Aspergillus niger*
**Plate 5: Antimicrobial activity of** ***Calotropis gigantea*** **and** ***Lactobacillus delbrueckii*** **against Fungi.**
**Plate 6: Antimicrobial activity of** ***C. gigantea*** **and** ***L. delbrueckii*** **against Bacteria**

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