

STUDY OF BIOLOGICAL PROPERTIES OF *CAESALPINIA* *DECAPETALA*

Shirish S. Pingale*

P.G. Department of Chemistry, Gramonnati Mandal's Arts, Com. & Sci. College,
Narayangaon, Pune 410504 (Affiliated to SP Pune University, Pune), India.

Article Received on
16 March 2021,

Revised on 05 April 2021,
Accepted on 26 April 2021

DOI: 10.20959/wjpr20215-20434

*Corresponding Author

Dr. Shirish S. Pingale

P.G. Department of
Chemistry, Gramonnati
Mandal's Arts, Com. & Sci.
College, Narayangaon, Pune
410504 (Affiliated to SP
Pune University, Pune),
India.

ABSTRACT

The aim of present research is to study antimicrobial activity of aqueous extract of *C. decapetala* leaves. Disc diffusion assay method was employed for antimicrobial activity study in which methanol was used as blank and Chloramphenicol (10 ml/mg) as a standard. In the antimicrobial study leaves extract of plant shows zone of inhibition against fungal species like *Penicillium chrysogenum* and *Aspergillus Niger* as well as bacterial species like *Escherichia coli*, *Kelbsiella aerogenes*, *Bacillus subtilis* and *Staphylococcus albus*. The extract obtained from *C. decapetala* gives minimum inhibitory concentration (MIC) in between 8.0 to 15.0 mg/ml. The results of this research work shows that *C. decapetala* is rich source of bioactive components and can be used as effective antimicrobial herbal drug and antioxidant reagent.

KEYWORDS: *Escherichia coli*, *Kelbsiella aerogenes*, *Bacillus subtilis* and *Staphylococcus albus*.

INTRODUCTION

Medicinal plant and plant products have been utilized for treatment of diseases from the beginning of civilization. Use of medicinal plant was mentioned in "Rigveda", which is written in between 4500 and 1600 B.C.^[1] Applications of herbal plants for treatment becoming more popular, as the herbal products are no or least side effects as compared to synthetic drugs.^[2]

Out of 300000 species of the plants only 5% species have been studied scientifically for their medicinal uses. India has 45000 diverse groups of species spread over 16 different agro

climatic zones, 10 vegetation zones, 25 biotic provinces, 426 habitats of specific species. Besides this, there are up to 18000 flowering plants, 2500 algae, 23000 fungi, 1600 types of lichen and 1800 varieties of bryophytes. Out of these 15000-20000 are of medicinal value. But only 7000 to 7500 plants are used for medicinal purposes.^[3,4]

About 80% of the people depend on traditional medicines for their primary healthcare problems according to WHO. In most of the Asian countries, people leaving in the rural areas mostly use herbal medicines for the treatment of all types of diseases including diarrhea, which shows good interaction between environment and human.^[5] In traditional medicines different types of ingredients found in plants are used to cure chronic and infectious diseases.^[6] Good knowledge of the different plant parts, which utilized against various diseases is most important for their applications. Bioactive compounds obtained from plant phytochemicals could be a potential source for food supplements as well as effective drugs and nutraceuticals.^[7]

Almost 61% new drugs obtained from 1981 to 2002 are based on herbal products. These new drugs are very effectively used in the area of cancer and infectious disease.^[8,9] The presence of certain secondary metabolites such as glycosides, resins, gums, alkaloids, volatile oil and tannins gives the medicinal value to the plants.^[10]

Most of the traditional medicinal plants showed strong antimicrobial activity and few of them are used to treat animals and peoples who suffer from infectious diseases with the help of infusion, decoction, tincture or herbal extract.^[11] Though, no scientific assessment of their effectiveness and mechanism of action have been find out in most cases, the active chemical constituents of these medicinal preparations have been beneficial.^[12] The growth of yeast, mould and bacteria in food and food products are sometime dangerous when it transforms into waste products. Different bacterial and fungal species not only ruin food products rendering them unfit for human consumption but also create poison by producing cyto-toxins during storage.^[13] Heating, refrigeration and addition of antimicrobial agents are used to decrease the risk of outbreaks of food poisoning and to preserve foods. These techniques sometimes shows harmful changes in organoleptic characterizations and loss of nutrients.^[14]

Different chemical preservatives can be applied to inhibit the microbial growth but most of the people arising the safety questions of this chemicals.^[13] In recent years, people demanding fresh, natural, chemical additive free and safe food products.^[15] There is need to

develop natural preservatives which obtained from medicinal plant extracts.^[16] Natural preservatives reduces the harmful effect on human, increases the life of food and food products. The properties of medicinal plants have to be studied by researchers to prevent the microbial spoilage because of their availability, less side effects and decreased toxicity.^[17]

Many plants are helpful in the treatment of gastrointestinal disorders, urinary tract infections, respiratory diseases and cutaneous infections.^[18] Many new natural and synthetic drugs have been discovered and developed by Science and Technology.^[19]

In the twentieth century, antimicrobial agents are used in relatively low concentrations against the microorganisms to prevent or treat specific infectious diseases without harming the host organism. Only one third synthetic drugs are used to treat infectious diseases.^[20]

Because of improper and regular use and misuse of antibiotic agents, developed the resistant pathogens against this antibiotics.^[21] Disadvantages such as the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics have been overcome by searching new antimicrobial agents from plant extracts. This problem has been overcome with the help of antibiotic resistance inhibition from plants extracts.^[22]

Medicinal plants found to be useful in the treatment of number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents.^[23] Since, the phytomedicines and biologically active compounds obtained from medicinal plants are used in herbal medicines with acceptable therapeutic index, hence it has been focused by scientists.^[24]

The bacteria and fungi are major disease causing agents to animals as well as plants. For the treatment of infectious diseases a variety of antibiotics are used.^[25] Along with good pharmaceutical activity, antibiotics sometime shows toxic effects such as hypersensitivity, immune-suppression and allergic reactions. The decrease in the pharmaceutical activity of the various antibiotics bacteria because of the growth of super resistant strains in bacteria and it is the serious concerns to the public health. Therefore it is essential to find out potential antimicrobial agent through screening of different medicinal plants to treat such diseases.^[26]

Existing broad spectrum antibiotics has number of pharmacological side effects according to the traditional healers. Traditional medicine is more effective and imparts least side effects as compared to synthetic antibiotics.^[26] For the alternate treatment on infectious diseases, most

of the time medicinal plants are used. New antimicrobial compounds were tried to be found out from various kinds of sources like microorganisms, animals and plants. Medicinal plants are used in development of new antibiotics which are environmentally friendly, sustainable and that do not present health risks to humans.^[27, 28] Antimicrobial natural products are in the plants to save them from microbial infection and deterioration.

Many higher plant extracts have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials. There is increasing demand of natural antimicrobial compounds derived from plants to preserve food. Besides, most of the customers have the tendency to question the safety of synthetic preservatives and to prefer natural foodstuffs. Hence it is important to screen the plants for their medicinal property.^[29]

The customers believe that complementary and alternative medicines such as herbal medicines are safe and effective forms of healing. Hence the most of the people show interest in herbal medicines. This enhances the medicinal scientist to explore different biological activity of medicinal plants.^[30]

About 75% people are use the number of newly identified chemicals derived from higher plants for therapeutic and preventive use. 25% of the pharmaceuticals agents are obtained by using chemicals derived from plants in the USA.^[31, 32]

Where the synthetic antibiotics are harmful to the environment, plant extracts are used to prevent bacterial infection because of low cost, environmental friendliness and effectiveness against some bacteria.

All the parts of plants namely roots, stem, bark, leaves, flowers and seeds are used as herbal medicine. It is commonly known as crested fever nut. In Ayurveda, *Caesalpinia decapetala* is used as anti-inflammatory, anti-malarial, anti-histamine, anti-asthma, anti-aging agent and anti-pyretic properties. It is also used to cure diseases like skin diseases, medicinal paste for treating poisonous snake bite, treating liver stagnation type reflux oesophagities, treating ecthyma and headache, for rabies, treating hyperosteo-geny. Different parts of it are used to prepared Chinese medicines.

Caesalpinia decapetala plant is used to cure diseases like skin, diabetic, bacterial, pyretic, diarrhea, asthma and malaria. Root extract of *C. decapetala* is used to treat sexually transmitted infections. Bark is poisons and used as fish poisoning. Fruit extract shows

inhibitory effect against candida albicans. It is used to prevent cold and treat bronchitis. Used in eye drop for treating trachoma caused by *Chlamydia trachomatis* in Chinese medicine. In Chinese medicinal paste for treating poisonous snake bite. Leaves of *C. decapetala* mixed with essential oil which shows antibacterial activity. Used for treating blood stasis type closed bone fracture. Used in Chinese medicine lotion for treating ecthyma with headache. It is used in Chinese medicine for treating scald and burn. For treating hyperlipemia caused by excessive uptake of meal. This plant is used in Chinese medicine for treating rabies. It has been used in treatment of jaundice, stomach disorder and biliousness. The leaves and roots are used as purgative and emmenagogue. It shows anti fertility activity.

Medicinal properties of plants are due to presence a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds (Biswas and Chattopadhyay 2002). Phyto-chemicals from medicinal plants serve as lead compounds in antimicrobial discovery. The crude extracts obtained from all parts of plant and phytochemicals of antimicrobial properties shows the important role in therapeutic treatment. *Caesalpinia decapetala* is originally from Asia and Malaysia. *C. decapetala* is generally known as Roth. It is a pantropical genus which belongs to the family of *Caesalpinaceae* having 120-150 species of trees, shrubs, and lianas. The genus consists of several members of species that are used traditionally for the treatment of inflammation, hepatotoxicity as well as diabetes.^[33]

The present study of antimicrobial activity of *Caesalpinia decapetala* focuses on scientific validation for their use in local communities and increases the scope of existing drug discovery programs.

MATERIALS AND METHODS

Biological activities of phyto constituents

Preparation of plant extract^[34, 35]

20 gm accurately weighed leaves powder of *Caesalpinia decapetala* was extracted continuously in Soxhlet apparatus for 24 hours. Methanol and water mixture in the volume ratio 4:1 was used as extractant of which the volume was 500 ml. The extract was cooled and filtered with the help of Whatmann filter paper no. 41 into a clean and previously weighed beaker. Residue was extracted with ethyl acetate solvent from which fats and waxes were separated. Separating funnel was used for further separation. The filtrate was added with 2M

H₂SO₄ till it become acidified .150 ml (3 x 50 ml) chloroform was used to extract above acidified filtrate in a separating funnel. The chloroform layer obtained was rather polar extract .From which terpenoids are separated .The aqueous layer obtained was basified with 2M NaOH till its P^H become 10. It was again extracted with chloroform: methanol mixture in volume ratio 3:1 of which volume was 120 ml (2 x 60 ml) followed by extraction with 80 ml (2 x 40 ml) chloroform in a separating funnel. The aqueous basic layer was collected in a clean previously weighed beaker. The methanol layer contains quaternary alkaloids and N-oxides and the chloroform extract was the basic extract. It consists of alkaloids.

The crude extracts of Fats and waxes, terpenoids, quaternary alkaloids and N- oxides, alkaloids are screened for antimicrobial activities.

Antimicrobial activity

Minimum inhibition concentration (MIC)^[36]

The Minimum Inhibitory Concentrations (MICs) was defined as the lowest concentration of the extract at which visible zone of inhibition was observed. The MIC is important in diagnostic laboratories to authenticate resistance of microorganisms to an antimicrobial agent as well as to monitor the activity of new antimicrobial agents. MIC is considered as the basic laboratory measurement to find out the antimicrobial activity of the lead compound. Agar well diffusion technique was used to determine the MIC. A serial dilution method was used for reconstituting the extracts. 100 mg of each extract was dissolved in 1 ml of dimethyl sulfoxide (DMSO) which is then diluted with sterile distilled water to achieve final decreasing concentration range of 100,500,1000 µg/ml. By using micropipette, 1 µl of each dilution was introduced in wells of nutrient medium plates which had been already seeded with standardized (0.6 optical density) of test bacterial and fungal cells. All the plates were incubated for 1 day at 37 °C. The minimum concentration of each extract showing a clear zone of inhibition was taken as MIC.

Antibacterial activity assay^[37]

Antimicrobial activity of the extracts was carried out by agar diffusion method. To determine the activity, the diameter of growth of inhibition zone surrounding the antibiotic disc was measured. According to this method, medium of Muller-Hinton agar and Potato Dextrose agar was used for bacteria and fungi respectively. The agar was melted and cool to 48-50⁰C, then medium of 20 ml was poured in a sterile petri plate and allowed to give solid plates.

Standard cultures were grown in freshly prepared nutrient solution during the assay. Forty eight hours old fungal and twenty four hours old bacterial culture were used. By using the sterile cotton swab the culture of bacteria and fungi were applied in a zigzag pattern to give the even distribution of the organism over the agar surface. The culture then allowed drying for 10 minutes. 5 mm diameter wells were bored using a well cutter. Pre-sterilized Whatmann No. 3 filter paper discs of 5 mm was picked by the outer edge with sterile forceps and dipped into prepared solution of the individual extracts separately with concentration 100 µg/ml and placed on the swabbed agar plates before incubation.

The incubation of the plates was carried out at 37°C for 48 hours for bacteria and at 20°C for 48-72 hours for fungi. The diameter of zone of inhibition was measured in mm around the each disc using transparent meter rule and the average was determined. The experiment was carried out in five replicates. Standard antibiotic Chloramphenicol and Clotrimazole were used as positive control with concentration 10 µg/ml for bacterial and fungal activity respectively.

Table 1: Bacterial culture.

| Sr. No. | Name | Type | ATCC No. |
|---------|-----------------------------|---------------|------------|
| 1 | <i>Bacillus subtilis</i> | Gram positive | ATCC 6051 |
| 2 | <i>Staphylococcus Albus</i> | Gram positive | ATCC 2178 |
| 3 | <i>Escherichia coli</i> | Gram negative | ATCC 10799 |
| 4 | <i>Klebsiella aerogenes</i> | Gram negative | ATCC 8329 |

Table 2: Fungal cultures.

| Sr. No. | Name | ATCC No. |
|---------|--------------------------------|------------|
| 1 | <i>Aspergillus niger</i> | ATCC 1207 |
| 2 | <i>Penicillium chrysogenum</i> | ATCC 10108 |

Procurement of cultures- All the microbial cultures were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune.

RESULTS

Table: 3. Zone of inhibition of different extracts of *Caesalpinia decapetala* against gram negative bacteria *E. coli* and *K.aerogens* in mm.

| Sr. No. | Compounds Name | Gram negative bacteria | | | | | | | | | | | |
|---------|--|------------------------|----------------|----------------|----------------|----------------|-------------|--------------------|----------------|----------------|----------------|----------------|-------------|
| | | <i>E. coli</i> | | | | | | <i>K. aerogens</i> | | | | | |
| | | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Mean | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Mean |
| 1 | D-Alkaloids | 13 | 13 | 14 | 13 | 13 | 13.2 | 11 | 12 | 12 | 11 | 12 | 11.6 |
| 2 | B(QA) | 13 | 14 | 15 | 13 | 13 | 13.6 | 13 | 12 | 12 | 12 | 12 | 12.2 |
| 3 | Terpenoids | 12 | 12 | 11 | 12 | 12 | 11.8 | 11 | 12 | 12 | 11 | 11 | 11.4 |
| 4 | Fats and Waxes | 11 | 11 | 11 | 11 | 10 | 10.8 | 11 | 12 | 12 | 11 | 11 | 11.4 |
| 5 | Standard antibiotic (<i>Chloramphenicol</i>) | - | - | - | - | - | 18.8 | - | - | - | - | - | 19.5 |

Table 4: Zone of inhibition of different extracts of *Caesalpinia decapetala* against gram positive bacteria *B. subtilis* and *S. Albus* in mm.

| Sr. No. | Compounds Name | Gram positive bacteria | | | | | | | | | | | |
|---------|--|------------------------|----------------|----------------|----------------|----------------|-------------|-----------------|----------------|----------------|----------------|----------------|-------------|
| | | <i>B. subtilis</i> | | | | | | <i>S. Albus</i> | | | | | |
| | | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Mean | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Mean |
| 1 | D-Alkaloids | 13 | 13 | 12 | 13 | 13 | 11.6 | 11 | 12 | 12 | 11 | 11 | 11.4 |
| 2 | B(QA) | - | - | - | - | - | 12.2 | 11 | 12 | 11 | 11 | 11 | 11.2 |
| 3 | Terpenoids | 10 | 11 | 10 | 10 | 10 | 11.4 | 11 | 11 | 12 | 11 | 11 | 11.2 |
| 4 | Fats and Waxes | - | - | - | - | - | 11.4 | 12 | 13 | 12 | 13 | 13 | 12.6 |
| 5 | Standard antibiotic (<i>Chloramphenicol</i>) | - | - | - | - | - | 20 | - | - | - | - | - | 20 |

Table 5: Zone of inhibition of different extracts of *Caesalpinia decapetala* against fungal species *A.niger* and *P.chrysogenium* in mm.

| Sr. No. | Compounds Name | Fungal species | | | | | | | | | | | |
|---------|---|-----------------|----------------|----------------|----------------|----------------|------|------------------------|----------------|----------------|----------------|----------------|------|
| | | <i>A. niger</i> | | | | | | <i>P. chrysogenium</i> | | | | | |
| | | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Mean | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | Mean |
| 1 | D-Alkaloids | 12 | 12 | 13 | 12 | 13 | 12.4 | 10 | 11 | 11 | 12 | 11 | 11 |
| 2 | B (QA) | 12 | 13 | 13 | 12 | 12 | 12.4 | 9 | 8 | 9 | 9 | 8 | 8.6 |
| 3 | Terpenoids | 11 | 12 | 11 | 11 | 11 | 11.2 | 12 | 11 | 12 | 12 | 11 | 11.6 |
| 4 | Fats and Waxes | 9 | 8 | 9 | 9 | 8 | 8.6 | 10 | 11 | 11 | 10 | 9 | 10.2 |
| 5 | Standard antibiotic (<i>Clotrimazole</i>) | - | - | - | - | - | 30 | - | - | - | - | - | 25 |

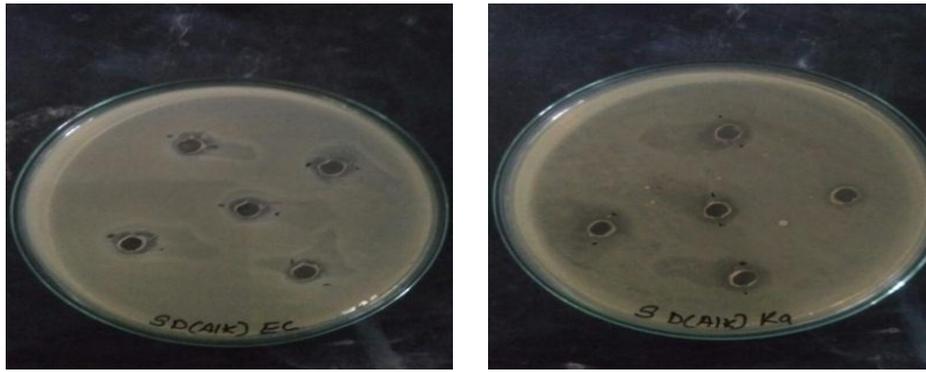


Fig: 1 Zone of inhibition of alkaloid of *Caesalpinia decapetala* against *E. coli* (A) and *K. aerogens* (B)

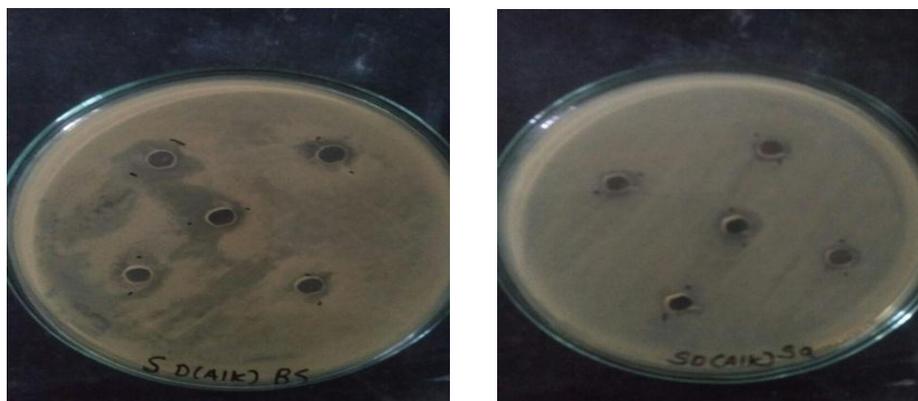


Fig: 2 Zone of inhibition of alkaloid of *Caesalpinia decapetala* against *B. subtilis* (C) and *S. Albus* (D)

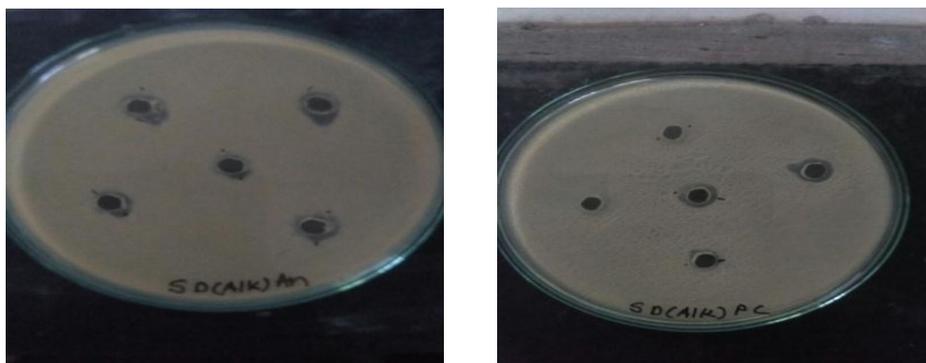


Fig: 3 Zone of inhibition of alkaloid of *Caesalpinia decapetala* against *A. niger* (E) and *P. chrysogenium* (F)



Fig: 4 Zone of inhibition of Quaternary alkaloid and N-Oxide of *Caesalpinia decapetala* against *E. coli* (A) and *K. aerogens* (B)

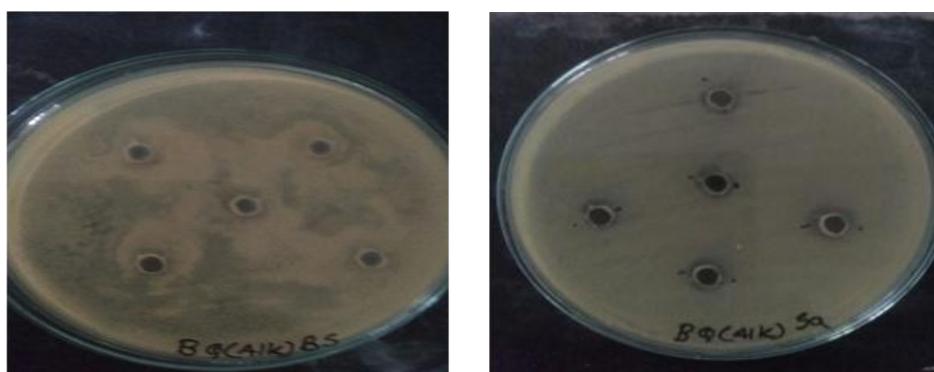


Fig: 5 Zone of inhibition of Quaternary alkaloid and N-Oxide extract of *Caesalpinia decapetala* against *B. subtilis* (C) and *S. Albus* (D)

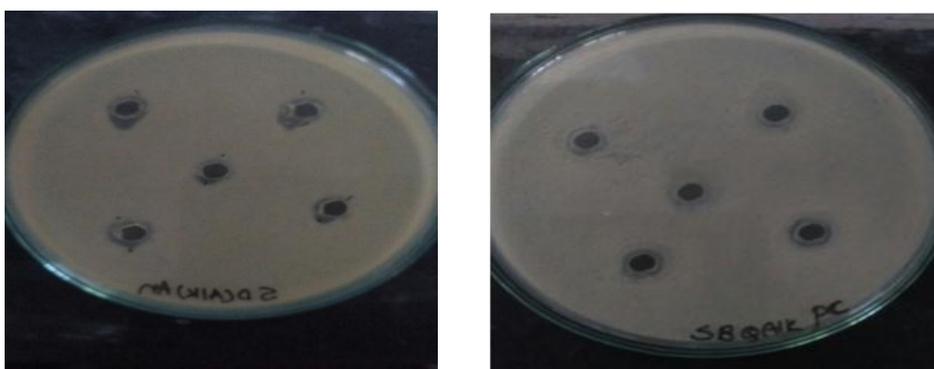


Fig: 6 Zone of inhibition of Quaternary alkaloid and N-Oxide extract of *Caesalpinia decapetala* against *A. niger* (E) and *P. chrysogenium* (F)

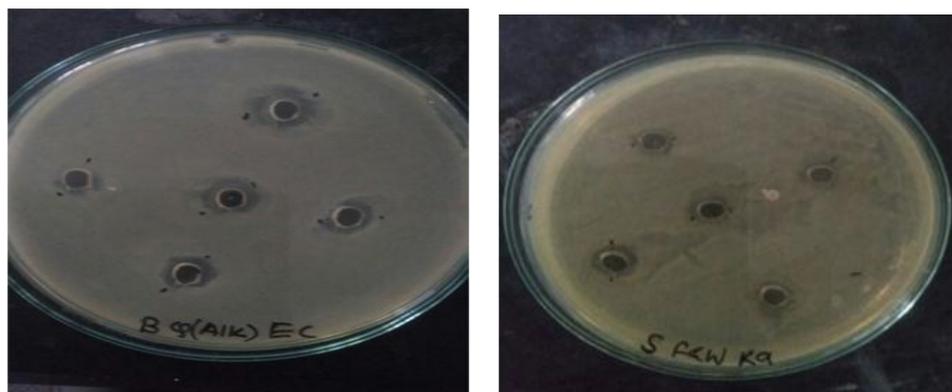


Fig: 7 Zone of inhibition of Terpenoids extract of *Caesalpinia decapetala* against *E-coli* (A) and *K.aerogens* (B)

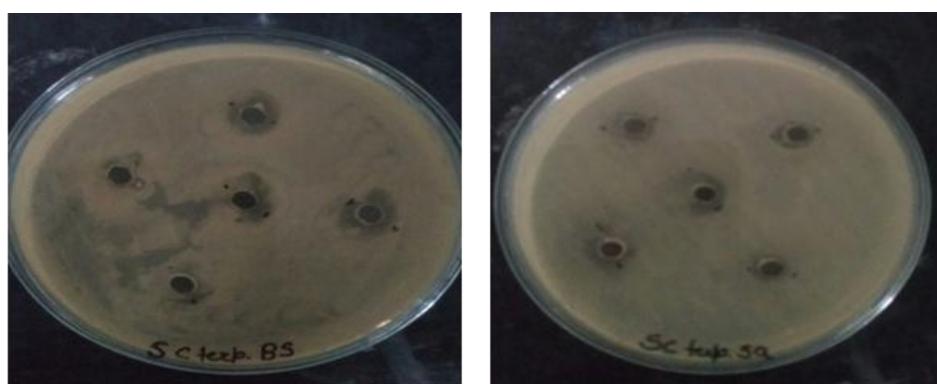


Fig: 8 Zone of inhibition of Terpenoids extract of *Caesalpinia decapetala* against *B.subtilis* (C) and *S. Albus* (D)

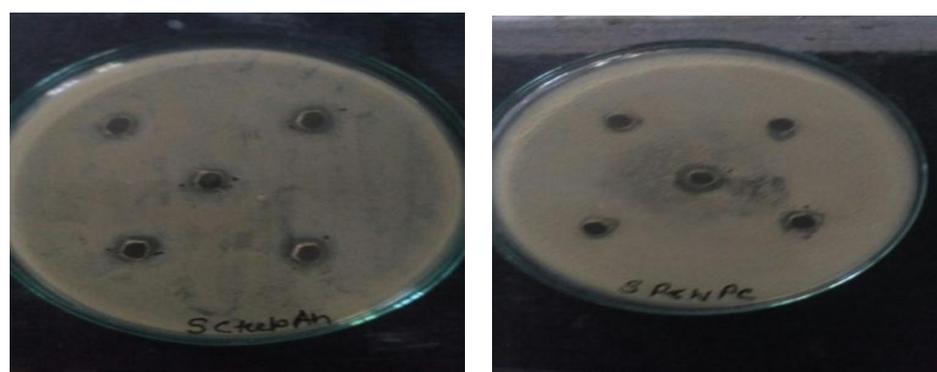


Fig: 9 Zone of inhibition of Terpenoids extract of *Caesalpinia decapetala* against *A.niger* (E) and *P.chrysogenium* (F)



Fig: 10 Zone of inhibition of Fats and Waxes extract of *Caesalpinia decapetala* against *E. coli* (A) and *K. aerogenes* (B)

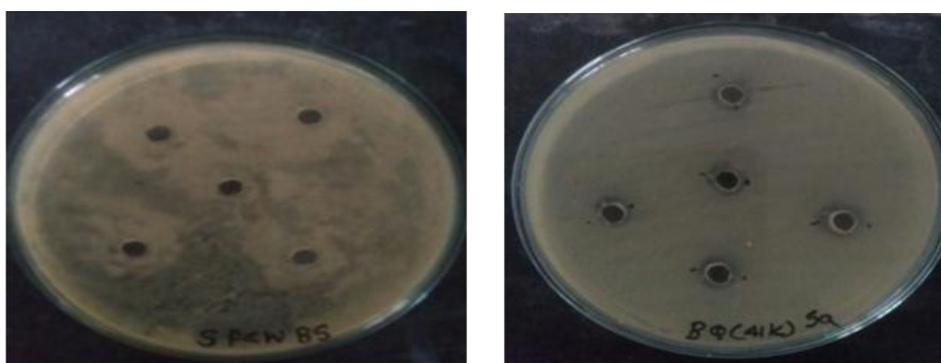


Fig: 11 Zone of inhibition of Fats and Waxes extract of *Caesalpinia decapetala* against *B. subtilis* (C) and *S. albus* (D)



Fig: 12 Zone of inhibition of Fats and Waxes extract of *Caesalpinia decapetala* against *A. niger* (E) and *P. chrysogenum* (F)

DISCUSSION

Antimicrobial activity

The antimicrobial activity of alkaloids, Quaternary alkaloids and N-oxides, terpenoids, fats and waxes of leaves of *Caesalpinia decapetala* against human pathogenic bacteria, *Bacillus subtilis*, *Staphylococcus albus*, *Escherichia coli*, *Klebsiella aerogenes*, and fungal species *Aspergillus niger* and *Penicillium chrysogenum* were studied by measuring the zone of inhibition in disc diffusion method.

The results of antibacterial and antifungal comparison of all the extracts of leaves of the plant under study against the two gram negative, two gram positive bacterial strains and two fungal species are interpreted in table 33 to 5 and figures 1 to 12.

➤ ***Bacillus subtilis***: *Bacillus subtilis* is having genus *Bacillus* and. These are gram-positive and catalase-positive bacterium. It is also recognized as grass bacillus or hay bacillus. They are found in soil as well as gastrointestinal part of humans and ruminant animals. The bacteria are rod-shaped. Environmental conditions can be tolerate by these bacteria because they produces hard and protective endospore. *B. subtilis* is a model organism for the study of bacterial cell differentiation and chromosome replication.^[38]

According to current investigation, Quaternary alkaloid and N-oxide fraction showed highest activity against Gram Positive bacteria *B. subtilis* with inhibition zone 12.2 mm followed by alkaloid and terpenoid, fat and wax fraction with zone of inhibition 11.4 to 11.6 mm. *B. subtilis* was found to be resistant against the fats and waxes fraction of leaves of *C. decapetala*.

➤ ***Staphylococcus albus***: *Staphylococcus albus* or epidermis is a member of Staphylococcaceae family and having genus staphylococcus. These are pathogenic bacteria parasitic to human. Bacterial cells (cocci) are spherical in shape typically occur in irregular clusters. The term *staphylococcus* means cluster arrangement. A color pigment produced by staphylococci gives different names to various strains. If color pigments are from orange to yellow are known as *S. aureus* while if they are white color, strains are designated as *S.albus*.

Staphylococci cause skin and lungs infection. It produces abscesses, boils and other infections of the skin, such as impetigo. Food poisoning occurs due to staphylococcus

contaminated food. Toxins and enzymes are produced from staphylococcus which destroys both red and white blood cells.^[39]

The results of antibacterial sensitivity of fat and wax fraction showed significant activity against *S. albus* with zone of inhibition 12.6 mm. But a Gram Positive bacterium *S. albus* was found to be resistant against the Quaternary alkaloid and N-oxide, fats and waxes and terpenoid fractions.

➤ ***Escherichia coli***: *Escherichia coli* is having Enterobacteriaceae family. They are gram-negative, rod shaped and do not produce spores. These are found in sewage water and soil contaminated with faecal substances. Dimension of it is 2 to 4 micron, generally found in coccobacillary form. They are having 4 to 8 mobile peritrichate flagella and grow in laboratory medium. These bacteria produces infection to the urinary tract, gastro intestine, bacteremia, septic wounds, peritonitis, cholecystitis, bedsores and meningial to all age group peoples. It may have adverse effect to lower part of respiratory passages to surgical patients.^[40-42]

The results of antibacterial sensitivity of Quaternary alkaloid and N-oxide fractions of *Caesalpinia decapetala* showed significant activity against Gram Negative bacteria *E-coli* with zone of inhibition 13.6 mm followed by alkaloid and terpenoid fraction with zone of inhibition 13.2 and 11.8 mm respectively.

➤ ***Klebsiella aerogenes*** : Enterobacteriaceae is a family of *K. aerogenes*. These are rod-shaped, non-motile and aerobic bacteria having a prominent polysaccharide shell. Mucoid polysaccharide shells are useful to built up them large moist colonies. Few bacteria generate an extracellular poisons complex which causes serious problem for lung in mice. *K.aerogenes* are present in all places and built up their colonies on the pharynx or gastrointestinal tract , skin in human.^[43,44]

In the present research, Quaternary alkaloid and N-oxide fraction showed highest activity against Gram Negative bacteria *K. aerogenes* with inhibition zone 12.2 mm followed by alkaloid and terpenoid fraction with zone of inhibition 11.6 - 11.4 mm. *B. subtilis* was found to be resistant against the fats and waxes fraction of leaves of *C. decapetala*.

➤ ***Aspergillus niger*** : *Aspergillus nigeris* is one of the most regular fungal species. It is member of Trichocomaceae family with genus *Aspergillus*. *A. niger* grow on fruits vegetables

and other substrate which provide food. Black mould disease on onions, apricots, peanuts and grapes is due to *A. niger*. Food is contaminated with it. It is present in soil and enclosed environment. Ochratoxins are produced from some species of *A. niger*.^[45] Ochratoxin A and isoflavone orobol are also produced from strains of true *A.niger*.^[46] It develop mycotoxins in food and food stuffs and decreases the quality of food. Mycotoxins are well known and powerful hepatocarcinogens in animals and humans.^[47]

In the research, the fungal strain *A.niger* was highly affected by alkaloid , Quaternary alkaloid and N-oxide fraction of leaves of *C. decapetala* with inhibition zone 12.4 mm followed by terpenoid fraction showed significant activity against *A.niger* with zone of inhibition 11.2 mm. *A.niger* was less affected by fat and wax fraction, *A. niger* was found to be resistant against it.

➤ ***Penicillium chrysogenum***: It is fungal species and member of *Trichocomaceae* family. It may be present on salty food materials in temperate and subtropical regions. It is mainly found in interior environments like buildings damaged by water or humid regions.^[48]

The compound terpenoid is reported to have strong antifungal activity against *P.chrysogenum* with inhibition zone 11.6 mm where as alkaloid and fat and wax fraction exhibited moderate antifungal activity with 10-11 mm zone of inhibition. *P.chrysogenum* was found to be resistant against Quaternary alkaloid and N-oxide fraction.

CONCLUSION

The present study supports use of *C. decapetala* leaf extracts as a source of antibacterial and antifungal agents that can afford protection against various diseases. It also justifies the predominance of the use of leaves in traditional medicine due to their interesting biological activities. It might be an alternative to synthetic antibiotics available in the market.

REFERENCES

1. Naqvi, S. B. S., Studies on antibacterial activity of compounds of plant origin and isolation of active components from *Sphaeranthus indicus* Linn., Department of pharmaceuticals, Ph.D. Thesis, University of Karachi, 1997.
2. Rajeswara Reddy Erva, Madhava Chetty K., Chandrasai Potla Durthi; Preliminary phytochemical analysis and extraction of crude drugs from medicinal plants and their antimicrobial activity; J. Pharm. Sci. & Res, 2019; 11(3): 726-732.

3. Lakshamanan, K. K.; Sankaranarayanan, A. S., Antifertility herbs used by the tribal's in Anaikatty, Coimbatore District, Tamilnadu, *Journal of Economic and Taxonomic Botany*, 1990; 14(1): 171-173.
4. Gupta, N. R.; Viswas K.; Pathak M.; Singh P. S.; Gupta A., Antibacterial activities of ethanolic extracts of plants used in folk medicine, *International Journal of Research in Ayurveda and Pharmacy*, 2010; 1(2): 529-535.
5. Bhutkar M.A., Wadkar G. H., Randive D.S., Shirwadkar B., Todkar S.S; Anti-microbial and Anti-diarrheal activity of ethanolic extract of *Caesalpinia decapetala* leaves; *International J. of Pharma Research & Life Sciences*; Jan-Feb. 2017; Volume I, Issue I.
6. Madhubala. S, Poongothai. M, Mahesh Kumar. E; Antibacterial and anti acne activity of *Caesalpinia sappan* L. and *Cinnamomum verum*; *J. Presl – A comparison*; *International Journal of Advanced Research in Biological Sciences*; 2018, 5(4);118-122.
7. Narintorn Rattanata, Sompong Klaynongsruang, Sakda Daduang and et.al; Inhibitory Effects of Gallic Acid Isolated from *Caesalpinia mimosoides* Lamk on Cholangiocarcinoma Cell Lines and Foodborne Pathogenic Bacteria; *Asian Pac J Cancer Prev*, 17(3): 1341-1345.
8. Cragg, G. M.; Newman, D. J., Biodiversity: A continuing source of novel drug leads, *Pure Appl. Chem*, 2005; 77(1): 7-24.
9. Bhalodia, N. R.; Shukla, V. J., Antibacterial and antifungal activities from leaf extracts of *Cassia fistula*: An ethnomedicinal plant, *J Adv Pharm Technol. Res*, 2011; 2(2): 104–109.
10. D.Anandhi, S.Kanimozhi, M. Anbarsan; Bioautography assay of *Caesalpinia coriaria* (Jacq) wild, as antifungal agent.; *Int. J. Curr. Res. Biol. Med*, 2016; 1(6): 1-6.
11. Patil Usha, Sharma M C; antiviral activity of *Lathakaranja* (*Caesalpinia Crista*) Crude extracts on selected animal viruses; *Global J Res. Med. Plants & Indigen. Med.*; Sept, 2012; 1(9): 440–447.
12. Wendakoon, C.; Calderon, P.; Gagnon, D., Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens, *Journal of Medicinally Active Plants*, 2012; 1(2): 60-68.
13. Shruti Shukla, Pradeep Mehta, Archana Mehta, Suresh Prasad Vyas, Vivek K. Bajpai; Preliminary phytochemical and antifungal screening of various organic extracts of *Caesalpinia bonducella* seeds; *Romanian Biotechnological Letters*, 2011; 16(4): 6384-6389.

14. Gaillard, Y.; Pepin, G., Poisoning by plant material: review of human cases and analytical determination of main toxins by high-performance liquid chromatography- (tandem) mass spectrometry, *J. Chromatography*, 1999; 733: 181-229.
15. Gould, G. W., Industry perspectives on the use of natural antimicrobials and inhibitors for food applications, *Journal of Food Protection Supplement*, 1996; 82-86.
16. Singh, A.; Sharma, P. K.; Garg, G., Natural products as preservatives, *International Journal of Pharmaceutical and Bio Science*, 2010; 1(4): 601-612.
17. Lee, S.B.; Cha, K.H.; Kim, S.N.; Altantsetseg, S.; Shatar, S.; Sarangerel, O.; Nho, C.W., The antimicrobial activity of essential oil from *Dracocephalum foetidum* against pathogenic microorganisms, *J. Microbiol*, 2007; 45(1): 53-57.
18. Khan, R.; Islam, B.; Akram, M.; Shakil, S.; Ahmad, A.; Ali, S. M.; Siddiqui, M.; Khan, A. U., Antimicrobial activity of five herbal extracts against multi drug resistant strains of bacteria and fungus of clinical origin, *Molecules*, 2009; 14: 586-597.
19. Preethi, R.; Devanathan, V. V.; Loganathan, M., Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens, *Advances in Biological Research*, 2010; 4(2): 122-125.
20. Harma, A., Antibacterial activity of ethanolic extracts of some arid zone plants, *International Journal of Pharm Tech Research*, 2011; 3(1): 283-286.
21. Enne, V. I.; Livermore, D. M.; Stephens, P.; Hall, L. M., Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction, *The Lancet*, 2001; 28: 1325-1328.
22. Kim, H.; Park, S. W.; Park, J. M.; Moon, K. H.; Lee, C. K., Screening and isolation of antibiotic resistance inhibitors from herb material resistant inhibition of 21 Korean plants, *Nat. Prod. Sci*, 1995; 1: 50-54.
23. B. Vinoth, R. Manivasagaperumal And M. Rajaravindran; phytochemical analysis and antibacterial activity of *Azadirachta Indica* A Juss.; *International Journal of Research in Plant Science*, 2012; 2(3): 50-55.
24. Sen, A.; Batra, A., Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach.*, *International Journal of Current Pharmaceutical Research*, 2012; 4(2): 67-73.
25. Davies, J., Inactivation of antibiotics and the dissemination of resistance genes, *Science*, 1994; 264(5157): 375-382.

26. Sowjanya Pulipati, G. Pallavi, B. Sujana, K. Anil Babu, P. Srinivasa Babu; evaluation of antibacterial activity of fresh and dry flower extracts of *Caesalpinia Pulcherrima* L.; International Journal of Biological & Pharmaceutical Research, 2012; 3(3): 360-365.
27. Gonzalez, J., Medicinal plants in Colombia, J Ethnopharmacology, 1980; 2(1): 43-47.
28. Mathur, A.; Bhat, R.; Prasad, G.B.K.S.; Dua, V. K.; Verma, S. K.; Agarwal, P. K., Antimicrobial activity of plants traditionally used as medicines against some pathogens, Rasayan J. Chem, 2010; 3(4): 615-620.
29. Chavan, P. A., Evaluation of antimicrobial activity of various medicinal plants extracts of Latur zone against pathogens, Int. J. Life. Sci. Scienti. Res, 2016; 2(5): 612-618.
30. Balunas, M. J.; Kinghorn, A. D., Drug discovery from medicinal plants, Life Sciences, 2005; 78: 431- 441.
31. Al-Snafi, A. E., Pharmacological effects of *Allium* species grown in Iraq-An overview, Int. J. Pharma Health Care Res, 2013; 4(1): 132-147.
32. Al-Snafi, A. E., Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants, Rigi Publication, Thi qar University, 2013.
33. Amna Parveen¹, Muhammad Sajid Hamid Akash^{2,3*}, Kanwal Rehman³, Qaisar Mahmood³, Muhammad Imran Qadir; Analgesic, anti-inflammatory and anti-pyretic activities of *Caesalpinia decapetala*; BioImpacts, 2014; 4(1): 43-48.
34. Shirish S. Pingale¹, Manohar G. Chaskar² and Nirmala R. Kakade; Phytochemical Analysis and Antimicrobial Activity of *Caesalpinia bonducella* leaves; J. Nat. Prod. Plant Resour, 2017; 7(1): 9-14.
35. Aneja, K. R., Experiments in microbiology, plant pathology and biotechnology, Sewage International Publisher, 2003; 390-391.
36. Mondher Boulaabaa, Mejdi Snoussi, Mariem Saada, Et.al; Antimicrobial activities and phytochemical analysis of *Tamarix gallica* extracts; Industrial Crops and Products, 2015; 1114–1122.
37. Akash B Mali, Dr Meenal Joshi and Versha Kulkarni; Phytochemical Screening and Antimicrobial Activity of *Stevia rebaudiana* Leaves; Int. J. Curr. Microbiol. App. Sci, 2015 4(10): 678-685.
38. Wikipedia, The free Encyclopedia.
39. The Columbia Encyclopedia, 6th ed.
40. Amenu, D., Antimicrobial activity of medicinal plant extracts and their synergistic effect on some selected pathogens, American Journal of Ethnomedicine, 2014; 1(1): 18-29.
41. Mahon, C. R.; Manuselis, G., Textbook of Diagnostic Microbiology, 1995.

42. Mackie, T. J.; McCartney, J. E., Microbial Infections. Medical Microbiology, 13th Edition Longman Group Limited, London, 1989.
43. Straus, D. C., Production of an extracellular toxic complex by various strains of *Klebsiella pneumonia*, Infection and Immunity, American Society for Microbiology, 1987; 55(1): 44-48.
44. Senior, P. J., Regulation of nitrogen metabolism in *Escherichia coli* and *Klebsiella aerogenes*: Studies with the continuous-culture technique, Journal of Bacteriology, American Society for Microbiology, 1975; 123(2): 407-418.
45. Abarca, M. L.; Bragulat, M. R.; Castellá, G.; Cabañes, F. J., Ochratoxin A production by strains of *Aspergillus niger* var. *niger*, Applied and Environmental Microbiology, 1994; 60(7): 2650-2652.
46. Schuster, E.; Dunn-Coleman, N.; Frisvad, J. C.; Van Dijck, P. W., On the safety of *Aspergillus niger*-a review, Applied microbiology and biotechnology, 2002; 59(4-5): 426-35.
47. Samson, R. A.; Houbraken, J.; Thrane, U.; Frisvad, J. C.; Andersen, B., Food and Indoor Fungi, CBS-KNAW- Fungal Biodiversity Centre, Utrecht, the Netherlands, 2010; 1-398.
48. Andersen, B.; Frisvad, J. C.; Søndergaard, I.; Rasmussen, I. S.; Larsen, L. S., Associations between fungal species and water damaged building materials, Applied and Environmental Microbiology, 2011; 77(12): 4180-4188.