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Phytochemical Study Of *Ficus Benghalensis, Ficus Krishnae, Calotropis Procera* And *Alstonia Scholaris* And Their Biomedical Characteristics

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Abstract

This study aimed to evaluate the phytochemicals and antimicrobial properties of leaf extracts of four plant species - Ficus Benghalensis, Ficus Krishnae, Calotropis Procera, and Alstonia Scholaris. Methanol and chloroform were used as solvents for extraction, and preliminary phytochemical investigation showed the presence of alkaloids, saponins, tannins, phobia tannins, flavonoids, carbohydrates, free amino acids, cardiac glycosides, steroids, and phytosterols and revealed the presence of various bioactive compounds. Antimicrobial activity was tested against six bacterial strains (Bacillus subtilis, Bacillus coagulants, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris) and two fungal strains (Candida tropicalis, Saccharomyces cerevisiae) using the agar well diffusion method. Results showed that methanolic extracts had better antimicrobial activity than chloroform, although all extracts showed activity against the microorganisms tested. These findings indicate that the leaf extracts of these plants contain natural products with potential pharmaceutical applications.

Keywords: Phytochemicals, antimicrobial activity, methanol, chloroform, microorganisms, pharmaceutical, vitamins, metabolites, Ayurveda, disease.

1. INTRODUCTION

Phytochemical studies have been conducted on *Ficus benghalensis, Ficus Krishnae, Calotropis Procera*, and *Alstonia Scholaris* to explore their biomedical characteristics. Bhuvaneswari M. Bhuvaneswari (2017) found that Ficus benghalensis roots contain various bioactive phytochemicals that could have potential therapeutic applications. Radhakrishnan and Venkatachalam Anupama Jayasree Radhakrishnan and Sivakumar Venkatachalam (2020) investigated the phytochemicals present in *Ficus benghalensis* fruits using a nonconventional technique of microwave-assisted solvent extraction. Ahirrao RA Ahirrao (2020) revealed that aqueous extract from *Ficus Benghalensis* roots contains high amounts of phenolic compounds with possible

antioxidant activity and antimitotic activity. Kanjikar and Londonkar Amarvani P Kanjikar and Ramesh L Londonkar (2017) studied the pharmacognostic, phytochemical and antimicrobial activity of stem bark of *Ficus Krishnae*. Similarly, Zuraida Zuraida (2019) identified phytochemical compounds of *Alstonia sp*. from different species and found flavonoids, tannins, saponins, alkaloids, steroids, and triterpenoids. Furthermore, Tadavi et. al., Samina K Tadavi, Shyam W Dafare, Sunil B Zanje et. al., (2023). conducted preliminary phytochemical analysis of *Ficus johanis* Subsp. *Afghanistanica* to identify the biological activities, such as anticancer, hepatoprotective, hypoglycemic, antitumor, antioxidant, anthelmintic, analgesic, antimicrobial activity, anti-parasitic, hypolipidemic, anti-inflammatory, antibacterial, anti-ulcerogenic, mucoprotective, gastroprotective, antifungal, antiviral, antimalarial, and antiparasitic activities associated with the genus *Ficus species*. Moreover, *Calotropis Procera* was studied by a group of researchers to validate the authenticity of correlationship of various phytoconstituents concentrations, antioxidant activity, and urease inhibitory potential of the same A. Shahzad (2016). The presence of phytochemical constituents in the selected species of Moraceae, such as *Ficus Krishnae*, and *Ficus Benghalensis* has been reported Amarvani P Kanjikar and Ramesh L Londonkar (2017). *F. Krishnae* was identified as an independent species based on their morphology and DNA barcoding differences, which distinguish it from Ficus benghalensis Senthil Kumar Umapathy, Jana Venkata Sudhakar et. al., (2021). Kumaresan et. al., Suriya Kumaresan, Rema Ramasamy and Philip Robinson Jayachandran (2018) investigated the combined crude methanolic extract of *Ficus religiosa* and *Ficus benghalensis* leaves and revealed their antioxidant, phytochemical, and cell proliferation activity against cervical cancer. The existence and survival of humankind is impossible without plant kingdom, as plants are primary producers and play an important role in sustaining the life forms on earth. A medicinal plant is any plant which, in one or more of its organs, contains substance that can be used for therapeutic purpose, or which is a precursor for synthesis of useful drugs. The plants possess therapeutic properties or exhibit Beneficial Pharmacological effects on the animal body are generally designated as Medicinal Plants. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, triglycerides, tannin, volatile oils, minerals, and vitamins possess medicinal properties. Each part of the plant contains distinct properties and is used for different purposes J. Azmir *et. al.,* (2013). India is known for its traditional medicinal systems Ayurveda, Siddha, and Unani. Medical systems are found mentioned even in the ancient Vedas and other scriptures. The Ayurveda concept appeared and developed between 2500 and 500 BC in India. The literal meaning of Ayurveda is science of life, because ancient Indian system of health care focused on views of man and his illness. It has been pointed out that positive health means metabolically well-balanced human beings. It offers programs to rejuvenate the body through diet and nutrition. It offers treatment methods to cure many common diseases such as food allergies, which have few modern treatments. Ayurveda is not a nutritional system for those seeking an escape or excuse to further abuse their body or mind. It is a system for empowerment, a system of freedom, and long-life Pandey, A.K., & Verma, N. (2013), Pandey A, Verma N. (2013). India is the largest producer of medicinal plants, so it widely known as the Botanical Garden of the world. Various plants have been used in traditional therapy throughout the world. Herbal remedied are obtained from a wide variety of natural resource including plant leaves,

bark, berries, flowers and roots Mahalakshmi R *et. al.*, (2010). India has about 8,000 species of known medicinal plants and about 1,000 plants have been used in the traditional system of medicine like Ayurveda, Unani and Siddha, while tribal use 7,500 plant species for medicinal purposes using the current global rates of species extinction at around 10 to 12 per cent of the plants (800-1,000 species) are likely to be threatened. According to the World Health Organization [WHO] estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts on their active Components Duraipandiyan, V., Ayyanar, M. & Ignacimuthu, S. (2006). Plants based bio-active compounds are gaining attention due to their multi-functional, and therapeutic properties. The most convincing evidence for protective benefits is attributed to their antioxidant property or multiple activities against free radicals. Therefore, drugs development from plants-based compounds could be useful in meeting this demand for newer drugs with minimal effects. KS Nagarsekar, *et. al.*, (2010). Moreover, medicinal plants are considered as rich source of new compounds possessing therapeutic value that can be further used for development of new drugs Chin, YW., Balunas, M.J., Chai, H.B. *et. al.*, (2006). However, plant remedies are effective and without side-effects, provided they are selected properly and taken under proper medical supervision. Infectious disease is one of the leading causes of death worldwide, particularly in developing countries, such as pathogenic *Escherichia coli, Vibrio cholera, Shigella sp., Salmonella species, Bacillus* Merrett, G.L. (2013). Thus, emergence of pathogenic microorganisms those resistant /multi drug to major class of antibiotics has been posing a threat in recent years, due to in discriminate use of synthetic antimicrobial drugs Esitken a., *et. al.*, (2003). There is a continuous development of resistant strains that create the need for search and advancement of new drugs to cure disease. There is possibility to modify the antibiotic activity by using compounds that can enhance the activity of the antibiotic, and plants are a rich source of these kinds of the Compounds Gibbons, S. (2005). Knowledge of the phytochemical constituents is necessary to enable investigation of the actual effectiveness of the plants in medicine. Most of their properties are due to essential oils and secondary metabolites. The ability to synthesis compounds by secondary metabolites possessing anti-microbial potential makes plants a valuable source of pharmaceutical and therapeutic products. Medicinal plants possess immune-modulator and antioxidant properties, leading to antibacterial activities. They are known to have versatile immune-modulator activity by stimulating both non-specific and specific immunity. It is well known that oxidative stress induced by oxygen free radicals and resultant tissue are the hallmarks of several chronic disorders, including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neuro-degenerative disease and others and cell death Shriwaikar A., A. Shirwaikar, R. Kuppusamy and I.S.R. Punitha, (2006). Oxidative process is one of the most important routes for producing free radical in foods, drugs and even in living System Barry Halliwell (1994). The use of plants extracts and phytochemicals, both with known antibacterial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in secondary metabolism of the plants Nascimento, Gislene G. F. *et. al.*, (2000). The following are some of the medicinal plants that have been studying their effect against some clinically

isolated bacteria. *Ficus bengalensis* is a woddy tree belonging to the family Musaceae. It is commonly known as a banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2000 varieties of Ficus species, most of which are native to the old-world tropics Kumar Ashok BS, Ranganayakulu D., Nandeesh R. *et. al.*, (2010). It endemic to Bangladesh, India, and Sri Lanka. It is also known as Bengal fig, Indian fig and East Indian fig, Indian Banyan or simply Banyan (English), also both, nyagrodha (Sanskrit), Bat, Bargad, and Bar (Hindi). The English name Banyan is given by the Britishers to this tree because under the tree Banias that is, the Hindu merchants used to assemble business. The triad Ganga, the Himalayas and the Banyan tree symbolize the images of India, for this reason it is considered as a National Tree. Ficus means fig and bengalensis means belonging to or is of Bengal Prafulla D. Patil, Shuguang Deng. (2009). Ficus plants are found throughout the world as moderate woody plants or trees. It is found throughout the year, grows in evergreen except in dry localities where it is leafless for a short time. It is hardy and drought-resistant; it withstands mild frost. It develops from seeds dropped by birds on walls or on other trees and is therefore considered destructive to forest trees, walls and buildings Vikas V Patil & Vijay R Pati. (2010). The plant is employed frequently with traditional medicine such as eye-disease, constipation, toothache, inflammation, leukoderma, headache, fever, rheumatic affections, and relieve stomach. In the traditional system of medicine, various plant parts of *Ficus Benghalensis* L. Such as stem bark, aerial roots, underground roots, vegetative buds, leaves, fruits and latex have been used in various nervous disorders i.e., seizure, insomnia, anxiety etc. Panday, D.R., Rauniar, G.P. (2016). *Ficus* species have also been used in Indian ayurvedic and traditional medicine Baby joseph and s. Justin raj. (2010). Extracts of *Ficus* species have also been documented to have antioxidant, anti-diabetes, antibacterial, anti-fungal, antiviral, anti-protozoal, anticancer, cytotoxic, anti-ulcer, anti-inflammatory, anti-hyperglycemia, anti-diarrhea, hepatocyte-protective, muco-protective and gastro-protective Activity Kuete, V., *et. al.*, (2009).

Ficus Krishnae belongs to the family Moraceae is also known as Krishna fig, krishna's butter cup and Makkhan Katori. It is mainly found in India, tropical Africa, and Sri Lanka. It is a large, fast growing, evergreen tree up to 30m tall, with spreading branches and Aerial roots Biswas, K. (1934). The unique feature of the tree is that leaves have a pocket –like fold at the base, behind which is a mythological story that Lord Krishna was very fond of butter and would even steal it. Once when he was caught by his mother, Yashoda, he tried to hide the butter by rolling it up in a leaf of this tree. Since then, the leaves of these trees have this shape Unnikrishnan, M.K., Rao, M.N.A. (1990). All parts are used to cure disease of K-apha. Its aerial roots are styptic, useful in syphilis, biliousness, dysentery, inflammation of liver etc. It has been proven to have anti–diabetic and antihyperlipidemic activity. Phenol compounds and hydrogenated coumarins have been isolated from Mahesh Mysore Shivananjappa & Manoj Kumar Joshi. (2012). *Calotropis Procera* is commonly known as Akk or Madar in Ayurveda. Calotopis is the Traditional medicinal plant it belongs to the family of *Asclepiadaceae.* It is a tropical plant growing wild in warm climate. It is a native plant of North Africa. This plant is well distributed throughout India Ranjit, Pusapati. (2012). It is commonly known as milkweed or swallows worth, crown flower. The leaves are very succulent in nature. The species of Asclepias and Calotropis which contain cardenolides toxic to vertebrates. The latex of this plant has been described for exhibiting diverse pharmacological properties

including antimicrobial activities Most of the Asclepiadaceae members are perennial herbs and their latex is exclusively used as a common remedy for wound healing and to stop bleeding on fresh cuts by traditional healers. Tribal people were using these plant parts to cure several illnesses such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea and mental disorders Raj Kumar Singh, *et. al.*, (2014). The latex of *Calotropis pocera* is used as purgative, while the flower and dried leaves are considered as digestive aids, useful in cough, asthma and anorexia. The root bark is useful in treating skin disease, intestinal worms also possess an analgesic, anticonvulsant and sedative effect. It's highly recommended for leprosy and different hepatitis. Oil extracted from leaves is very efficacious in treating cases of paralysis Verma, V.C., Singh, S.K. and Prakash, S. (2011). The extracts of C. procera extracts possess good larvicide activity against mosquitoes. *Alstonia Scholaris* belongs to the family Apocynaceae It is also known as Devils tree; Dita Bark tree has long been used as a traditional medicine to cure various human and livestock ailments. The plant is growing throughout the humid regions of India, especially in West Bengal and west-coast forests of south India. The plant is used in Ayurvedic, Unani and Sidhha /Tamil types of alternative medicinal systems Khare, S., Singh, N.B., Singh, A. *et al.* (2020). Thus, the present study was undertaken to assess the antimicrobial activity of various leaf extract of *Calotropis pocera*, *Ficus Benghalensis*, *Alstonia schcolaris*, *Ficus Krishnae* under following parameter. Preparation of various leaf extract dissolved in organic solvents. Crude dried leaf extract dissolved in methanol, Crude dried leaf extract dissolved in chloroform, Preliminary Photochemical screening of leaf extracts. To study the antimicrobial activity of extracts by Agar well diffusion method.

2. MATERIALS AND METHODS

2.1. Materials

2.2. Collection of plant material

In this study, the materials used were leaves of different plants. The leaves were collected from two different locations. The first location was the Herbal Garden of Post Graduate Government College for Girls in sector 42, and the second location was the Botanical Garden of Punjab University Chandigarh. The collection of plant material was done during the month of March, as specified in the study. It is important to note that proper care and precautions were taken during the collection of plant material to ensure that the samples were not contaminated and were in optimal condition for analysis.

2.3. Chemicals

The chemicals used in the present study include nutrient agar, Muller Hinton agar, peptone, yeast extract, and malt extract, which were obtained from Hi-Media Laboratories Limited located in India. Additionally, beef extract, agar, sodium hydroxide, sodium carbonate, and ferric chloride (FeCl2) were purchased from NICE Chemical Pvt. Ltd. in India. The chemicals such as sulphuric acid, chloroform, hydrochloric acid, methanol, ethanol, and glacial acetic acid were acquired from Qualigens Fine Chemicals Ltd. in India. Moreover, iodine, potassium iodine, Molish reagent, and Ninhydrin were supplied from Rankem Industries Pvt. Ltd. located in India. The present study, uses a few other routine chemicals that were procured from local commercial sources. It is notable that all the chemicals that were used in this study were of analytical grade, indicating that they were of

exceptionally high purity to render the intended results and avoid inference caused by impurities or contamination.

2.4. Microorganisms and culture conditions

he five bacterial strains and one fungal strain were obtained from MTCC (Microbial Type Culture Collection) Centre located in IMTECH (Institute of Microbial Technology) in Chandigarh. The bacterial strains used were Bacillus subtilis (MTCC No.: 121), *Bacillus coagulans* (MTCC No. 429), *Escherichia coli* (MTCC No.:40), *Staphylococcus aureus* (MTCC No.: 87), *Pseudomonas aeruginosa* (MTCC No.: 424), and *Proteus vulgaris* (MTCC No. 426). Additionally, the fungal strain used was *Candida tropicalis* (MTCC No.184), and *Saccharomyces cereviisae* (MTCCC No. 36) was also used in the present study.

Figure A Bacillus subtillus

Figure B Escherichia coli

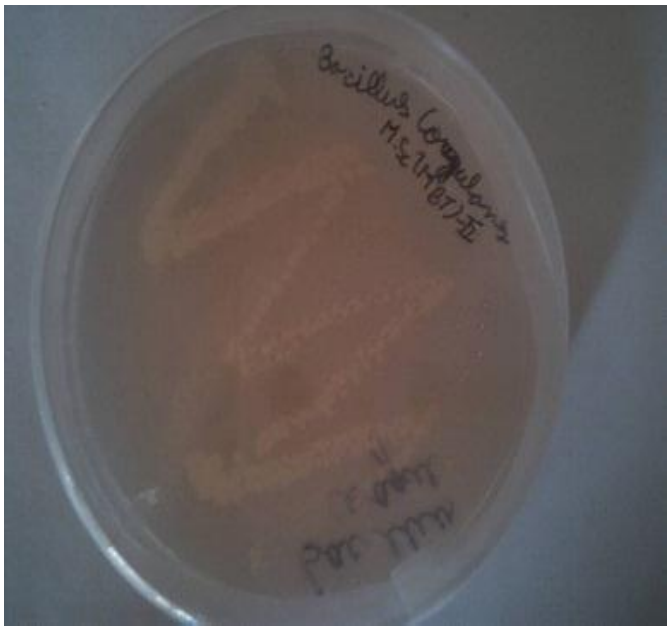


Figure C Bacillus coagulan

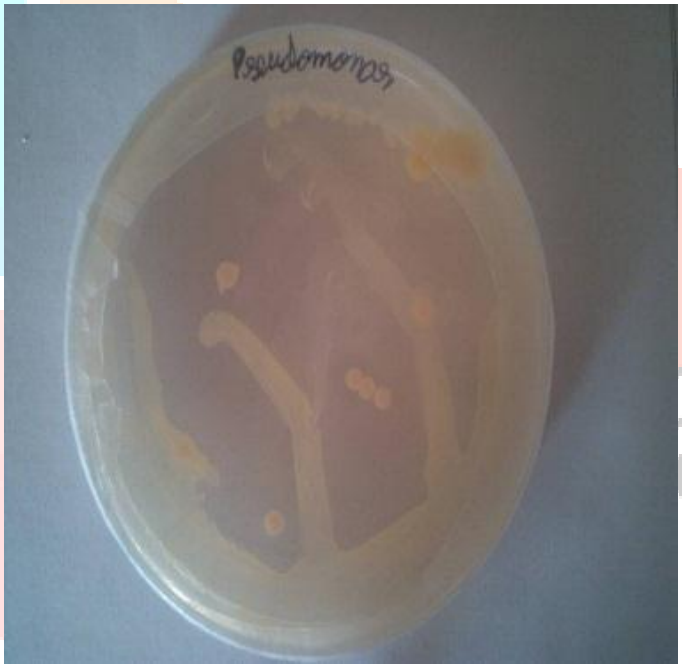


Figure D Staphylococcus aureus

Figure E Candida tropicalis

(E) ***Seudomonas aeruginosa***
(*F*) ***Candida tropicalis***

2.5. Culture conditions *Bacillus subtilius*

To culture the different strains specific nutrient agar and growth conditions were used. For *Bacillus subtilius* strain (MTCC No.: 121), the strain was first revived on Nutrient agar, composed of 1.0 g beef extract, 2.0g yeast extract, 5.0g peptone, 5.0g NaCl, and distilled water (1L) and then incubated at 30°C for 48 hours. The culture was then stored at 4°C until further use. *Escherichia coli strain* (MTCC No.:40) was revived on Nutrient agar at 37°C for 48 hours, composed of 1.0 g beef extract, 2.0g yeast extract, 5.0g peptone, 5.0g NaCl, agar (15.0g), and distilled water (1L). After incubation, it was then stored at 4°C. *Staphylococcus aureus strain* (MTCC No.: 87) was revived on Nutrient agar composed of 1.0 g beef extract, 2.0g yeast extract, 5.0g NaCl, agar (15.0g), and distilled water (1L) at 37°C for 24 hours and stored at 4°C. Like *Staphylococcus aureus* strain, *Pseudomonas aeruginosa* strain (MTCC No.: 424) was revived on Nutrient agar and incubated at 37°C for 24 hours, followed by storage at 4°C. *Bacillus coagulans* strain (MTCC No.: 492) was also revived on Nutrient agar and incubated at 37°C for 24 hours, followed by storage at 4°C. Candida tropicalis strain (MTCC No.: 184) was revived on YEPDA composed of yeast extract (3.0g), peptone (10.0g), dextrose (20.0g), agar (15g), and distilled water (1L) and incubated at 28°C for 48 hours, followed by storage at 4°C on YEPDA plates. Lastly, *Saccharomyces cerevisiae* strain (MTCC No.:36) was revived on Malt yeast agar composed of malt extract (3.0g), yeast extract (3.0g), peptone (5.0g), glucose (10.0g), agar (20.0g), and distilled water (1L) and then incubated at 30°C for 48 hours. It was then stored at 4°C until further use.

2.6. Growth media

The growth media used in this study were carefully selected based on their specific nutritional requirements for the growth of bacterial, fungal, and yeast cells. The following are the details of the different growth media and their composition:

(A) YEPD medium: This medium is used for the growth of yeast cells and contained 0.3% (w/v) yeast extract, 1.0% (w/v) peptone, and 2.0% (w/v) dextrose. Additionally, 1.5% (w/v) agar was used as a solidifying agent. The medium was sterilized by autoclaving at 15 psi for 20 minutes.

(B) Nutrient Broth medium: This medium is used for the growth of bacterial cells and contained 1.0 g beef extract, 2.0 g yeast extract, 5.0 g peptone, and 5.0 g NaCl dissolved in 1 L of distilled water. Moreover, 1.5% agar was used as a solidifying agent. The medium was sterilized by autoclaving at 15 psi for 20 minutes.

(C) Muller Hinton agar medium: This medium is used for antimicrobial susceptibility testing of bacterial cells and contained 30% beef infusion, 1.75 g casein hydrolysate, 0.15 g starch, and 17.0 g agar. The pH was adjusted to neutral at 25°C, and the medium was sterilized by autoclaving at 15 psi for 20 minutes.

(D) Sabouraud Dextrose Agar medium (SDA): This medium is used for the growth of fungal and yeast cells and contained 10 g peptone, 40 g dextrose, and 15 g agar dissolved in 1 L of distilled water. The pH was adjusted to 5.6 at 25°C, and the medium was sterilized by autoclaving at 15 psi for 20 minutes.

Each of these growth media was used for the specific purpose of maintaining the optimal growth conditions for the different types of microorganisms used in this experiment.

3. Experimental design

3.1. Growth conditions

The study utilizes bacterial strains such as *Pseudomonas aeruginosa, Escherchia coli, Styaphylococcus aureus, Bacillus subtilis, Bacillus coagulans, Proteus vulgaris* and yeast strains *Saccharomyces cerevisiae* and *candida tropicalis*. To ensure optimal growth of these strains, inoculums were prepared by transferring single colonies from nutrient agar plates of respective bacterial strains to 30 ml Nutrient broth media and grown at 37°C and 30°C, respectively for overnight at 200 rpm. Similarly, inoculums for Saccharomyces cerevisiae and candida tropicalis were prepared by transferring a single colony from YEPDA plates in 30 ml YEPD media and grown at 30˚C and 28˚C for overnight at 190 rpm. For each strain, 200 micro litre of the inoculum was transferred to 20 ml of respective growth media, and the cells were grown till mid-log phase (O.D 620 = 0.1-0.3) at 200 rpm for 24 hours at 37°C and 30°C. For *Saccharomyces cerevisiae* and *candida tropicalis*, the cells were grown till mid-log phase (O.D 620=0.1-0.3) at 200 rpm for 17-18 hours at 30°C and 28°C, respectively. These cells were then stored at 4°C for further use.

3.2. Collection and processing of plants leaves.

In this study, fresh leaves were collected from the Herbal Garden of P.G.G.C.G Sector-42 and from Punjab University Chandigarh. The leaves were collected in clean plastic bags and sealed to prevent any contamination or damage during transportation. Upon arrival at the laboratory, the leaves were processed for further analysis. First, the leaves were shade dried to prevent any degradation of their active constituents. Afterward, the dried leaves were ground in a mixer grinder to obtain fine particles that could be used for further experiments. The use of standardized procedures ensured that the leaves were processed consistently, and any changes observed in the bioactivity were likely attributed to the test compounds instead of sample variability.

3.3. Preparation of various leaf extracts of Plants

In this study, various leaf extracts of plants were prepared using different solvents, and their bioactive compounds were tested using different chemical tests.

1 ml of test solution, 1 ml of distilled water was added and shaken well. Formation of foamy leather indicates the presence of saponins.

i. Preparation of crude leaf extracts in Methanol and chloroform solvent for the presence of alkaloids: The dried and ground leaves were extracted using methanol and chloroform solvents to obtain crude leaf extracts. The presence of alkaloids in these extracts was tested.

ii. Test for Saponins (Frothing test): To 1 ml of test solution, 1 ml of distilled water was added, and the solution was shaken well. The formation of a foamy leather indicated the presence of saponins.

iii. Test for Tannins: To 1 ml of test solution, 1 ml of distilled water was added, followed by a few drops of FeCL3. The formation of a green precipitate indicated the presence of tannins.

iv. Test for Paleobotanies: To 1 ml of test solution, 1 ml of 1% HCl was added, and the mixture was boiled. The deposition of red precipitates indicated the presence of paleobotanies.

v. Test for Flavonoids (Alkaline reagent test): To 1 ml of test solution, a few drops of NaOH were added carefully. The formation of an intense yellow precipitate that became colorless with the addition of a few drops of dilute acid indicated the presence of flavonoids.

vi. Test for Terpenoids: To 1 ml of test solution, 1 ml of chloroform was added, evaporated to dryness, and then 1 ml of conc. H2SO4 was added and heated for 2 minutes. The formation of a greyish color indicated the presence of terpenoids.

vii. Test for Carbohydrates (Molisch test): To 1 ml of test solution, a few drops of Molisch reagent were added, and then a few drops of H2SO4 were added carefully. The appearance of a reddish-violet ring at the interface indicated the presence of carbohydrates.

viii. Test for Free amino acids (Ninhydrin test): To 1 ml of test solution, a few drops of ninhydrin were added. The appearance of blue color indicated the presence of amino acids.

ix. Test for Cardiac glycosides (Keller kill ani test): To 5 ml of test solution, 2 ml of glacial acetic acid containing 1-2 drops of 2% ferric chloride solution were added, and then it was under layered carefully with conc. H2SO4. A brown ring of the interface indicated the presence of cardiac glycosides.

x. Test for Steroids: To 1 ml of test solution, 1 ml of chloroform was added, and then a few drops of conc. H2SO4 were added carefully. A red color produced in the lower layer. The chloroform layer indicated the presence of steroids. Alternatively, to 1 ml of test solution, 1 ml of chloroform was added and treated with H2SO4 and acetic acid. The development of a greenish color indicated the presence of steroids.

xi. Test for Phytosterols: To 1 ml of test solution, a few drops of conc. H2SO4 were added carefully from the walls of the test tube. The appearance of a reddish-brown color indicated the presence of phytosterols.

These chemical tests were performed on the various leaf extracts to identify their chemical composition and the presence of bioactive compounds.

3.4 Antimicrobial activity in different leaf extracts by using agar well diffusion Method.

In this study, the antimicrobial activity of crude leaf extracts obtained from methanol and chloroform solvents was tested using the agar well diffusion method. This method involved inoculating bacterial and fungal strains onto agar medium and creating wells using a sterile cork borer. Then, the crude leaf extracts were added to each well, and the plates were incubated at their respective temperatures for 24 hours. Afterward, the inhibition zone around each well was measured using a scale. A larger inhibition zone indicated stronger antimicrobial activity and then zone of inhibition was calculated.

3.4.1 Agar well diffusion method

Principle

The antibacterial present in the plants extract is allowed to diffuse out into the medium and interact in the plate freshly inoculated with the test organisms Muhammad Evy Prastiyanto, Fandhi Adi Wardoyo and Wildiani Wilson et. al., (2020). The resulting zones of inhibition will be uniformly circular as there will be a confluents lawn of growth. The diameter of inhibition can be measured in millimeters to determine antimicrobial activity of plant leaf extracts.

The activity was determined by measuring the diameter of the inhibition zone in millimeters, which is proportional to the strength of the antimicrobial activity. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined using microdilution methods in Mueller-Hinton broth. To perform the agar well diffusion method, freshly prepared bacterial or fungal cultures were evenly spread

on Mueller-Hinton agar plates. Using a sterile cork borer, wells were created in the agar, and a specific volume of plant extract was filled in each well Leni Nurhayati, Nadhira Yahdiyani and Akhmad Karim Hidayatulloh (2020). The plates were then incubated at appropriate temperature for bacteria and fungi. Following incubation, the diameter of the inhibition zone around each well was measured in millimeters, and the results were compared to the control plates. A larger inhibition zone indicated stronger antimicrobial activity.

3.4.2. Procedure

Muller Hinton Agar (MHA) for bacterial Strains and YEPDA for fungus strain plates were inoculated with100µl of microbial inoculum of each bacterium and fungus and was spread. Three wells (10mm) were made in each of these plates with sterile pipettes. About 0.1ml of 100mg/ml concentration of plant extract were added using auto pipettes into the wells and allowed to diffuse. The plates were incubated at 37˚C for bacterial strain and 28˚C for fungus strain for 18-24 hours. Norfloxin, Tetracycline, Ampicillin, Penicillin-G, Streptomycin and Nystatin, Fluconazole, and Cefuroxime were used as a positive control for bacterial and fungal strain respectively. If antibacterial activity was present on the plate, it was indicated by the zone of inhibition surrounding the well containing the plant extract. The zone of inhibition was measured in millimeters to evaluate the antimicrobial activity.

The following procedure was used to test the antimicrobial activity of plant extracts using the agar well diffusion method:

1. Prepare Muller Hinton Agar (MHA) plates for bacterial strains and YEPDA plates for fungal strains.

2. Inoculate 100µl of microbial inoculum of each bacterium and fungus and spread it evenly on the agar plates.

3. Using a sterile pipette, make three wells (10mm) in each of these plates.

4. Prepare a 100mg/ml concentration of plant extract.

5. Add 0.1ml of the plant extract using an auto pipette into each well and allow it to diffuse.

6. Incubate the plates at 37˚C for bacterial strains and 28˚C for fungal strains for 18-24 hours.

7. Use Norfloxin, Tetracycline, Ampicillin, Penicillin-G, Streptomycin, and Nystatin, Fluconazole, and Cefuroxime as positive controls for bacterial and fungal strains, respectively.

8. After incubation, observe the plates for the presence of an inhibition zone surrounding the well containing the plant extract. If antibacterial or antifungal activity is present, it will be indicated by the zone of inhibition.

9. Measure the diameter of the zone of inhibition in millimeters to evaluate the antimicrobial activity of the plant extract.

Calculation of Percentage inhibition

The percentage inhibition and activity index of plant extracts were calculated to evaluate their antimicrobial activity.

A. The percentage inhibition was calculated using the following formula:

% Inhibition = (1 - Zone of inhibition / Plate diameter) × 100%

The zone of inhibition was measured in millimeters, and the plate diameter was 90mm, as growth occurs all over the agar plate.

B. The activity index was determined using the following formula:

Activity index = Zone of inhibition of extract / Zone of inhibition of standard

C. For statistical analysis, all experiments were carried out in triplicates, and the data was represented as mean ± SD (standard deviation). This approach helped to assess the reproducibility and variability of the results.

4. RESULTS AND DISCUSSION

The results of the study showed that the leaves of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* contained various bioactive compounds, including alkaloids, saponins, tannins, paleobotanies, flavonoids, terpenoids, carbohydrates, amino acids, cardiac glycosides, steroids, and phytosterols. These compounds have been previously reported to have various therapeutic properties, such as anti-inflammatory, antimicrobial, antifungal, analgesic, astringent, and immunomodulatory effects.

In addition, the antimicrobial activity of the crude leaf extracts obtained from methanol and chloroform solvents was tested using the agar well diffusion method. The results showed that these extracts exhibited significant antimicrobial activity against various bacterial and fungal strains, including *Escherichia coli, Staphylococcus aureus, Aspergillus niger, and Candida albicans*. The zone of inhibition was measured, and the percentage inhibition and activity index were calculated.

Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris are important medicinal plants, and they are used for treatment of a wide range of health disorders in traditional and folk medicine. Every part of these plants such as leaves, bark, stem, and seeds are used for various fields of medicine. Leaf is considered to be one of the highest accumulatory parts of plants containing bioactive compounds which are synthesized as secondary metabolites. In the present study, the leaves of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* were dried and processed to extract methanol and chloroform.

Among the four plants tested, *Ficus Krishnae* and *Ficus Benghalensis* extracts showed the highest antimicrobial activity against the tested microorganisms. The results suggested that these plants could be a good source of natural antimicrobial agents and have the potential to be used as alternative therapies to synthetic antimicrobial agents.

The extraction was based on polarity of the solvents where highly polar methanol polarity index (5.1) and chloroform polarity index (4.1). solvents were used. Thus, the aim of the present study was to evaluate the preliminary phytochemicals, and antimicrobial properties of different leaves extract of *Ficus Krishnae, Ficus Benghalensis, Calotropis procera, Alstonia Scholaris.*

4.1 Preliminary phytochemical testing of various extracts of leaves *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris.*

There has been observed a correlation of the phytochemical constituent of a medicinal plant with its pharmacological activity. Screening the active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases. The preliminary phytochemical tests were performed for identifying different chemical groups present in each extract using standard methods of Haslam, E., & Cai, Y. (1994). with some modifications as described earlier. The results obtained in this study suggested that the presence of phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plant studied. The phytochemical characteristics of the leaf extracts of *Ficus krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris.* investigated are summarised in Table 1 and Table 2. The phytochemical screening carried out on different leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* revealed the presence of alkaloids, saponins, tannins, phlobatannins, flavonoids, terpenoids, phytosterols etc., which are known to exhibit medicinal as well as physiological activities.

These findings are consistent with previous reports on the phytochemical composition of these plants and support their traditional use in the treatment of various ailments. Alkaloids, for example, have been reported to have analgesic and anti-inflammatory properties, while saponins have antifungal and antiviral activities. Tannins, phlobatannins, and flavonoids are known for their anti-inflammatory, antioxidant, and anticancer properties. Terpenoids and phytosterols have been reported to have antimicrobial and anti-inflammatory activities.

The table 1 shows the preliminary phytochemical testing of methanolic extract of leaves of *Ficus benghalensis, Ficus krishnae, Calotropis procera, and Alstonia scholaris*. The presence or absence of various phytochemicals was observed, and the intensity of the colour was noted. All four plants tested positive for alkaloids, with a high intensity of colour, indicating a high concentration of alkaloids in the methanolic extract. Saponins, tannins, carbohydrates, and free amino acids were also present in all four plants but with varying degrees of intensity. Phytosterols were present in Ficus benghalensis and Calotropis procera with a high intensity of colour, while Alstonia scholaris showed a moderate intensity. Terpenoids were absent from all four plants.

Cardiac glycosides were present in Calotropis procera and Alstonia scholaris, while steroids were present in all four plants with a moderate to high intensity of colour.

Table 1: Preliminary phytochemical testing of Methanolic extract of Leaves of *Ficus benghalensis, Ficus krishnae, Calotropis procera and Alstonia scholaris.*

S. No	Phytochemicals	Plants			
		Ficus benghalensis	***Ficus krishnae***	***Calotropis procera***	***Alstonia scholaris***
1.	Alkaloids	++	++	++	++
2.	Saponins	+	+	++	++
3.	Tannins	++	++	++	+
4.	Phlobatannins	+	+	+	++
5.	Flavonoids	+	+	++	+
6.	Terpenoids	-	-	-	-
7.	Carbohydrates	++	++	+	+
8.	Free amino acids	++	+	+	+
9.	Cardiac glycosides	-	-	+	++
10.	Steroids	+	+	+	+
11.	Phytosterols	++	-	+	+

(+) indicates presence (-) indicates absence.

++ = High intensity of colour; + =moderate intensity of colour, - = No colour

The table 2 shows the preliminary phytochemical testing of chloroform extract of leaves of Ficus benghalensis, Ficus krishnae, Calotropis procera, and Alstonia scholaris. The presence or absence of various phytochemicals was observed, and the intensity of the colour was noted. All four plants tested positive for tannins, with a high intensity of colour, indicating a high concentration of tannins in the chloroform extract. Alkaloids, saponins, carbohydrates, and free amino acids were also present in varying intensities in all four plants. Flavonoids were present in Ficus benghalensis, Ficus krishnae, and Calotropis procera with a high intensity of colour. Phytosterols were present in Ficus benghalensis and Alstonia scholaris, while steroids were present in Ficus krishnae and Calotropis procera with a moderate to high intensity of colour. Phlobatannins were present in Ficus benghalensis and Calotropis procera, with a moderate intensity of colour. Cardiac glycosides were present in Calotropis procera and Alstonia scholaris, while terpenoids were absent from all four plants.

Table 2: Preliminary phytochemical testing of Chloroform extract of Leaves of *Ficus benghalensis, Ficus krishnae, Calotropis procera and Alstonia scholaris.*

S. No	Phytochemicals	Plants			
		Ficus benghalensis	*Ficus krishnae*	*Calotropis procera*	*Alstonia scholaris*
1.	Alkaloids	++	++	+	+
2.	Saponins	+	+	++	++
3.	Tannins	++	++	++	++
4.	Phlobatannins	+	+	+	+
5.	Flavonoids	++	+	+	+
6.	Terpenoids	-	-	-	-
7.	Carbohydrates	+	+	+	+
8.	Free amino acids	+	+	-	+
9.	Cardiac glycosides	-	-	+	+
10.	Steroids	+	++	+	++
11.	Phytosterols	++	-	-	++

(+) indicates presence (-) indicates absence.

++ = High intensity of colour; + =moderate intensity of colour, - = No colour

The alkaloids contained in plants are used in medicine as anesthetic Pandey, A.K., & Verma, N. (2013), Pandey A, Verma N. (2013), analgesic Antherden LM (1969) A.J. Harborne. (1998), antispasmodic and antibacterial Agents Stary F. (1998). The presence of tannins also suggests that it might have anti-viral and anti-bacterial activities and can help in healing wounds and burns Haslam, E., & Cai, Y. (1994). The plant extracts contained saponins are known to produce inhibitory effect on inflammation; tonic and stimulating activities as observed in Chinese and Japanese medical herbs Edeoga HO, Okwu DE, Mbacbie BO (2005). Saponins are also very important classes of secondary metabolites that are used in treatment of heart conditions. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness Sodipo, O. A., *et al.,* (1991).

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganisms *in vitro*. Their antimicrobial action is probably due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall. The presence of flavonoids suggests that the plant might have an antioxidant, anti-allergic, anti-microbial, anti-cancer Activity Marjorie Murphy Cowan, (1999). Flavonoids, a group of polyphenolic compounds with known properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and anti- inflammatory action Pourmorad F, *et. al.,* (2006). has been isolated from plants. They were also effective antioxidants and show strong anticancer activities Salah N, *et. al.,* (1995), Del-Rio A, *et. al.,* (1997). Steroids have been reported to have antibacterial properties [40] and they were very important compounds especially due to their relationship with compounds such as sex hormones. In the

present study, the number of phytochemicals screened in the leaf extracts of *Ficus krishnae, Ficus bengalensis, Calotropis Procera, Alstonia Scholaris.*In the methanolic and chloroform leaf extract of *Ficus Benghalensis* showed alkaloids, saponins, tannins, phlobatannis, flavonoids, glycosides, carbohydrates, free amino acid, steroids, and phtyosterols. In *Ficus Krishnae* leaf extract showed alkaloids, saponins, tannins, phlobatannis, flavonoids, glycosides, carbohydrates, free amino acid, and steroids are present whereas terpenoids, cardiac glycosides and phytosterol were absent Pandya, D.J., Joshi, M.K., & Gorwadiya, H.C. (2012). In leaf extract of *Calotropis Procera* showed alkaloids, saponins, tannins, paleobotanies, flavonoids, glycosides, carbohydrates, free amino acid, steroids, and phytosterols are present whereas terpenoid was absent. In the leaf extract of *Alstonia Scholaris* showed alkaloids, saponins, tannins, paleobotanies, flavonoids, glycosides, carbohydrates, free amino acid, steroids, and phytosterols. The presence of different groups of phytochemicals, produced by plants such as flavonoids, alkaloids, tannins, and saponins etc. has been directly linked with the medicinal values of a particular plant. The presence of these compounds has also been confirmed to have antimicrobial activity Jain SC, Jain R, Vlietinck AJ (2004).

4.2. Antimicrobial properties of different leaf extract of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris*

The antimicrobial activity of different leaf extracts was screened in the study against the six bacterial strains (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis, Bacillus coagulans, Proteus vulgaris)* and two fungus strain *(Saccharomyces cerevisiae* and *Candida tropicalis)* for possible antimicrobial activity. Antimicrobial activity is defined as the activity that kills or slows down the growth of microorganisms. It was determined by the agar well diffusion method.

Calotropis procera leaves on selected pathogenic microorganisms was investigated using the disc diffusion method. The results showed that the methanolic and aqueous extracts of *Calotropis procera* had significant antimicrobial activities against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans*.

Reference [14]: Singh et al. (2013). Antibacterial activity of Ficus krishnae and Ficus benghalensis. Journal of Chemical and Pharmaceutical Research, 5(7), 180-183.

The antibacterial activity of methanolic and aqueous extracts of leaves of *Ficus krishnae* and *Ficus benghalensis* was investigated using the agar well diffusion method against four bacterial strains: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa*. The methanolic extract of *Ficus krishnae* showed the highest antimicrobial activity against all tested bacteria, followed by the methanolic extract of *Ficus benghalensis*.

In the present study, the antimicrobial activity of different leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, and Alstonia Scholaris* was evaluated using the agar well diffusion method

against six bacterial strains and two fungal strains. The results showed that all the extracts had significant antimicrobial activities against some or all of the tested microorganisms.

Among the four plants tested, *Ficus Krishnae* and *Ficus Benghalensis* extracts showed the highest antimicrobial activity against the tested microorganisms. *Calotropis Procera* and *Alstonia Scholaris* extracts also exhibited significant antimicrobial activities against some of the tested microorganisms.

4.3. Antimicrobial activity is determined by measuring the zone of inhibition.

Antimicrobial studies of different leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera and Alstonia Scholaris* by agar well diffusion method have shown considerable antibacterial and antifungal activity against all microbial strains. The chemical kills the microbial cells to form a clear or an area without the growth of microbes referred to as the zone of inhibition. As the area covered under the zone of inhibition has increased that indicated better the capability of chemical/extract to kill the microbes.

The results of the study showed as in Table 3 that the methanolic extract of the leaves of *Ficus Benghalensis, Ficus Krishnae, Calotropis Procera,* and *Alstonia Scholaris* had considerable antibacterial activity against *Escherichia coli, Bacillus subtilis,* and *Bacillus coagulans*.

In *Escherichia coli,* the zone of inhibition observed for the tested extracts ranged from 8.67±1.15mm to 13±1.73mm, while the standard ampicillin showed a zone of inhibition of 18.67±1.15mm. The highest zone of inhibition was observed for *Ficus Krishnae* extract.

In *Bacillus subtilis,* the zone of inhibition observed for the tested extracts ranged from 11.00±1.00mm to 14.33±2.08mm, while the standard ampicillin showed a zone of inhibition of 22.00±2.00mm. The highest zone of inhibition was observed for *Ficus Benghalensis* extract.

In *Bacillus coagulans*, the zone of inhibition observed for the tested extracts ranged from 14.67±1.15mm to 17.67±2.52mm, while the standard penicillin G showed a zone of inhibition of 17.00±2.65mm. The highest zone of inhibition was observed for *Ficus Benghalensis* extract.

In *Pseudomonas aeruginosa* (Table 3), the diameter of the zone of inhibition was 15.67±2.08mm, 18.00±2.00mm, 19.00±1.73mm, 18.33±0.58mm, where standard cefuroxime (30µg/well) showed the zone of inhibition of 20.67±1.15mm, which was used as a positive control.

In *Staphylococcus aureus* (Table 3), the diameter of the zone of inhibition was 12.67±1.15mm, 15.33±0.58mm, 12.67±1.15mm, 12.67±1.15mm, where standard tetracycline (30µg/well) showed the zone of inhibition of 20.67±1.15mm that was used as a positive control.

In *Proteus vulgaris* (Table 3), the diameter of the zone of inhibition was 17.33±0.58mm, 17.33±0.58mm, 16.00±1.73mm, 13.33±1.53mm, where standard norfloxacin (10µg/well) showed the zone of inhibition of 22.00±2.00mm, which was used as a positive control.

In *Candida tropicalis* (Table 3) the diameter of zone of inhibition was 11.00±1.00mm,12.67±1.15mm,11.67±0.58mm,12.00±2.00mm, where standard fluconazole (10µg/well) showed the zone of inhibition of14.00±1.73mm that used as a positive control.

In *Saccharomyces cerevisiae* (Table 3) the diameter of zone of inhibition was16.00±2.00 mm, 14.33±1.53mm, 11.67±0.58mm,11.33±1.15mm, where standard nystatin (50µg/well) showed the zone of inhibition of 20.67±1.15mm that used as a positive control.

The results of the study demonstrate substantial antimicrobial activity of the different leaf extracts against the tested bacterial strains. The zone of inhibition observed for the methanolic extract of *Ficus Krishnae, Ficus Benghalensis*, and *Calotropis Procera* was higher than that of *Alstonia Scholaris*. Moreover, the reported zone of inhibition in the study is comparable or even higher than the standard antibiotics used in the study, signifying the potency of the tested extracts as natural antimicrobial agents.

Table 3: Effect of antimicrobial activity of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris* Leaf extracts in Methanol

Test microorganisms	Zone of inhibition (mm)				
	Ficus benghalensis	Ficus krishnae	Calotropis procera	Alstonia scholaris	Standard*
Escherichia coli	12.67±1.15	13±1.73	12.67±1.15	8.67±1.15	18.67±1.15
Bacillus subtilis	14.33±2.08	13.00±1.73	11.00±1.00	12.00±2.00	22.00±2.00
Bacillus coagulans	17.67±2.52	16.00±2.00	14.67±1.15	16.67±3.06	17.00±2.65
Pseudomonas aereginosa	15.67±2.08	18.00±2.00	19.00±1.73	18.33±0.58	20.67±1.15
Staphylococcus aureus	12.67±1.15	15.33±0.58	12.67±1.15	12.67±1.15	20.67±1.15
Proteus vulgaris	17.33±0.58	17.33±0.58	16.00±1.73	13.33±1.53	22.00±2.00
Candida tropicalis	11.00±1.00	12.67±1.15	11.67±0.58	12.00±2.00	14.00±1.73
Saccharomyces cerevisiae	16.00±2.00	14.33±1.53	11.67±0.58	11.33±1.15	20.67±1.15

Values represent mean ±SD (n=3) Standard*; *Escherichia coli* = Ampicillin(10µg/disc), *Bacillus subtilis* = Ampicillin (10µg/disc), *Bacillus coagulant* = Penicillin-G(10µg/disc), *Pseudomonas aeruginosa*= Cefuroxime(30µg/disc), *Staphylococcus aureus* =tetracycline (30µg/disc), *Proteus vulgaris* = Norfloxacin (10µg/disc), *Saccharomyces cerevisiae*=Nystatin (50µg/disc).

Amum indicum and Azadirachta indica are used to treat oral diseases, while Ficus benghalensis was used to treat dental problems by traditional healers in Tamil Nadu, India, as reported [15]. However, the current study focused on the antimicrobial activity of the leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis*

Procera, and Alstonia Scholaris. [16] discusses the developmental and reproductive biology of Planococcus minor, a species of scale insect that feeds on various plant hosts, including Ficus benjamina, Ficus carica, Ficus elastica, Ficus religiosa, and Ficus sycomorus, among others.

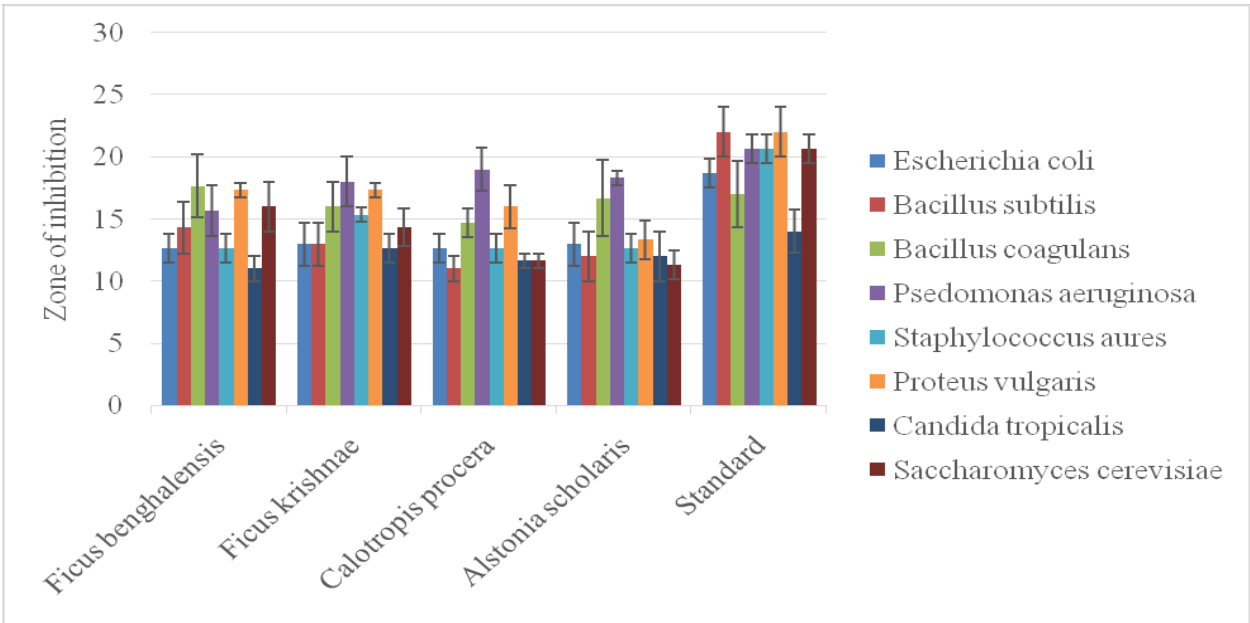


Figure 1: Zone of inhibition against *Escherichia Coli, Bacillus subtilis, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Candida tropicalis and Saccharomyces cerevisiae* by Methanolic extracts of leaves of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris.*

The results presented in Table 4 show the antimicrobial activity of the chloroform extract of the leaves of four different plant species against various microbial strains.

In *Escherichia coli*, the chloroform extract of *Ficus Benghalensis, Ficus Krishnae, Calotropis Procera,* and *Alstonia Scholaries* showed a zone of inhibition ranging from 8.33±0.58mm to 8.67±1.15mm. The standard ampicillin (10µg/well) showed a zone of inhibition of 18.67±1.15mm, which is much higher than the observed inhibition by the plant extracts.

In *Bacillus subtilis*, the chloroform extract of the four plant species showed a zone of inhibition ranging from 9.33±1.15mm to 12.33±2.52mm, while the standard ampicillin showed a zone of inhibition of 22.00±2.00mm.

In *Bacillus coagulans*, the chloroform extract of the four plant species showed a zone of inhibition ranging from 12.00±2.00mm to 15.00±1.00mm, while the standard penicillin-G showed a zone of inhibition of 17.00±2.65mm.

In *Pseudomonas aeruginosa*, the chloroform extract of the four plant species showed a zone of inhibition ranging from 11.33±1.15mm to 14.67±2.52mm, while the standard cefuroxime showed a zone of inhibition of 20.67±1.15mm.

In *Staphylococcus aureus*, the chloroform extract of the four plant species showed a zone of inhibition ranging from 10.67±0.58mm to 13.67±1.53mm, while the standard tetracycline showed a zone of inhibition of 20.67±1.15mm.

In *Proteus vulgaris*, the chloroform extract of the four plant species showed a zone of inhibition ranging from 13.67±1.53mm to 17.33±0.58mm, while the standard norfloxacin showed a zone of inhibition of 22.00mm.

In *Candida tropicalis* (Table 4) the diameter of zone of inhibition was 11.00±1.00mm,11.33±1.15mm,11.67±0.58mm,10.33±0.58 mm, where standard fluconazole (10µg/well) showed the zone of inhibition of14.00±1.73mm that used as a positive control and

In *Saccharomyces cerevisiae* (Table 4) the diameter of zone of inhibition was 11.67±0.58mm,11.33±0.58mm10.67±1.15mm, ,10.67±1.15mm, where standard nystatin (50µg/well) showed the zone of inhibition of 20.67±1.15mm that used as a positive control. The maximum zone of inhibition by methanol extract (table 3) as compared to chloroform extract (table 4).

Table 4: Effect of antimicrobial activity of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaries* Leaf extracts in Chloroform.

Test microorganisms	Zone of inhibition (mm)				
	Ficus benghalensis	Ficus rishnae	Calotropis procera	Alstonia scholaris	Standard*
Escherichia coli	8.67±1.15	8.67±1.15	8.33±0.58	8.67±1.15	18.67±1.15
Bacillus subtilis	12.33±2.52	11.33±1.15	10.67±0.58	9.33±1.15	22.00±2.00
Bacillus coagulans	15.00±1.00	15.00±1.00	12.00±2.00	13.67±1.53	17.00±2.65
Pseudomonas aereginosa	14.00±1.00	12.67±1.15	11.33±1.15	14.67±2.52	20.67±1.15
Staphylococcus aureus	10.67±1.15	13.67±1.53	11.33±1.15	10.67±0.58	20.67±1.15
Proteus vulgaris	14.67±0.58	17.33±0.58	13.67±1.53	15.3±3.06	22.00±2.00
Candida tropicalis	11.00±1.00	11.33±1.15	11.67±1.15	10.33±0.58	14.00±1.73
Saccharomyces cerevisiae	11.67±0.58	11.33±0.58	10.67±1.53	10.67±1.15	20.67±1.15

Values represent mean ±SD(n=3).) Standard*; *Escherichia coli* = Ampicillin(10µg/disc), *Bacillus subtilis* = Ampicillin(10µg/disc), *Bacilluscoagulans* = Penicillin-G (10µg/disc), *Pseudomonas aeruginosa* = Cefuroxime(30µg/disc), *Staphylococcus aureus*= tetracycline (30µg/disc), *Proteus vulgaris*=Norfloxacin(10µg/disc), *Saccharomyces cerevisiae*=Nystatin(50µg/disc).

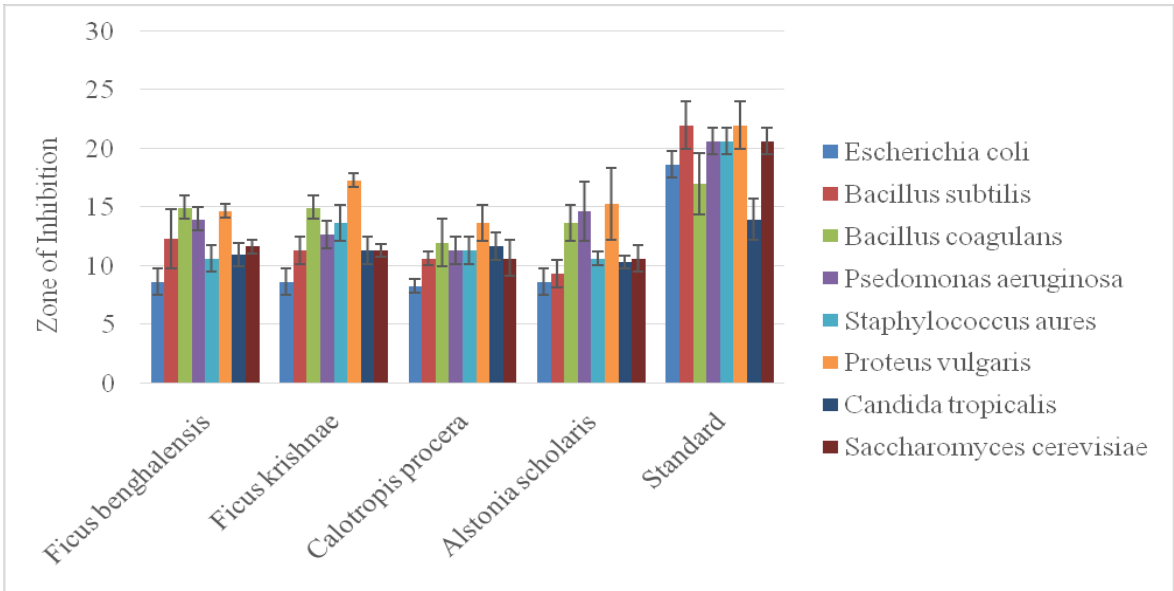


Figure 2: Zone of inhibition against *Escherichia coli, Bacillus subtilis, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Candida tropicalis* and *Saccharomyces cerevisiae* by Chloroform extracts of leaves of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris.*

4.4. The antimicrobial activity expressed as percent inhibition.

The study also carried out percent inhibition analysis to determine the antimicrobial activity of various leaf extracts against the test organisms. The results showed that all the extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, and Alstonia Scholaris* leaves exhibited higher percentage inhibition compared to their respective controls or solvents against all the bacterial strains.

The methanol extract showed high inhibitive activity against *Staphylococcus aureus, Bacillus coagulans, Proteus vulgaris,* and *Pseudomonas aeruginosa*. The methanol extract also exhibited high percent inhibition against *Saccharomyces cerevisiae*. On the other hand, chloroform extracts showed high percentage inhibition against *Bacillus coagulans, Proteus vulgaris,* and *Pseudomonas aeruginosa*. In particular, the methanol extract showed high percent inhibition against *Pseudomonas aeruginosa*, while the chloroform extract showed high percent inhibition against Proteus vulgaris.

Bacillus subtilis was found to be more sensitive towards both methanol and chloroform extracts, while Escherichia coli exhibited higher sensitivity towards methanol extract.

The results of the study indicate that both methanolic and chloroform extracts of the leaves of *Ficus Benghalensis, Ficus Krishnae, Calotropis Procera, and Alstonia Scholaris* possess significant antimicrobial activity against various bacterial and fungal strains. The diameter of the zone of inhibition observed ranged from 6mm to 18mm, more than 8mm indicating that the plant extracts were effective in inhibiting the growth of these microorganisms. The study found that all the tested microorganisms, including *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Saccharomyces cerevisiae, Bacillus coagulans, Proteus*

vulgaris, and *Candida tropicalis* were highly sensitive towards the methanolic and chloroform extracts. This suggests that the plant extracts have broad-spectrum antimicrobial activity. Among the tested microorganisms, the methanolic and chloroform extracts showed maximum inhibition against *Staphylococcus aureus, Bacillus coagulans, Proteus vulgaris,* and *Pseudomonas aeruginosa*. This suggests that the plant extracts may be particularly effective against these bacterial strains. The standard antibiotic has shown maximum inhibition against all the microbes, so they are highly sensitive to it. All the extracts were better antimicrobial agent against *Staphylococcus aureus*. *Bacillus coagulans, Proteus vulgaris, Pseudomonas aeruginosa*.

4.5. The antimicrobial activity expressed as percent inhibition.

Percent inhibition was also carried out to determine the antimicrobial activity of various leaf extract against test organisms. It showed the ability of extracts to which extent it was better compared to others against test microorganism used in the present study. In the present study, all the extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* leaves showed higher percentage inhibition as compared to their respective controls or solvents in all the bacterial strains. The results indicated that methanol extract showed high inhibitive activity for *Staphylococcus aureus, Bacillus coagulans, Proteus vulgaris, Pseudomonas aeruginosa. Saccharomyces cerevisiae* Methanol extract had shown high percent inhibition against *Staphylococcus aureus, Bacillus coagulans, Proteus vulgaris, Pseudomonas aeruginosa. Saccharomyces cerevisiae* but Chloroform extracts showed high percentage inhibition against *Bacilluscoagulans, Proteus vulgaris, Pseudomonas aeruginosa. Methanol* extract showed high percent inhibition against *Pseudomonas aeruginosa* whereas Chloroform extract showed high percent inhibition against and *Proteus vulgaris* whereas *Bacillus subtilis* showed maximum sensitivity towards both methanol and chloroform whereas *Escherichia coli* found to be more sensitive towards methanol.

The *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera,* and *Alstonia Scholaris* leaf extracts were evaluated for their percent inhibition against various microorganisms in the present study. The results of the study are presented in Table 5.

The methanolic leaf extracts of all four plant species showed higher percent inhibition compared to their respective controls or solvents against all the tested microorganisms. *Ficus Benghalensis* and *Ficus Krishnae* extracts exhibited similar percent inhibition against *Escherichia coli*, while *Calotropis Procera* and *Alstonia Scholaris* extracts showed slightly lower percent inhibition.

In *Bacillus subtilis, Ficus Benghalensis* extract exhibited maximum percent inhibition, followed by *Calotropis Procera* and *Alstonia Scholaris*, while *Ficus Krishnae* extract showed comparatively lower percent inhibition.

In *Bacillus coagulans*, all four plant extracts exhibited high percent inhibition, with *Ficus Benghalensis* extract showing the highest percent inhibition.

In *Pseudomonas aeruginosa*, *Calotropis Procera* and *Alstonia Scholaris* extracts exhibited the highest percent inhibition, while *Ficus Krishnae* and *Ficus Benghalensis* extracts showed slightly lower percent inhibition.

In *Staphylococcus aureus*, *Ficus Krishnae* and *Calotropis Procera* extracts exhibited higher percent inhibition, while *Ficus Benghalensis* and *Alstonia Scholaris* extracts showed comparatively lower percent inhibition.

In *Proteus vulgaris*, all four plant extracts exhibited high percent inhibition, with *Ficus Benghalensis* and *Ficus Krishnae* extracts showing the highest percent inhibition.

In *Candida tropicalis*, *Ficus Benghalensis* and *Ficus Krishnae* extracts exhibited similar percent inhibition, while *Calotropis Procera* and *Alstonia Scholaris* extracts showed slightly lower percent inhibition.

In *Saccharomyces cerevisiae*, *Ficus Benghalensis* and *Pseudomonas aeruginosa* extracts exhibited the highest percent inhibition, while *Ficus Krishnae and Calotropis Procera* extracts showed relatively lower percent inhibition.

Table 5: Percent inhibition against microorganisms by Methanolic leaf extracts of*Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris*

Test microorganisms	Percent inhibition (mm)				
	Ficus benghalensis	**Ficus krishnae**	**Calotropis procera**	**Alstonia scholaris**	**Standard***
Escherichia coli	14.07±1.28	14.44±1.92	14.07±1.28	14.44±1.92	20.74±1.28
Bacillus subtilis	15.92±2.31	14.44±1.92	12.22±1.11	13.33±2.22	24.44±2.22
Bacillus coagulans	19.62±2.79	17.77±2.22	16.29±1.28	18.51±3.39	18.88±2.94
Pseudomonas aereginosa	17.40±2.31	19.99±2.22	20.74±1.28	20.37±0.64	22.96±1.28
Staphylococcus aureus	14.07±1.28	17.03±0.64	14.07±1.28	14.07±1.28	22.96±1.28
Proteus vulgaris	19.25±0.64	19.25±0.64	17.77±1.69	14.81±1.69	24.44±2.22
Candida tropicalis	12.22±1.11	14.07±1.28	12.96±0.64	13.33±2.22	15.55±1.92
Saccharomyces cerevisiae	17.77±2.22	15.92±1.69	12.96±0.64	12.59±1.28	22.96±1.28

Values represent mean ±SD(n=3) Standard*; *Escherichia coli* = Ampicillin(10µg/disc), Bacillus *subtilis*=Ampicillin(10µg/disc), Bacilluscoagulans =Penicillin-G(10µg/disc), *Pseudomonas aeruginosa* = Cefuroxime(30µg/diisc), *Staphylococcus aureus*= tetracycline(30µg/disc), *Proteus vulgaris*=Norfloxacin(10µg/disc), *Saccharomyces cerevisiae*=Nystatin(50µg/disc).

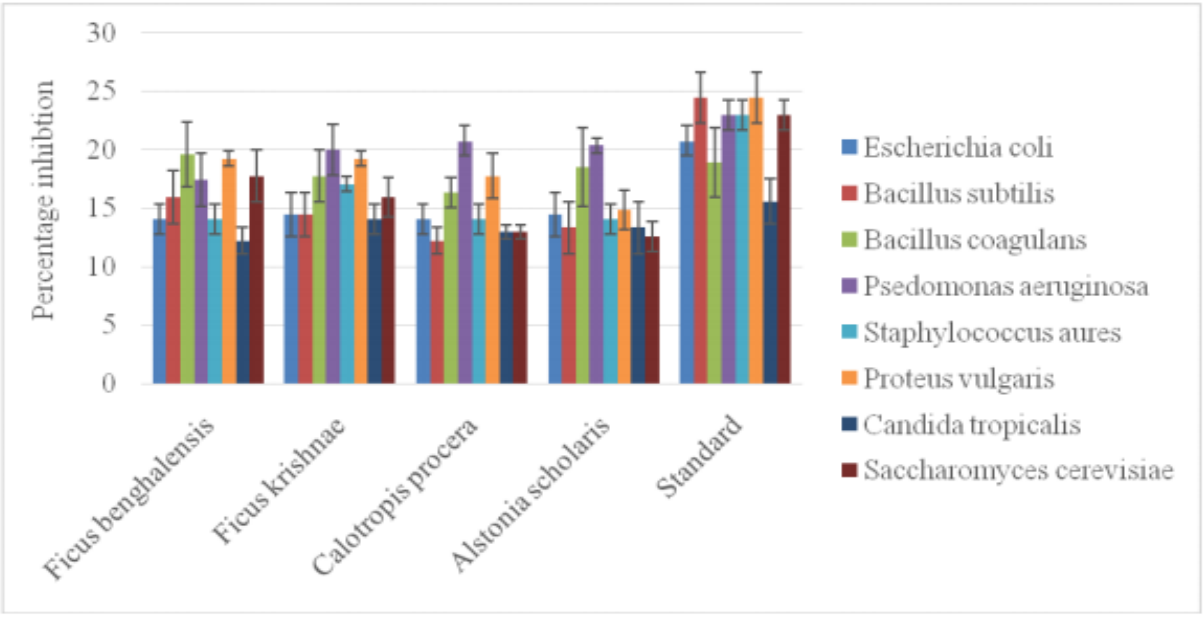


Figure 3: Percent inhibition against *Escherichia coli, Bacillus subtilis, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Candida trpoicalis and Saccharomyces cerevisiae* by Methanolic extracts of leaves of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris.*

4.6. Antimicrobial activity as expressed in terms of activity index.

Activity index determines which extract is showing better activity as compared to positive control. Ampicillin, Penicillin G, Cefuroxime, Tetracycline, Norfloxacin and Nystatin was used as positive control for bacterial strains and fungal strains, respectively. Although all extracts showed less or equal percentage inhibition and zone of inhibition as compared to standards but In methanolic leaf extract *Ficus Benghalensis* showed high activity index for *Bacillus coagulans, Ficus Krishnae* for *Staphylococcus aureus. Alstonia Scholaris* showed high activity index for *Bacillus coagulans. Pseudomonas aeruginosa, Calotropis Procera* showed higher activity index for *Escherichia coli, Staphylococcus aureus* as compared to others and their respective solvents. Antibacterial, antifungal activity, percentage inhibition and activity index of various leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia scholars* have been evaluated in the present research work. The *in vitro* antimicrobial activity of different leaf extracts is the first step towards the development of new potential drugs. Therefore, different extracts were tested for their antimicrobial activity against different microbial strains viz., *Escherichia coli, Bacillus subtilis, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Candida tropicalis and Saccharomyces cerevisiae.* Antimicrobial activity of the test extracts was tested by using agar well diffusion method. Inhibitory zone formation was observed with all extracts at a considerable value. Furthermore, all the extracts showed higher percentage inhibition and activity indices as compared to their respective solvents/controls against the bacterial strains and fungal strains. The results revealed the presence of inhibiting potentiality in all the test extracts at various degrees against the studied microorganisms.

The study also determined the activity index of the methanolic leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera,* and *Alstonia Scholaris* against the tested microorganisms. The results of the activity index determination are presented in Table 6.

In *Escherichia coli*, all four plant extracts showed a similar activity index, indicating that they are equally effective in inhibiting the growth of this bacterial strain.

In *Bacillus subtilis*, Ficus Benghalensis and Ficus Krishnae extracts showed similar activity index values, while Calotropis Procera and Alstonia Scholaris extracts showed comparatively lower activity index values.

In *Pseudomonas aeruginosa*, all four plant extracts exhibited similar activity index values, indicating that they are equally effective in inhibiting the growth of this bacterial strain.

In *Staphylococcus aureus*, *Ficus Krishnae* extract showed a higher activity index compared to the other three plant extracts.

In *Proteus vulgaris*, Ficus benghalensis extract showed the highest activity index, followed by Alstonia Scholaris and Calotropis Procera, while Ficus Krishnae extract showed a comparatively lower activity index.

In *Candida tropicalis, Ficus Krishnae* extract showed the highest activity index, followed by *Alstonia Scholaris* and *Ficus Benghalensis*, while *Calotropis Procera* showed a comparatively lower activity index.

In *Saccharomyces cerevisiae*, all four plant extracts exhibited similar activity index values, indicating that they are equally effective in inhibiting the growth of this fungal strain.

Table 6: Activity index determination against microorganisms by Methanolic extract of leaves of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris.*

Test microorganisms	**Activity index**			
	Ficus benghalensis	***Ficus krishnae***	***Calotropis procera***	***Alstonia scholaris***
Escherichia coli	0.67±0.08	0.69±0.05	0.82±0.04	0.66±0.07
Bacillus subtilis	0.64±0.06	0.66±0.07	0.53±0.05	0.56±0.05
Bacillus coagulans	1.04±0.14	0.95±0.09	0.99±0.06	1.16±0.22
Pseudomonas aeruginosa	0.78±0.11	0.87±0.11	0.92±0.09	0.95±0.05
Staphylococcusaureus	0.61±0.08	0.74±0.06	0.61±0.08	0.65±0.05
Proteus vulgaris	0.89±0.04	0.78±0.07	0.77±0.11	0.74±0.12
Candida tropicalis	0.80±0.10	0.92±0.07	0.84±0.14	0.92±0.07

Saccharomyces cerevisiae	0.78±0.10	0.69±0.10	0.59±0.04	0.54±0.05

Values represent mean ±SD(n=3)

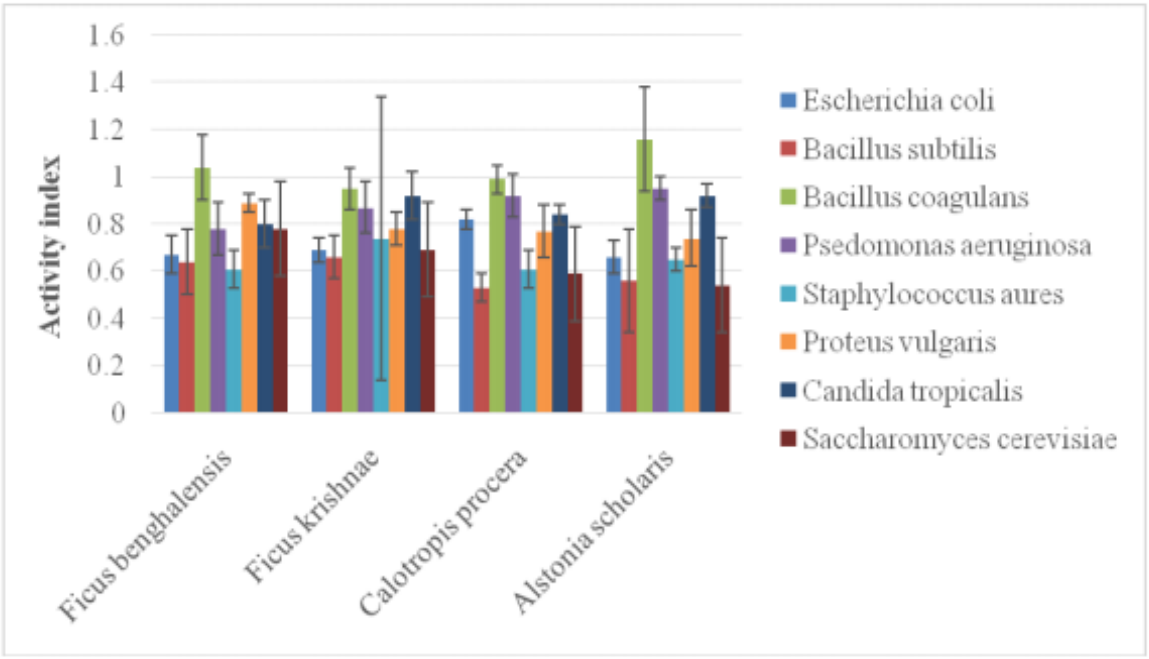


Figure 5: Activity index determination against *Escherichia coli, Bacillus subtilis, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Candida trpoicalis* and *Saccharomyces cerevisiaeby* Methanolic extract of leaves of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris.*

The chloroform extracts of Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, and Alstonia Scholaris leaves were also evaluated for their activity index against the tested microorganisms. The results of the activity index determination are presented in Table 7.

The results showed that all four plant extracts possess antimicrobial activity against the tested microorganisms. However, their effectiveness varied depending on the bacterial strain.

In *Escherichia coli*, all four plant extracts showed a similar activity index value, indicating that they are equally effective in inhibiting the growth of this bacterial strain.

In *Bacillus subtilis, Ficus Benghalensis* extract showed the highest activity index, followed by *Ficus Krishnae,* while *Calotropis Procera and Alstonia Scholaris* showed comparatively lower activity index values.

In *Bacillus coagulans*, all four plant extracts exhibited high activity index values, indicating that they are particularly effective against this bacterial strain.

In *Pseudomonas aeruginosa*, *Ficus Benghalensis* and *Alstonia Scholaris* extracts showed higher activity index values, while *Ficus Krishnae* and *Calotropis Procera* showed comparatively lower activity index values.

In *Staphylococcus aureus, Ficus Krishnae* extract showed the highest activity index, followed by *Calotropis Procera* and *Alstonia Scholaris*, while *Ficus Benghalensis* showed a comparatively lower activity index value.

In *Proteus vulgaris, Alstonia Scholaris* and *Ficus Krishnae* extracts showed the highest activity index values, followed by *Ficus Benghalensis* and *Calotropis Procera*.

In *Candida tropicalis, Ficus Krishnae* extract showed the highest activity index, followed by *Saccharomyces cerevisiae*, while *Ficus Benghalensis* and *Calotropis Procera* showed comparatively lower activity index values.

In *Saccharomyces cerevisiae, Ficus Krishnae* and *Ficus Benghalensis* extracts showed higher activity index values, while *Calotropis Procera* and *Alstonia Scholaris* showed comparatively lower activity index values.

Table 7: Activity index determination against microorganisms by chloroform extract of leaves of *Ficus krishnae, Ficus benghalensis, Calotropis procera, Alstonia scholaris.*

Test microorganisms	**Activity index**			
	Ficus benghalensis	***Ficus krishnae***	***Calotropis procera***	***Alstonia scholaris***
Escherichia coli	0.46±0.03	0.46±0.07	0.54±0.05	0.44±0.05
Bacillus subtilis	0.55±0.06	0.57±0.04	0.51±0.02	0.43±0.05
Bacillus coagulans	0.89±0.12	0.90±0.08	0.81±0.13	0.95±0.08
Pseudomonas aeruginosa	0.70±0.06	0.61±0.08	0.55±0.01	0.75±0.09
Staphylococcus aureus	0.51±0.07	0.66±0.05	0.54±0.05	0.55±0.05
Proteus vulgaris	0.76±0.06	0.79±0.05	0.66±0.05	0.84±0.12
Candida tropicalis	0.80±0.02	0.83±0.14	0.84±0.20	0.81±0.17
Saccharomyces cerevisiae	0.57±0.02	0.55±0.05	0.54±0.07	0.51±0.07

Values represent mean ±SD(n=3).

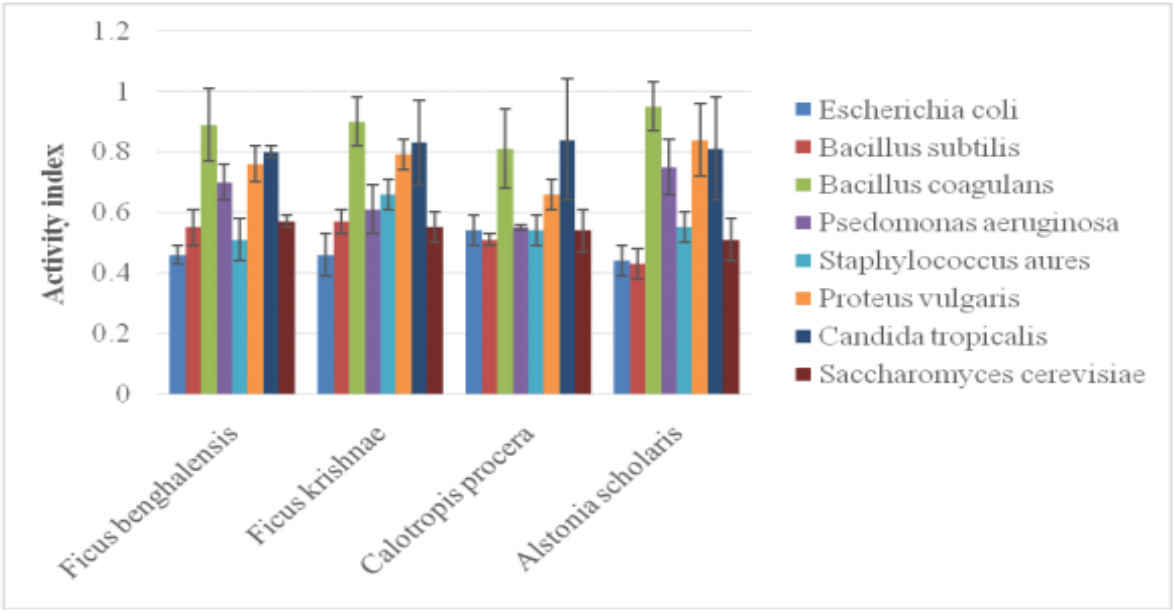
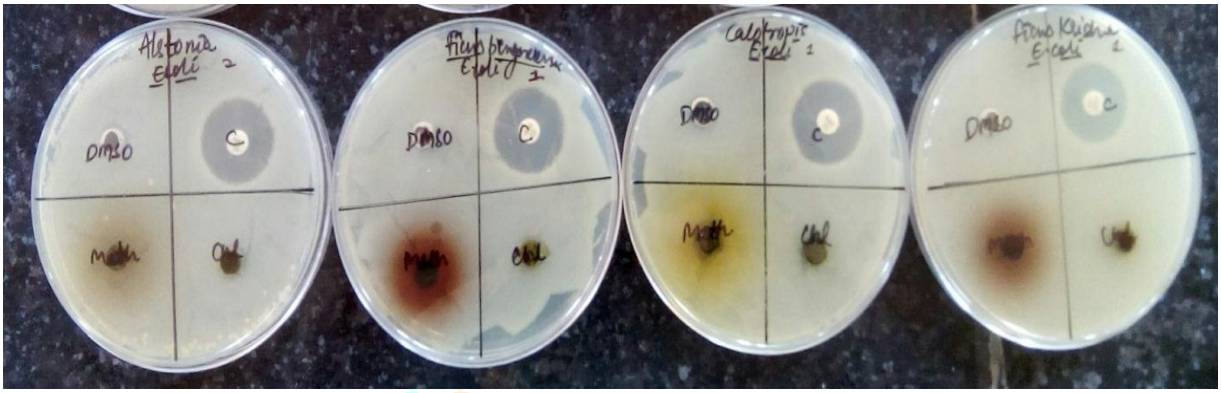


Figure 6: Activity index determination against *Escherichia coli, Bacillus subtilis, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Candida trpoicalis and Saccharomyces cerevisiae*by Chloroform extract of leaves of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris.*

Usually, medicinal plants contain several phytochemical compounds, which are very necessary to control the growth of microorganisms. The findings observed that the screening of the leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* for phytochemicals showed the presence of phyto-constituents such as saponins, tannins, flavonoids, alkaloids, steroids, phytosterols etc. in them. In the present study, it was also observed that *Staphylococcus aureus, Bacillus coagulans, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, Saccharomyces cerevisiae* whereas *Bacillus subtilis, Candida trpoicalis* was least sensitive towards methanol extract. Among these extracts, was most sensitive towards the chloroform extract *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vuglaris, and Saccharomyces cerevisiae* whereas *Escherichia coli, Bacillus subtilis, Candida tropicalis* was least sensitive towards chloroform extract. Among these extracts, Methanol extracts have shown better inhibition against all the test microorganisms and the inhibitory effect was comparatively less as compared to standards (Figure 5, 6). In the present study, all the solvents controls (Methanol and chloroform) used for extraction showed no inhibitory activity towards all the microorganisms. Although the concentration of standard antibiotics was high as compared to concentration of different extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia scholar's* leaves used in the present study. So, there is a possibility that these extracts can act as better antimicrobial agents as compared to antibiotics. They can be used to further isolate the specific compounds from it. The antimicrobial activities of the leaf extracts might be contributed by the presence of these photochemical. The results showed that methanol extract contained high intensity of alkaloids, tannins, and steroids but flavanoids, saponins and phytosterols were present in moderate intensity had been reported that aqueous and methanolic extracts from plants used in

allopathic medicines were potential sources of antiviral, antitumor and antimicrobial agents. It had also been demonstrated that the antimicrobial activity of the plant is mainly due to the presence of essential oils, flavonoids, terpenoids, tannins, alkaloids, saponins and other natural polyphenolic compounds or free hydroxyl groups in plant extracts.

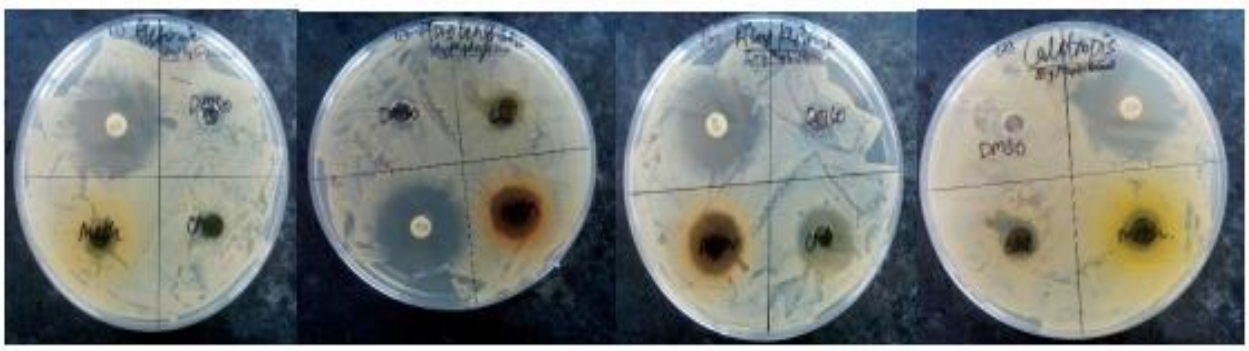


A B C D

Figure 7; Zone of inhibition *of Escherichiacoli* (A) *Alstonia scholaris* (B) *Ficus Benghalensis* (C) *Calotropis procera* (D) *Ficus krishnae*.

A B C D

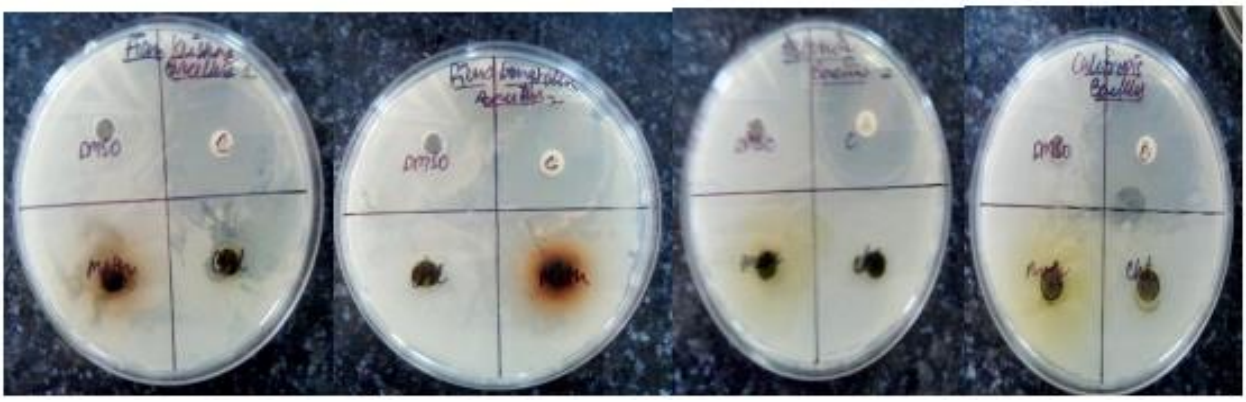
Figure 8; Zone of inhibition of Bacillus coagulans (A)Ficus Benghalensis (B)Alstonia scholaris (c)Ficus Krishnae (d) Calotropis Procera.



A B C D

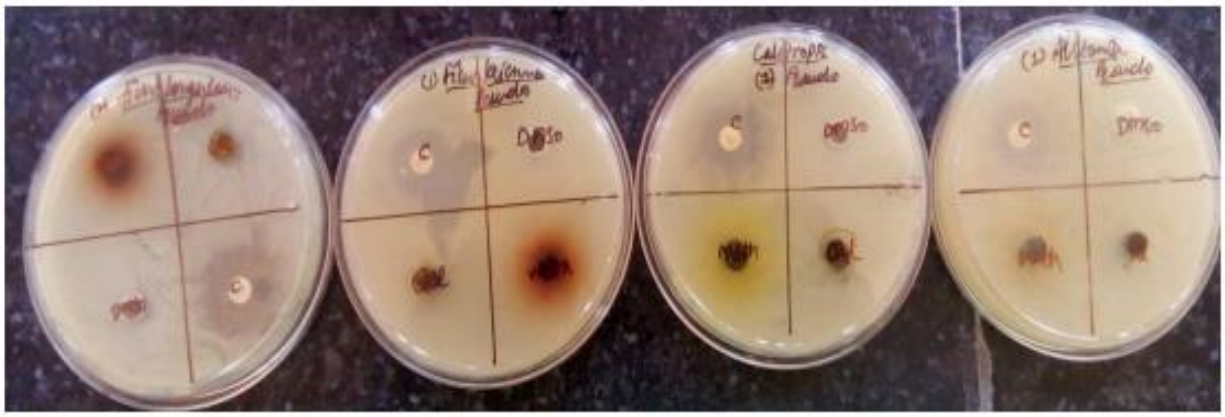
Figure 9; Zone of inhibition of Staphylococcus aureus (A)Ficus Benghalensis (B)Alstonia

scholaris (C)Ficus Krishnae (D)Calotropis Procera



A B C D

Figure 10; Zone of inhibition of Bacillus subtilis (A) Ficus Benghalensis (B) Alstonia Scholaris (C) Calotropis Procera (D)Ficus Krishnae

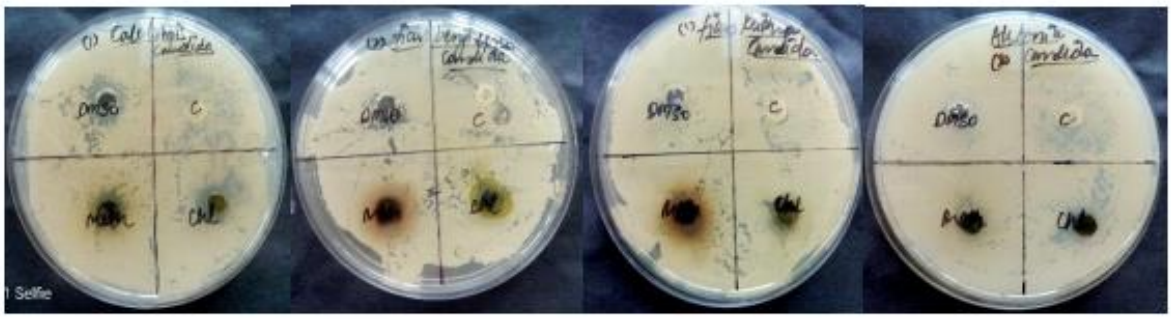


A B C D

Figure 11; Zone of inhibition of Pseudomonas aeruginosa (A) Ficus Benghalensis (B)Ficus krishnae (C)Calotropis Procera (D)Alstonia Scholaris.

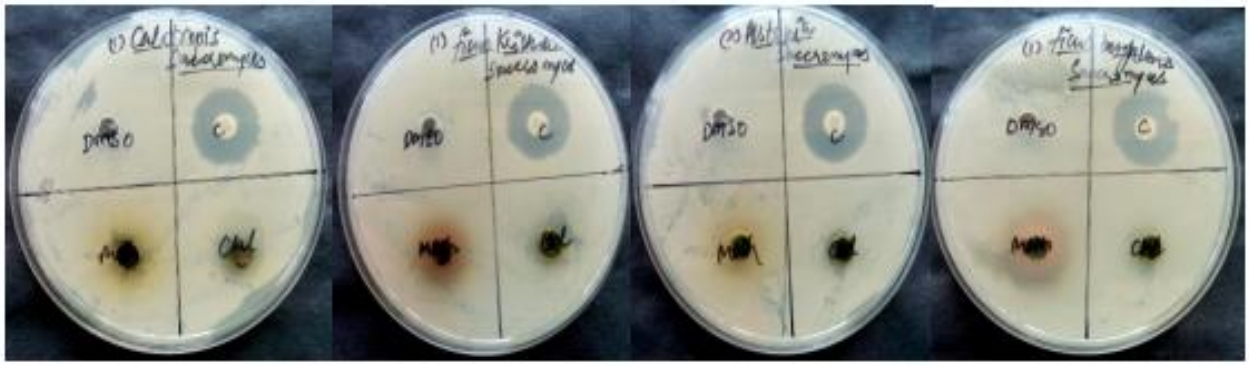
A B C D

Figure 12; Zone of inhibition of Proteus vulgaris (A)Alstonia Scholaris (B)Ficus Krishnae (C) Calotropis Procera (D) Ficus Benghalensis.



A B C D

Figure 12; Zone of inhibition of Candida tropicals (A) Calotropis Procera (B) Ficus benghalensis (C) Ficus Krishnae (D) Alstonia Scholaris



A B C D

Figure 13; Zone of inhibition of Saccharomyces cerevisiae (A) Calotropis Procera (B) Ficus krishnae (C) Alstonia Scholaris (D) Ficus Benghalensis

The extraction was done by using different solvents as Methanol and Chloroform from the leaves of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia scholaris* producing Crude leaf extract in methanol and chloroform further dissolved in DMSO (Dimethyl sulphoxide), These extracts were then analyzed for various following as

a) Antimicrobial Activity Crude leaf extract in Methanol and chloroform showed maximum inhibitory activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Bacillus coagulans, Proteus vulgaris.* Although crude leaf extract in methanol was effective against all the test microorganisms used in the study but showed less activity as compared to chloroform extracts. All the extracts were effective against *Proteus vulgaris* and *Pseudomonas aeruginosa*. Percent inhibition was greater in *Pseudomonas aeruginosa* and *Proteus vulgaris* by crude extract in methanol but crude extract in chloroform extract showed better percent inhibition against *Bacillus coagulans and Proteus vulgaris.* In the case of *Candida tropicals* crude extract in methanol and chloroform showed the lowest inhibitory effect.

b) Phytochemical constituents Test Phytocomponents such as alkaloids, tannins, and steroids were present in high amounts in crude extract in methanol. Crude extract in chloroform showed the presence of flavonoids, tannins, and sterols in moderate amounts. Other phytocomponents including phlobatannins, carbohydrates were found in trace amounts in all the extracts. The data presenting indicates that all the leaf extracts of *Ficus Krishnae, Ficus benghalensis, Calotropis Procera, Alstonia Scholaris* in methanol and chloroform confirmed the presence of phyto-constituents such as saponins, alkaloids, tannin, phlobatannins, flavonoid, steroids, phytosterols etc. in high amount which are known to exhibit medicinal as well as physiological activities. Methanolic leaves extracts of *Ficus krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* showed good antibacterial capability and can be used in many herbal formulations to ease human and animal ailments. Moderate anti-fungal activity was recorded against *Saccharomyces cerevisiae* in methanol and chloroform extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia Scholaris* The study indicated that methanolic extract was found to be better antimicrobial agent as compared to other extracts tested. Even chloroform also showed antimicrobial activity. But their efficiency was lower than methanol. The less antimicrobial activity has shown by leaf extracts of *Ficus Krishnae, Ficus Benghalensis, Calotropis Procera, Alstonia scholaris* can be due to absence of biochemicals with antibiotic properties or less concentration of antibacterial constituents which is not enough to make it effective. Although all the extracts have shown activity against microorganisms tested, which indicates that these extracts contain biologically active ingredients, still further studies need to be undertaken to establish the efficacy of leaves extracts of *Ficus Krishnae, Ficus benghalensis, Calotropis Procera, Alstonia Scholaris* as antimicrobial agents.

Conclusion

In conclusion, the use of plants for their medicinal and therapeutic benefits has been a common practice for thousands of years. This is due to their potential to produce a diverse range of bioactive compounds such as alkaloids, flavonoids, and tannins, which have shown to possess antimicrobial, antioxidant, anti-inflammatory, and anti-cancer properties. Various extraction techniques and solvents such as methanol and chloroform have been employed to extract these plant-based bioactive compounds. The efficacy of the crude leaf extract in methanol and chloroform against different microorganisms varies and is dependent on the type of organism and the extraction method used.

5. References

Ahirrao, R. (2020). Effect Of Aqueous Extract On Total Phenolic Content and Antimitotic Activity Of Ficus Benghalensis Root On Allium Cepa Root Meristamatic Cells. ABEB, 4(4). https://doi.org/10.33552/abeb.2020.04.000593

Antherden LM (1969). Textbook of Pharmaceutical Chemistry, 8th edn. Oxford University Press, London pp. 813-814.

A.J. Harborne. (1998). Phytochemical Methods A Guide to Modern Techniques of Plant Analysis Springer Dordrecht. 3, XIV, 302.

Barry Halliwell. (1994). Free Radicals and Antioxidants: a Personal View. Nutrition Reviews, 52, 253–265.

Bhuvaneswari, M. (2017). Untitled. IJGP, 2(11). https://doi.org/10.22377/ijgp.v11i02.925

BABY JOSEPH AND S. JUSTIN RAJ. (2010). PHYTOPHARMACOLOGICAL AND PHYTOCHEMICAL PROPERTIES OF THREE FICUS SPECIES - AN OVERVIEW. IJPBS, 1, 246-253.

Biswas, K. (1934). Some Foreign Weeds and their Distribution in India and Burma. Current Science, 2(11), 422–425.

Chin, YW., Balunas, M.J., Chai, H.B. *et al. (2006).* Drug discovery from natural sources. *AAPS J* 8, E239–E253.

Del-Rio A, Obdululio BG, Casfillo J, Marin FG,Ortuno A (1997). Uses and Properties of citrus flavonoids. J. Agric Food Chem. 45:4505-4515.

Duraipandiyan, V., Ayyanar, M. & Ignacimuthu, S. (2006) Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern Med* 6, 35.

Esitken a., Karlidag h., Ercisli s., (2003). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (Prunus armeniaca L. cv. Hacihaliloglu). Aust. J. Agric. Res. 54, no.4,377-380.

Edeoga HO, Okwu DE, Mbacbie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7): 685-688.

Epand, R. F., Savage, P. B., & Epand, R. M. (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochimica et biophysica acta, 1768(10), 2500–2509.

Gibbons, S. (2005) Plants as a Source of Bacterial Resistance Modulators and Anti-Infective Agents. Phytochem Rev 4, 63–78.

Haslam, E., & Cai, Y. (1994). Plant polyphenols (vegetable tannins): gallic acid metabolism. *Natural product reports*, *11*(1), 41–66.

J. Azmir et al. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. J. Food Eng., 117, Issue 4, 426-436.

Jain SC, Jain R, Vlietinck AJ (2004). *In vivo* and *in vitro* antimicrobial efficacy of *Mimosa hamata* Ind. J.Biotechnol., 3: 271-273.

KS Nagarsekar, MS Nagarsenker, SR Kulkarni. (2010). Evaluation of Composition and Antimicrobial Activity of Supercritical Fluid Extract of Leaves of Vitex negundo. Indian J. Pharm. Sci. 72 (5): 641-643.

Kuete, V., Nana, F., Ngameni, B., Mbaveng, A. T., Keumedjio, F., & Ngadjui, B. T. (2009). Antimicrobial activity of the crude extract, fractions and compounds from stem bark of Ficus ovata (Moraceae). J. Ethnopharmacol., 124(3), 556–561.

Kumar Ashok BS, Ranganayakulu D., Nandeesh R. et al. (2010). Comparative in vitro anthelmintic activity of three plants from the amaranthaceae family. Arch. Biol. Sci., Belgrade, 62, 185-189.

Khare, S., Singh, N.B., Singh, A. *et al.* (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. *J. Plant Biol.* 63, 203–216.

Kanjikar, A., Londonkar, R. (2017). Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity Of Stem Bark Of Ficus Krishnae. Phyto, 5(9). https://doi.org/10.25258/phyto.v9i5.8156

Kumaresan, S., Ramasamy, R., Jayachandran, P. (2018). Antioxidant and Cytotoxic Activity Of Combined Extracts Prepared Using Ficus Religiosa And Ficus Benghalensis Leaves Against Cervical Cancer Cell Line (Hela). Asian J Pharm Clin Res, 12(11), 407. https://doi.org/10.22159/ajpcr.2018.v11i12.26948

Kanjikar, A., Londonkar, R. (2017). Gc-ms Analysis Of Bioactive Compounds In Petroleum Ether Extract Of Ficus Krishnae. Int. Res. J. Pharm., 10(8), 178-181. https://doi.org/10.7897/2230-8407.0810204

Mahalakshmi R et al. (2010). Hepatoprotective activity on Vitex negundo Linn. (verbanaceae) by using wistar albino rats in ibuprofen induced model. *International journal of pharmacology*. 6-5, 658-663.

Mahesh Mysore Shivananjappa & Manoj Kumar Joshi. (2012). Influence of Emblica officinalis aqueous extract on growth and antioxidant defense system of human hepatoma cell line (HepG2), Pharmaceutical Biology, 50:4, 497-505.

Marjorie Murphy Cowan, (1999). Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 12, 564-582.

Merrett, G.L. (2013). Tackling Antibiotic Resistance for Greater Global Health Security. GHSA

Marjorie Murphy Cowan, (1999). Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 12, 564-582.

Nurhayati, L., Yahdiyani, N., Hidayatulloh, A. (2020). Perbandingan Pengujian Aktivitas Antibakteri Starter Yogurt Dengan Metode Difusi Sumuran Dan Metode Difusi Cakram. jthp, 2(1), 41. https://doi.org/10.24198/jthp.v1i2.27537

Nascimento, Gislene G. F. et al. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol. 31,247-256.

Prastiyanto, M., Wardoyo, F., Wilson, W., Darmawati, S. (2020). Antibacterial Activity Of Various Extracts Of Averrhoa Bilimbi Against Multidrug Resistant Bacteria. J Bio Bio Edu, 2(12), 163-168. https://doi.org/10.15294/biosaintifika.v12i2.23600

Pandey, A.K., & Verma, N. (2013). A Study of Solvent Optimization and Phytochemical Analysis for Antibacterial Activity of Nyctanthus arbortristis and Ficus bengalensis. Int. j. pharm. chem. sci. 2.

Pandey A, Verma N. (2013) Evaluation of antibacterial activity of *Euphorbia hirta* and *Calotropisprocera* against MDR pathogens. Int J Plant Anim Environ Sci ;3(3):17-24.

Prafulla D. Patil, Shuguang Deng. (2009). Optimization of bio diesel production from edible and non-edible vegetable oils, Fuel, 88,1302-1306.

Panday, D.R., Rauniar, G.P. (2016). Effect of root-extracts of *Ficus Benghalensis* (Banyan) in memory, anxiety, muscle co-ordination and seizure in animal models. *BMC Complement Altern Med* 16, 429.

Pourmorad F, Hosseinimehr SJ, Shanabi majd N (2006). Antioxidant activity, Phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 5(11): 1142-1145.

Pandya, D.J., Joshi, M.K., & Gorwadiya, H.C. (2012). PHYTOPHARMACOGNOSTICAL STUDY ON MAKKHAN KATORI: *Ficus Krishnae*. International journal of biomedical research, 3, 427-430.

Ranjit, Pusapati. (2012). AN OVERVIEW OF PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF *Calotropis Procera*. FS J Pharm Res. 1. 18-25.

Raj Kumar Singh, Pradeep Kumar Mittal, Gaurav Kumar, Ramesh Chandra Dhiman. (2014). Evaluation of mosquito larvicidal efficacy of leaf extract of a cactus plant, Agave sisalana. J Entomol Zool Stud, 2(1), 83-86.

Radhakrishnan, A., Venkatachalam, S. (2020). A Holistic Approach For Microwave Assisted Solvent Extraction Of Phenolic Compounds From ficus Benghalensis Fruits and Its Phytochemical Profiling. J Food Process Eng, 11(43). https://doi.org/10.1111/jfpe.13536

Shahzad, A. (2016). Analysis Of Antimicrobial Potential Of Some Ficus Taxa From District Bhimber Azad Jammu and Kashmir, Pakistan. Appl Ecol Env Res, 5(14), 159-176. https://doi.org/10.15666/aeer/1405_159176

Shriwaikar A., A. Shirwaikar, R. Kuppusamy and I.S.R. Punitha, (2006), In-vitro Antioxidant Studies on the Benzyl Tetra Isoquinoline Alkaloid Berberine, Biol. Pharm. Bull., 29(9), pp. 1906-1910.

Stary F. (1998). The Natural Guide to Medicinal Herbs and Plants. Tiger Books International, London p. 12-16.

Sodipo, O. A., Awanji, M. A., Kolawole, F. B. and Oduntuga, A. A. 1991. Saponin is the active fungal principle in Garcinia kola, Hekle seed. BioSci.Res.Commun., 3:171.Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, Evans C (1995). Polyphenolic flavonoils as seavenger of aqueous phase radicals as chain breaking antioxidant. Arch Biochem. Broph. 2: 339- 346.

Tadavi, S., Dafare, S., Zanje, S., Rathod, Y., Gadpayale, J. (2023). Preliminary Phytochemical Analysis of Ficus Johanis Subsp. Afghanistanica From Moraceae Family. J Pharmacogn Phytochem, 1(12), 41-43. https://doi.org/10.22271/phyto.2023.v12.i1a.14546

Umapathy, S., Umapathy, S., Sudhakar, J., Ramalingam, S. (2021). Systematic Reinstatement Of Highly Sacred Ficus Krishnae Based On Differences In Morphology and Dna Barcoding From Ficus Benghalensis (Moraceae). PK, (186), 121-138. https://doi.org/10.3897/phytokeys.186.74086

Unnikrishnan, M.K., Rao, M.N.A. (1990). Anti-inflammatory activity of methionine, methionine sulfoxide and methionine sulfone. *Agents and Actions*. 31, 110–112.

Vikas V Patil & Vijay R Pati. (2010). EVALUATION OF ANTI-PYRETIC POTENTIAL OF FICUS CARICA LEAVES. IJNPR. 2, 151-155.

Verma, V.C., Singh, S.K. and Prakash, S. (2011), Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss.. J. Basic Microbiol., 51: 550-556.

Zuraida (2019). Phytocemical Compounds Of Different Species Of Alstonia Sp. From Palembang- South Sumatera. IOP Conf. Ser.: Earth Environ. Sci., 1(308), 012027. https://doi.org/10.1088/1755-1315/308/1/012027