

Antibacterial Activity of Five Plant Stem Bark And Leaves Extracts in Different Solvent Against Pathogenic Bacteria

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Abstract- Antibacterial activity of Acetone, Chloroform and Aqueous extracts prepared from five plants Stem bark and Leaves (*Manilkara zapota*, *Manilkara hexandra*, *Mangifera indica*, *Madhuka indica*, *Annona squamosa*) used in traditional folk medicine of India were screened against four pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* and *Salmonella enterica*). The highest antibacterial potentiality was exhibited by the Aqueous extract of *Manilkara hexandra* leaves, Chloroform extract of *Annona squamosa* leaves, Acetone extract of *Annona squamosa* leaves and *Manilkara hexandra* stembark, Aqueous extract of *Mangifera indica* leaves can be as equally potent as the most effective antibiotic Ampicillin. A comparison of data in the inhibition zones of pathogenic bacteria showed that Ampicillin was effective against all of the bacterial strains tested.

Keywords- Antibacterial activity, *Annona squamosa*, *Madhuka indica*, *Manilkara hexandra*, *Manilkara zapota*

I. INTRODUCTION

Antibiotics are one of four most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illness not, only because of many of them produce toxic reactions, but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented.

Herbal medicines have known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorder has been described by practitioners of traditional medicine. Antimicrobial properties of medicinal plants are being increasingly reported from different part of the world.

The World Health Organization estimate that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.

In recent years, there has been an increasing awareness about the importance of medicinal plants. Drugs from these plants are easily available, inexpensive, safe, efficient and rarely accompanied by side effects. Plants which have been selected for medical use over thousands of years constitute the most obvious starting point for new therapeutically effective drugs such as anticancer drugs and antimicrobial drugs. Recently, medicinal plants usage has increased in spite of the advances made in the field of chemotherapy. The reason proposed are the use of medicinal plant as materials for the extraction of active pharmacological agents or as precursors for chemicopharmaceutical hemisynthesis. There is also the increased use of medicinal plants in industrialized countries for galenic preparations and herbal medicines. Plants have an amazing ability to produce a wide variety of secondary metabolites like alkaloid, glycoside, terpenoid, saponin, steroid, flavonoid, tannin, quinone and coumarins. These biomolecules are the source of plant-derived antimicrobial substances. Some natural products are highly efficient in the treatment of bacterial infections.

Medicinal plants are effective in the treatment of infectious diseases and infections of various types of external wounds (chronic, deep suppurative, open, lacerated, incised and ulcerated) and have been used for these purposes in humans and different species of animals. The use of medicinal plants has the added benefit of reducing many of the side effects often associated with synthetic antimicrobials.

There are numerous plants and natural products which have antibacterial, antifungal and antiprotozoal effect that could be used either systematically or locally. Medicinal properties of plants have also been preferred throughout the world, due to their potent pharmacological activities, low toxicity and economic viability when compared with synthetic drugs. Medicinal plants rich in a wide variety of bioactive

secondary metabolites such as tannins, alkaloids, saponins, flavonoids and phenolic compounds that can produce definite physiological action on the human body.

II. MATERIAL AND METHOD

Four bacterial strain in which Two were Gram positive: *Staphylococcus aureus*, *Bacillus subtilis* and Two were Gram negative *Escherichia coli*, *Salmonella enterica* were used in this study. All the bacterial strains were clinically isolates obtained from Microbial Type culture collection, Pune.

Collection of Plant materials

The Medicinal Plants used for the experiment were *Manilkara zapota*, *Manilkara hexandra*, *Mangifera indica*, *Madhuka indica*, *Annona squamosa*. The Plant material were collected from the surrounding area of Talod taluka, North Gujarat, India.

Preparation of Plant Extracts

Collected Plant parts were washed thoroughly and chopped into small pieces, shade dried and grinded into powdered form with the help of mechanical grinder. 2gm of powdered plant material was dissolved in 10 ml of three different solvent like Acetone, Chloroform and Water respectively for 24 hours. This solution was sonicated for 10 min and filter it and used for further Antibacterial activity test.

Determination of Antibacterial activity

Antibacterial activity of different Plant extracts was determined by Agar well diffusion method. 0.1 ml of Freshly grown culture of test organisms (10^6 CFU/ml) was aseptically introduced and spread on surface of sterile agar plate. Wells of 6mm diameter were made in agar plate with help of sterile cork-borer. 50 μ l of different plant extract and same volume of extraction solvent for negative control were filled in the wells with the help of micro pipette. Standard reference antibiotic like Ampicillin was used as positive control for the test organisms. Plates were left for some times at 4 $^{\circ}$ C till the extracts diffuses in the medium with the lid closed and incubated at 37 $^{\circ}$ C for 24 hr. The plates were observed for zone of inhibition. Antibacterial activity was evaluated by measuring the diameter of zone of inhibition against the tested bacterial pathogens. (Shinde A.B et al.2015)

III. RESULT AND DISCUSSION

The Acetone, Chloroform and Water extracts of *Manilkara zapota* Stem bark and Leaves, *Manilkara hexandra* Stem bark and Leaves, *Mangifera indica* Stem bark and Leaves, *Madhuka indica* Stem bark and Leaves, *Annona squamosa* Stem bark and Leaves were screened for Anti-bacterial activity against two Gram positive; *Staphylococcus aureus* and *Bacillus subtilis* and two Gram negative bacteria; *Salmonella enterica* and *Escherichia coli*. Culture dilution of bacteria is 10^6 CFU/ml. Spreading of culture on petri dish 100 μ l of 10^6 CFU/ml. Petri dish keep for incubation at 37 $^{\circ}$ C for 24 hours. After 24 hours zone of inhibition major in mm.

Table 1: Anti-bacterial activity against *Staphylococcus aureus* bacteria.

Sr. No.	Plant Name	Solvent for Extraction	Zone of inhibition in mm	
			Sample	Ampicillin
1	<i>Annona squamosa</i> Leaf	Chloroform	13	13
2	<i>Annona squamosa</i> Stem bark		11	
3	<i>Manilkara hexandra</i> Leaf		00	
4	<i>Manilkara hexandra</i> Stem bark		12	
5	<i>Manilkara zapota</i> Leaf		00	
6	<i>Manilkara zapota</i> Stem bark		00	
7	<i>Madhuka indica</i> Leaf		00	
8	<i>Madhuka indica</i> Stem bark		00	
9	<i>Mangifera indica</i> Leaf		00	
10	<i>Mangifera indica</i> Stem bark		11	
11	<i>Annona squamosa</i> Leaf	Water	00	13
12	<i>Annona squamosa</i> Stem bark		00	
13	<i>Manilkara hexandra</i> Leaf		14	
14	<i>Manilkara hexandra</i> Stem bark		12	
15	<i>Manilkara zapota</i> Leaf		00	
16	<i>Manilkara zapota</i> Stem bark		13	
17	<i>Madhuka indica</i> Leaf		00	
18	<i>Madhuka indica</i> Stem bark		00	
19	<i>Mangifera indica</i> Leaf		00	
20	<i>Mangifera indica</i> Stem bark		00	
21	<i>Annona squamosa</i> Leaf	Acetone	13	13
22	<i>Annona squamosa</i> Stem bark		11	
23	<i>Manilkara hexandra</i> Leaf		12	
24	<i>Manilkara hexandra</i> Stem bark		13	
25	<i>Manilkara zapota</i> Leaf		00	
26	<i>Manilkara zapota</i> Stem bark		00	
27	<i>Madhuka indica</i> Leaf		00	
28	<i>Madhuka indica</i> Stem bark		11	
29	<i>Mangifera indica</i> Leaf		00	
30	<i>Mangifera indica</i> Stem bark		11	

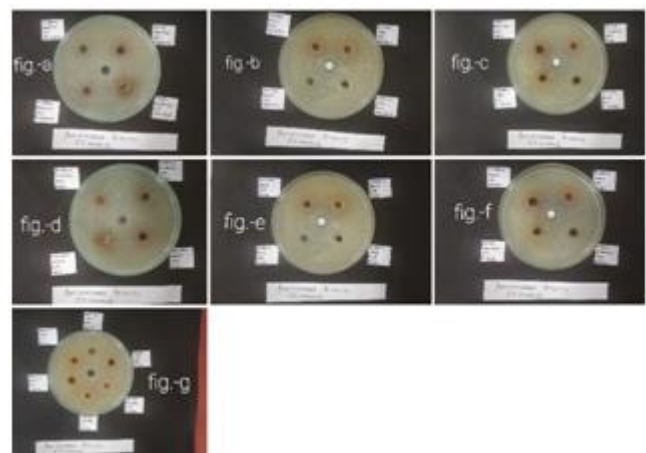


Figure 1:- *S. aureus* Antibacterial activity

The Antibacterial activity result was noted in above Table no-1. The Chloroform extract of Leaves of *Annona squamosa* showed the best zone of inhibition (13mm) which was equivalent to the test antibiotic Ampicillin against *Staphylococcus aureus*, while the Stem extract of the same plant species against the test pathogen showed a zone little has i.e.,11mm and the zone size of 12mm observed for the *Manilkara hexandra* Stem bark and 11mm observed in *Mangifera indica*. While none of the other extract showed any inhibition.

The test organism *Staphylococcus aureus* was effectively inhibited by three plant extracts viz. Aqueous extract of *Manilkara hexandra* Leaf, *Manilkara hexandra* Stem bark and *Manilkara zapota* Stem bark with zone diameter 14mm, 12mm and 13mm respectively. *Manilkara hexandra* Leaf showed 14mm zone of inhibition. This was higher than the inhibition by test antibiotic Ampicillin inhibitory zone diameter of 13mm. The aqueous extract of *Manilkara zapota* Stem bark showed the best zone of inhibition 13mm which was equivalent to the test antibiotic Ampicillin against *Staphylococcus aureus*. The Aqueous extract of *Manilkara hexandra* Stem bark showed 12mm zone of inhibition which was less than the test antibiotic Ampicillin. While none of the other plant extract showed any inhibition zone.

The Acetone extract of Leaf of *Annona squamosa* and *Manilkara hexandra* Stem bark showed the best zone of inhibition(13mm) which was equivalent to the test antibiotic Ampicillin against *Staphylococcus aureus*. Acetone extract of *Manilkara hexandra* Leaf showed 12mm zone of inhibition. This was less than the test antibiotic Ampicillin(13mm). While another three plants Acetone extracts of *Annona squamosa* Stem bark, *Madhuka indica* Stem bark and *Mangifera indica* Stem bark showed zone of inhibition 11mm which was less than the Ampicillin. While none of the other plant extract showed any inhibition.

Table 2: Anti-bacterial activity against *Bacillus subtilis* bacteria.

Sr. No.	Plant Name	Solvent for Extraction	Zone of inhibition in mm	
			Sample	Ampicillin
1	<i>Annona squamosa</i> Leaf	Chloroform	11	33
2	<i>Annona squamosa</i> Stem bark		12	
3	<i>Manilkara hexandra</i> Leaf		00	
4	<i>Manilkara hexandra</i> Stem bark		00	
5	<i>Manilkara zapota</i> Leaf		00	
6	<i>Manilkara zapota</i> Stem bark		00	
7	<i>Madhuka indica</i> Leaf		00	
8	<i>Madhuka indica</i> Stem bark		00	
9	<i>Mangifera indica</i> Leaf		00	
10	<i>Mangifera indica</i> Stem bark		00	
11	<i>Annona squamosa</i> Leaf	Water	00	25
12	<i>Annona squamosa</i> Stem bark		00	
13	<i>Manilkara hexandra</i> Leaf		11	
14	<i>Manilkara hexandra</i> Stem bark		12	
15	<i>Manilkara zapota</i> Leaf		00	
16	<i>Manilkara zapota</i> Stem bark		00	
17	<i>Madhuka indica</i> Leaf		00	
18	<i>Madhuka indica</i> Stem bark		00	
19	<i>Mangifera indica</i> Leaf		00	
20	<i>Mangifera indica</i> Stem bark		00	
21	<i>Annona squamosa</i> Leaf	Acetone	11	21
22	<i>Annona squamosa</i> Stem bark		11	
23	<i>Manilkara hexandra</i> Leaf		00	
24	<i>Manilkara hexandra</i> Stem bark		00	
25	<i>Manilkara zapota</i> Leaf		00	
26	<i>Manilkara zapota</i> Stem bark		00	
27	<i>Madhuka indica</i> Leaf		00	
28	<i>Madhuka indica</i> Stem bark		00	
29	<i>Mangifera indica</i> Leaf		00	
30	<i>Mangifera indica</i> Stem bark		00	

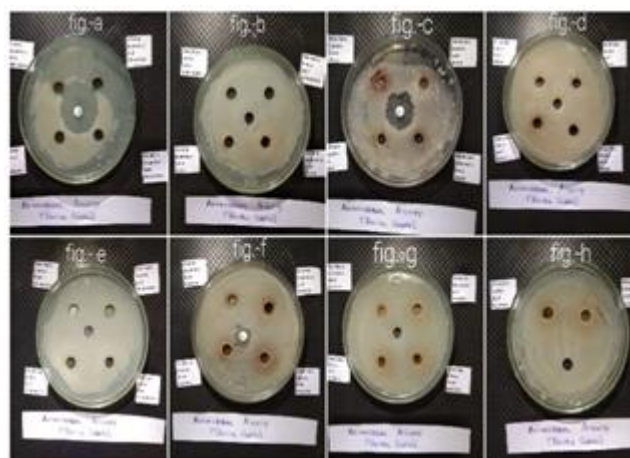


Figure 2:- *B. subtilis* Antibacterial activity

The Result was noted in above Table 2. The test organism *Bacillus subtilis* was effectively inhibited by two plant extracts viz. Chloroform extract of *Annona squamosa* Leaf and Stem bark with zone diameter 11mm and 12mm respectively. This was very lower than the inhibition by Ampicillin inhibitory zone diameter of 33mm against *Bacillus subtilis*. While none of the other plant extract showed any inhibition.

The Aqueous extract of *Manilkara hexandra* Leaves and Stem bark showed the zone of inhibition 11mm and 12mm respectively which was very lesser than the inhibition by Ampicillin inhibitory zone 25mm against *Bacillus subtilis*. While none of the other plant extract showed any inhibition. The Acetone extract of *Annona squamosa* Leaves and Stem bark showed the zone of inhibition 11mm respectively which

was very lower than the inhibition by Ampicillin inhibitory zone 21mm. While none of the other plant extract showed any inhibition.

Table 3: Anti-bacterial activity against *Escherichia coli* bacteria.

Sr. No.	Plant Name	Solvent for Extraction	Zone of inhibition in mm	
			Sample	Ampicillin
1	<i>Annona squamosa</i> Leaf	Chloroform	00	33
2	<i>Annona squamosa</i> Stem bark		14	
3	<i>Manilkara hexandra</i> Leaf		00	
4	<i>Manilkara hexandra</i> Stem bark		00	
5	<i>Manilkara zapota</i> Leaf		00	
6	<i>Manilkara zapota</i> Stem bark		00	
7	<i>Madhuka indica</i> Leaf		00	
8	<i>Madhuka indica</i> Stem bark		14	
9	<i>Mangifera indica</i> Leaf		00	
10	<i>Mangifera indica</i> Stem bark		00	
11	<i>Annona squamosa</i> Leaf	Water	00	21
12	<i>Annona squamosa</i> Stem bark		00	
13	<i>Manilkara hexandra</i> Leaf		00	
14	<i>Manilkara hexandra</i> Stem bark		00	
15	<i>Manilkara zapota</i> Leaf		00	
16	<i>Manilkara zapota</i> Stem bark		00	
17	<i>Madhuka indica</i> Leaf		00	
18	<i>Madhuka indica</i> Stem bark		00	
19	<i>Mangifera indica</i> Leaf		16	
20	<i>Mangifera indica</i> Stem bark		00	
21	<i>Annona squamosa</i> Leaf	Acetone	12	21
22	<i>Annona squamosa</i> Stem bark		15	
23	<i>Manilkara hexandra</i> Leaf		11	
24	<i>Manilkara hexandra</i> Stem bark		00	
25	<i>Manilkara zapota</i> Leaf		11	
26	<i>Manilkara zapota</i> Stem bark		14	
27	<i>Madhuka indica</i> Leaf		00	
28	<i>Madhuka indica</i> Stem bark		12	
29	<i>Mangifera indica</i> Leaf		00	
30	<i>Mangifera indica</i> Stem bark		00	

The organic extracts of the sample plant species of *Madhuka indica* Stem bark highly effective in inhibiting the growth of test organism *E-coli* compared to their aqueous extract. The Acetone and Chloroform extract had zone size of 14mm and 12mm respectively whereas aqueous extract showed no inhibition at all.

Aqueous extract of *Mangifera indica* Leaf showed the best zone of inhibition(16mm) which was lower than the inhibition by Ampicillin inhibitory zone diameter 21mm against *E-coli*. While another plant extract showed none of the inhibition. Acetone extract of *Annona squamosa* leaf, *Manilkara hexandra* Leaf, *Manilkara zapota* Leaf, *Manilkara zapota* Stem bark showed the zone of inhibition 12mm, 11mm, 14mm respectively which was lesser than the inhibition by Ampicillin inhibitory zone diameter 21mm against *E-coli*. Whereas other plants not showed any zone of inhibition.

Table 4: Anti-bacterial activity against *Salmonella enterica* bacteria.

Sr. No.	Plant Name	Solvent for Extraction	Zone of inhibition in mm	
			Sample	Ampicillin
1	<i>Annona squamosa</i> Leaf	Chloroform	00	23
2	<i>Annona squamosa</i> Stem bark		00	
3	<i>Manilkara hexandra</i> Leaf		00	
4	<i>Manilkara hexandra</i> Stem bark		00	
5	<i>Manilkara zapota</i> Leaf		00	
6	<i>Manilkara zapota</i> Stem bark		00	
7	<i>Madhuka indica</i> Leaf		00	
8	<i>Madhuka indica</i> Stem bark		00	
9	<i>Mangifera indica</i> Leaf		00	
10	<i>Mangifera indica</i> Stem bark		00	
11	<i>Annona squamosa</i> Leaf	Water	00	21
12	<i>Annona squamosa</i> Stem bark		00	
13	<i>Manilkara hexandra</i> Leaf		12	
14	<i>Manilkara hexandra</i> Stem bark		00	
15	<i>Manilkara zapota</i> Leaf		00	
16	<i>Manilkara zapota</i> Stem bark		00	
17	<i>Madhuka indica</i> Leaf		00	
18	<i>Madhuka indica</i> Stem bark		00	
19	<i>Mangifera indica</i> Leaf		00	
20	<i>Mangifera indica</i> Stem bark		11	
21	<i>Annona squamosa</i> Leaf	Acetone	00	21
22	<i>Annona squamosa</i> Stem bark		00	
23	<i>Manilkara hexandra</i> Leaf		00	
24	<i>Manilkara hexandra</i> Stem bark		00	
25	<i>Manilkara zapota</i> Leaf		00	
26	<i>Manilkara zapota</i> Stem bark		11	
27	<i>Madhuka indica</i> Leaf		00	
28	<i>Madhuka indica</i> Stem bark		11	
29	<i>Mangifera indica</i> Leaf		00	
30	<i>Mangifera indica</i> Stem bark		11	



Figure 3:- *E. coli* Antibacterial activity

The result was noted in above Table 3. The organic extract of the sample plant species of *Annona squamosa* Stem bark highly effective in inhibiting the growth of test organism *E-coli* compared to their aqueous extract. The Acetone and Chloroform extract had zone size of 14mm and 15mm respectively whereas aqueous extract showed no inhibition at all.

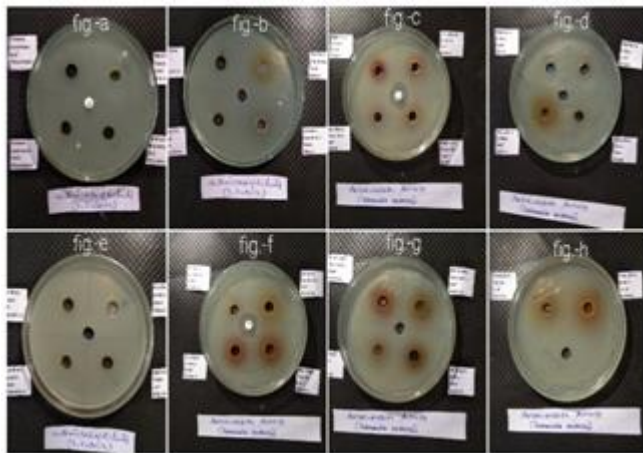


Figure 4:- *S. enterica* Antibacterial activity

The Result was noted in below Table 4. The Chloroform extract of all the plant test against the Gram-negative bacteria *Salmonella enterica* whereas none of inhibition seen at all the organic extract of the sample plant species of *Mangifera indica* Stem bark highly effective in inhibiting the growth of test organism *Salmonella enterica* compared to their Chloroform extract the Acetone and Aqueous extract had zone size of 11mm whereas Chloroform extract showed no inhibition at all. The aqueous extract of *Manilkara hexandra* Leaf and *Mangifera indica* Stem bark showed the zone of inhibition 12mm and 11mm respectively which was lesser than the inhibition by Ampicillin inhibitory zone diameter 21mm against *S. enterica* test organism. While another plant extract showed none of the inhibition.

IV. CONCLUSION

The current study was initiated because of the interesting resistance to antibiotics including bacteria and fungi. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents. As a result, the antibacterial activity of different medicinal plant parts extract of five plants was screened against the most common pathogens. In general, *Annona squamosa* leaf, *Manilkara hexandra* leaf and stem bark appeared to be effective source of active antimicrobial agents. However, extracts of *Annona squamosa* stem bark and *Mangifera indica* leaf possess higher antimicrobial activity among the other tested medicinal herbs.

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