Evaluation of Phytochemical Analysis and Antibacterial Activities of *Acacia auriculiformis* A. Cunn. ex Benth.

By

Nithya. M

Reg.No. 20PBO012

A Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the

Degree of Master of Science in Botany

MAY 2022

Evaluation of Phytochemical Analysis and Antibacterial Activities of

***Acacia auriculiformis* A. Cunn. ex Benth.**

By

Nithya. M

Reg.No. 20PBO012

A Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the

Degree of Master of Science in Botany

MAY 2022

Signature of the Head of the Department

Signature of the Supervisor

ACKNOWLWDGEMENT

ACKNOWLEDGEMENT

First and foremost, I wish to thank **God Almighty** for endorsing the investigator with immense blessings which helped to overcome the hurdles, paving way for the successful completion of the study.

I express my profound thanks to **Dr. T.S.Avinashilingam** ,the Founder and First Chancellor, **Dr.Rajammal P.Devadas**, Avinashilingam institute for Home science and Higher Education for women, Coimbatore, for providing the opportunity to undertake the present research programme.

I grateful acknowledge **Honourable Dr. T. S. K. Meenakshisundaram** Avl, Managing trustee, and **Prof. S.P. Thyagarajan, M.Sc., Ph.D., M.D**. Chancellor, for providing all necessary amenities for the completion of my work.

I also extent my sincere thanks to Vice Chancellor **Dr. (Mrs.) V. Bharathi Harishankar Vice Chancellor** for providing the needed facilities during the study period.

I wish to extend my thanks to **Dr. (Mrs.) S. Kowsalya, M.Sc., M.Phil., Ph.D**. Registrar for providing the academic needs during the study period.

My Sincere thanks to **Dr. (Mrs). A. Vijayalakshmi, M.Sc., M.Phil., Ph.D.,** Professor,,Dean and Head Department of Botany, Avinashilingam institute for Home science and Higher Education for women, Coimbatore, for her encouragement towards the successful completion of the study.

My profound and heartfelt thanks to my guide **Dr. (Mrs.) G. Sakthivigneswari,M.Sc., M.phil., Ph.D,** Assistant professor, Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her encouragement, constant support and guidance towards the successfully completion of the study.

I wish to thank my **Parents and to My Relatives** for their constant prayers and moral support and motivation and without their support; I would not have been able to reach this height.

I also offer my sincere and deep thank to my friends **T. Joys Ememmal, Shanthni. J**, **A.P. Allen Princy** and **all my classmates** for the endless help, discussion and suggestion to perform the work. I would like to thank all lab assistant at Department of Botany for continuous help.

M. Nithya

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

INTRODUCTION

CHAPTER-I

INTRODUCTION

We are well aware of the value of plants and their substantial contribution to human health. The plant kingdom is a treasure trove of potential medications, and there has been a growing understanding of the relevance of medicinal plants in recent years. Medicinal plants, according to the World Health Organization (WHO), are the best source of a wide range of medications. Traditional medicines, which contain substances derived from medicinal plants, are used by almost 80% of people in developed countries.

The chemicals rich in nutrients present in the plants are biologically active and are termed phytochemicals. Currently, phytochemicals are becoming more popular due to their innumerable medicinal uses. These phytochemicals are present in several parts of the plants, such as leaves, flowers, seeds, barks, roots and pulps (Banu and Cathrine, 2015). World Health Organization (WHO) reported that medicinal plants are a significant source of obtaining various drugs. About four-fifths of the individuals from developed countries use traditional medicines that have compounds derived from medicinal plants. Medicinal plants must be investigated better to understand their property, safety and efficiency.

Medicinal plants have some organic compounds, including tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids, which provide various physiological effects on the human body. Those compounds are synthesized either by primary or secondary metabolism of the particular living organisms. Secondary metabolites are extremely diverse compounds with a variety of functions which can be used for various therapies for humans, animals, plants and research investigation (Yadav and Agarwala .,2011).

Medicinal plants are the best source of those agents rich in antimicrobial properties. Life-saving drugs have been a part of the evolution of humans and healthcare. In this century, 88% of humans concentrate on plant-derived medicines as their primary defence for maintaining health. About 119 secondary metabolites derived from plants are used as drugs (Vinoth *et al.*, 2012).

The term "phytochemicals" refers to plant components studied for their potential health benefits but are not yet formally designated as essential nutrients. Plants generate phytochemicals to help them resist diseases from fungi, bacteria and plant viruses and be consumed by insects and other animals.

Compounds considered necessary nutrients, which are naturally found in plants and required for proper physiological processes, must be received from the diet in humans and fall into the phytochemical group. Humans are poisoned by some phytochemicals, which are known as phytotoxins. Primary metabolite is a type that plays a direct role in normal development, growth and reproduction. It usually serves a physiological purpose in the body. It is also known as a central metabolite, which is even more confined like ethanol, lactic acid, and core amino acids.

Plant compounds are classified as secondary metabolites since the plants that make them only need a small amount to carry out their metabolic functions. Plants have shaped the foundations of elegant traditional medicinal techniques that have been practiced for thousands of years in China, India, and other nations (Nandagoapalan *et al.,* 2016).

Secondary metabolites are composites that are not required for a cell's survival but have a part in its interaction with its surroundings. To protect themselves, plants use these chemicals from biotic and abiotic stressors. Secondary metabolites are members of several metabolite families that can be induced in large amounts in response to stress. Primary metabolites play an essential role in metabolism by contributing to feeding and reproduction. Many secondary metabolites are employed as primary compounds in pharmaceuticals, flavours, fragrances, insecticides, and dyes and consequently have a high economic value (Pagare *et al*., 2015).

Recently, pharmaceutical companies have developed several novel antibiotics in the last three decades, microorganism resistance to these medications has increased. In general, bacteria have the genetic potential to transfer and acquire drug resistance, which is used as a therapeutic agent. Microbial resistance is becoming more of an issue and the future usage of antimicrobial treatments is still questionable. As a result, steps must be made to mitigate the problem, such as limiting antibiotic use, conducting research to better understand the genetic causes of resistance and continuing research to discover new antibiotics, both synthetic and natural.

Plant extracts and phytochemicals, both of which have been shown to have antibacterial characteristics, can be very useful in medicinal treatments. Several plants have been employed for their antibacterial properties related to chemicals produced in the plant's secondary metabolism. These goods are identified by their active ingredients, such as the phenolic compounds found in essential oils and tannin. (Nascimento *et al*.,2000).

Flavonoids, alkaloids, tannins and terpenoids are phytochemicals found in medicinal plants with antibacterial and antioxidant activities. Some plant species have been studied extensively for their antibacterial properties. The most significant feature of medical textiles is antibacterial activity, which ensures adequate protection against germs, biological fluids, aerosols and disease transmission. For example, Cinnamon, garlic, basil, curry, ginger, sage, mustard, and other plant crude extracts have antibacterial effects against a broad spectrum of Gram-positive and Gram-negative bacteria.

Staphylococcus aureus is a bacterial species. It is a non-moving tiny round-shaped or non-motile cocci that stain Gram-positive. *Staphylococcus* is the name given to it because of this. Bacterial cells of the bacteria (*Staphylococcus aureus*), one of the mastitis-causing pathogens in dairy cows.

Escherichia coli is a non sporulating coliform bacteria that are Gram-negative and facultatively anaerobe. Rod-shaped cells measure around 2.0 m long and 0.25–1.0 m in diameter, with a cell volume of 0.6–0.7m. Antibiotics can effectively treat *E. coli* infections outside the digestive tract and most intestinal infections. However, they are not used to treat infections caused by a single strain of these bacteria in the intestine. The flagella that allow bacteria to swim are arranged in a peritrichous pattern. It also adheres to the microvilli of the intestines and effaces them using an adhesion protein called intimin. Most *E. coli* that lives in the gut do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, newborn meningitis, hemorrhagic colitis and Crohn's disease. Severe stomach cramps, diarrhoea, hemorrhagic colitis, vomiting and fever are common symptoms.

The primary intention of this analysis was to highlight the updated pharmacological, phytochemical and antibacterial investigations of *Acacia auriculiformis*

PLATE-1

HABIT- *Acacia auriculiformis* A. Cunn ex Benth.

Acacia auriculiformis A. Cunn. ex Benth, a member of the Fabaceae family, is a straight, medium-sized, deciduous or evergreen tree that may grow up to 30 metres tall and is commonly found along India's roadsides and parks. The generic name Acacia is attained from the Greek word 'akis,' which signifies a spike or a point. In contrast, the Latin word 'auricula' refers to a creature's external ear, while the word 'form' refers to a frame, figure, or shape. The tree is an Australian native introduced initially to India in West Bengal in 1946. Glucuronic acid, methyl glucuronic acid, arabinose, rhamnose, and galactose are abundant in the tree. Plants have been documented to have antioxidant, antimicrobial, antimalarial, antifilarial, and antimutagenic pharmacological properties.

The flowering and fruiting seasons of *A. auriculiformis* differ by area; for example, in Australia, the flowering season is from April to July, and ripened seeds are accessible 4-5 months later, from August to October. Flowering occurs in Malaysia from February to May, while ripened fruit seeds can be gathered from October to April. The flowering season of *A. auriculiformis* occurs in India from December to January, with fruiting occurring from February to March. Flowering and fruiting occur in some parts of India from March to December, but more so in September and October. *A. auriculiformis* bark, leaves, and fruits (pods with seeds and funicles) are all used in various biological processes.

*Acacia auriculiformis*is native to Australia, Indonesia, and Papua New Guinea and prefers terrestrial habitat. Except for Jammu and Kashmir, Sikkim, and Arunachal Pradesh, it is scattered throughout India (Rangra *et al.*, 2019).

Acacia auriculiformis is one of the most common tree species utilized in social forestry in the tropics and subtropics, alongside eucalyptus, casuarina, and leucine. Acacia's appeal has been boosted by its high survival rate, rapid growth rate, and very deep canopy achieved by the trees after a few years of planting. *Acacia* trees are also considerably better at growing in poor soils than most trees. As a result *Acacia* trees are widely planted across the tropics and subtropics (Ganesh *et al*.,2009).

OBJECTIVES

The review's major goal was to highlight the most recent pharmacological, phytochemical and antibacterial activities of leaf extract of *Acacia auriculiformis* A.Cunn. ex Benth. The present study focused with the following objectives

1) To collect the *Acacia auriculiformis* A.Cunn. ex Benth. leaves and shade dried
2) To prepare the selected leaf extracts using aqueous, ethanol and methanol extracts
3) To investigate selected pharmacognostic and preliminary phytochemical characteristics of *Acacia auriculiformis* leaf samples.
4) To investigate the antibacterial activity of selected plant leaf extracts against microbes such as *Escherchia.coli* and *Staphylococcus aureus*.

REVIEW OF LITERATURE

CHAPTER -II

REVIEW OF LITERATURE

The available literature pertaining to the medicinal, antibacterial and antioxidant properties of plant extracts as relevant to present investigation is presented in this chapter.

This chapter reviews the literature on the selected plant medicinal properties, phytochemical analysis and antibacterial properties Herbal pharmaceuticals are gaining popularity as a source of new antibacterial drugs, and they are thought to be harmless for both humans and the environment.

The antibacterial activity of various extracts of *Bauhinia variegata* L. at three concentrations was reported by Parekh *et al*.(2006). They stated that defeated extracts showed better activity than those without defeating and that the polarity of the solvent appears to play an important role in exhibiting potential antibacterial activity

Bhuvaneshwari *et al*. (2007) investigated the phytochemical composition of *Nerium Indicum* and discovered the presence of alkaloids, terpenoids, cardiac glycosides, saponins, tannins and carbohydrates in all solvents. They tested the antibacterial activity of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhimurium* found that they had a better zone of inhibition against the selected microbes.

Yisa (2009) studied the phytochemical analysis and antimicrobial activity of *Scoparia Dulcis* and *Nymphaea lotus* and discovered that *Scoparia dulcis* had bioactive compounds such as saponins, glycosides and polysaccharides. In contrast, *Nymphaea lotus* contained saponins, tannins and alkaloids. They revealed that ethanolic and aqueous extracts of *Nymphaea lotus* and *Scoparia dulcis* possess antibacterial activity

Devi *et al.* (2009) tested the antibacterial activity of the essential oil of *Clausena anisate* leaf against five Gram-positive and six Gram-negative bacteria isolated from clinical samples. They discovered that essential oil has substantial antibacterial action against all pathogens tested, with the lowest inhibitory concentration (M.I.C.) values for *Salmonella typhimurium* and *Pseudomonas aeruginosa*

Arokiyaraj *et al*. (2009) investigated the leaves of *Vitex agnus-castus* for *in vitro* antibacterial activity using hexane, ethyl acetate, methanol and an aqueous medium. They found that active ethyl acetate had the highest antibacterial activity. Terpenoids, steroids, flavonoids and carbohydrates were found in their phytochemical analysis.

Roy *et al*. (2010) compared the antimicrobial activity of two samples of *Andrographis paniculata* extracts obtained by extraction in chloroform and HCl respectively. They found that Chloroform was a suitable organic solvent to extract the active compounds responsible for *A. paniculata* antimicrobial activity

Viji *et al.* (2010) found a wide range of secondary metabolites in the leaf and stem of *Cardiospermum helicacabum*. According to their antibacterial activity data, acetone and chloroform extracts of the leaf demonstrated more decisive inhibitory action against *Salmonella typhi* and *Streptococcus subtilis*. They noticed that acetone extracts of the stem had the most potent inhibitory effect against *S. typhi.* In contrast, benzene extracts of the stem had a moderate inhibitory effect against *Escherichia coli.*

Ibrahim *et al*. (2010) found phlorotannins, carbohydrates, tannins, glycosides, volatile oils, resins, balsams, flavonoids, and saponins in *Commelinabenghalensis L*., but no terpenes, sterols, phenols, or anthraquinones. They concluded that the existence of some of these secondary metabolites implies that the plant could have therapeutic value and supports some ethnic uses.

Gulfraz *et al*. (2011) underwent a Phytochemical investigation of *Eruca sativa* seeds and found that seeds included several nutrients aspects which is necessary for food or feed purposes. They discovered that it is a suitable source of feed supplement because it contains crude protein (29.58 per cent). They reported on the antibacterial activity of several *Eruca sativa* test samples of Seed oil inhibited the growth of all antibiotic-resistant bacteria to the greatest extent possible.

Pandey *et al*. (2011) investigated the antimicrobial activity of ethanolic, methanolic, ethyl acetate and hot water extracts of *Citrus Limon fruit* parts such as peels and seeds. They found that methanolic extract of lemon peels had the highest zone of inhibition against *Pseudomonas aeruginosa*, while hot water extract of lemon peels showed the zone of inhibition. They also discovered that the largest zone of inhibition against *Pseudomonas* *aeruginosa* was the ethanolic extract of lemon seeds, while the most miniature zone of inhibition was hot water extract

Doss *et al*. (2011) observed the antibacterial activity of successive solvent extracts of *Medicago sativa* (petroleum ether, Chloroform, benzene, methanol, ethanol and water extracts of *Medicago sativa*) against seven significant bacterial strains. They discovered that methanol extract showed significant activity against all the tested bacteria, followed by chloroform and ethanol extract. Their research findings back up the usage of *Medicago sativa* as an ethnomedicine.

According to Arekemase *et al*. (2011), The extracts and latex of *Jatropha curcas* were found to have potent antimicrobial activity against *Staphylococcus aureus, Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus flavus*, with a minimum inhibitory concentration as low as 0.5 ml. These findings confirmed the plant's potency in treating human infections, including sexually transmitted diseases.

Phytochemical analyses, antibacterial activities and antifungal activities on *Couroupita guianensis* leaf were examined using water and methanol solvents by Kavitha *et al*. (2011). In the medicinal plant's leaf extracts, they discovered alkaloids, phenolics, flavonoids, saponins, and tannins. They observed that the methanol extract of *C. guianensis* had a greater spectrum of antibacterial and antifungal activity than the aqueous extract.

Yadav *and* Agarwala(2011) examined the phytochemical studies of different medicinal plants in various extracts. They discovered that the aqueous extracts of the plant produce positive results for flavonoid and phenolic components

The antibacterial and antifungal properties of *Cassia fistula* leaves were investigated by Gaushala *et al*.(2011). They stated that it demonstrates the presence of several phytochemicals with biological activity that may have therapeutic value and that the plant has similar components such as saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. They found that for all sensitive bacteria, the growth inhibition zone measured varied from 11 to 20 mm, and for fungal strains, it ranged from 14 to 20 mm.

Sumathy *et al*. (2011) analysed the phytochemicals found in *Musa acuminata* flowers. Phytochemical screening revealed the presence of active chemicals such as glycosides, tannins, saponins, phenols, steroids and flavonoids in the methanolic extract of *M. acuminate*

Sridhar *et al*. (2011) investigated the phytochemical analyses of *Wrightia tinctoria* using the solvents methanol and water. The plant contained steroids, alkaloids, carbohydrates, flavonoids, tannins & phenols, saponins, and proteins. They also tested antibacterial activity against six bacteria: three Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*, and *Leuconostoc lactis*) and three Gram-negative (*Staphylococcus aureus, Streptococcus pyogenes*, and *Leuconostoc lactis*) (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi)*. Other bacterium types such as *Staphylococcus aureus, Leuconostoc lactis*, *Salmonella typhi, Pseudomonas aeruginosa*, and *Streptococcus pyogenes* were inhibited by both aqueous and methanol extracts, apart from Escherichia coli, which was inhibited by methanol extract.

The Nutritional and Phytochemical Screening of *Garcinia kola* was examined by Adesuyi *et al.* (2012). They stated that *Garcinia* phytochemical composition indicates that it could be used in pharmaceutical and medical research to develop vaccines and supplements to prevent diseases. It can also be used as a raw material in various manufacturing flower industries.

Vinoth *et al*. (2012) investigated on leaf extract of *Azadirachta indica*. This plant extracts showed steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, flavonoids and tannin. They concluded that the leaf extract of *Azadirachta indica* possesses significant antibacterial activity against dental infections and various dieseases.

The phytochemical composition, antioxidative action and antibacterial activity of the methanolic leaf extract of locally accessible *Mentha piperita* was examined by Pramila *et al.* (2012). They discovered that the crude methanolic mint leaf extract contained tannins and flavonoids. The mint leaf methanolic extract exhibited significant antibacterial and antifungal activity against various bacteria and fungi.

Ethanol, methanol, Ethyl acetate, acetone, Chloroform, Petroleum ether, hexane, hot water, and extracts of *Withania somnifera* were tested for antibacterial activity and phytochemical screening by Velu and Baskaran (2012). Their research found that all of the extracts had antibacterial activity comparable to the standard against all of the species tested and positive phytochemical analysis results.

Deepti *et al*. (2012) assessed antimicrobial efficacy. They identified the phytoconstituents responsible for the biological activities of different solvent extracts of *Morinda tinctoria* leaves, finding that Methanol extract was more effective against all organisms, followed by Ethyl acetate, chloroform, and hexane extracts. They stated that their research on the antibacterial efficacy of *M. tinctoria* leaves using light is a valuable source for discovering antibacterial alternatives to currently available medications

Saranya et *al.(*2013) looked at the antibacterial activity of *Abutilon indicum* and *Phyllanthus niruri* against human pathogenic microorganisms. Their findings revealed the positive results of phenolics, saponins, and flavonoids, as well as that these species have good antagonistic activity against human pathogenic microorganisms

Manjula *et al.* (2013) investigated the phytochemical composition of *Clitoria ternatea* and discovered the presence of alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids and phenols. According to their studies, this plant possesses more bioactive compounds.

Deshpande *et al.* (2013) tested the antibacterial activity of ethanol and petroleum ether extracts of *Acacia nilotica* stem bark. They discovered that ethanol extract showed more significant activity against *Streptococcus mutans* than petroleum ether extract.

Design *et al*. (2014) examined the Phytochemical Analysis and Anti-microbial Activity of *Eupatorium glandulosum.* They revealed that phytochemical analysis of plant extracts revealed the presence of numerous components such as alkaloids, tannins, glycosides, saponins, carbohydrates, and phenolic compounds and that the dried leaf extract of *Eupatorium glandulosum* has antibacterial action. They found that plant extracts have antibacterial properties, with acetone extract having the highest activity.

Ganesh *et al.* (2014) investigated the antibacterial activity of pepper (*Piper nigrum L.)* against *Staphylococcus aureus, Salmonella typhi*, *Escherichia coli*, *Proteus sp*., and *Pseudomonas aeruginosa*. They found that the chloroform extract of *Piper nigrum* showed high antibacterial activity against *Escherichia coli* and *Salmonella typhi.*

In aqueous and ethanol extracts, Patel *et al*. (2014) investigated the antibacterial activity of *Moringa oleifera* leaf extracts against *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutans, Bacillus subtilis* and *Staphylococcus epidermidis* bacteria. They discovered that ethanol leaf extract had the highest activity against *Streptococcus mutants,* whereas aqueous extract had the highest activity against *Proteus Vulgaris*.

Ehiowemwenguan *et al*. (2014) tested the antibacterial activity of ethanolic and aqueous extracts in banana (*Musa sapientum)* peels on gram-positive and gram-negative bacteria and found that the ethanol extract inhibited growth more effectively than the water extracts. Their phytochemical investigation revealed that ethanol is a better solvent for extracting bioactive compounds such as glycosides, alkaloids, saponins, tannins, flavonoids, and volatile oil from banana peels.

Khodja *et al*. (2014) investigated antioxidant and antibacterial properties and polyphenol content in methanolic extracts of four Lamiaceae species (*Ajuga iva, Marrubium vulgare, Mentha pulegium* and *Teucrium polium)*. They found a significant positive association between the antioxidant activity of extracts and total phenolics and total flavonoids and an antibacterial impact of extracts against *Escherichia coli* and *Staphylococcus aureus.* According to their findings, *Escherichia coli* was more responsive to the extracts than *S. aureus,* and *M. vulgare* was the most effective extract.

According to Okereke *et al.* (2015) the extracts of *Hibiscus sabdariffa* demonstrated the presence of secondary plant metabolites in the form of phytochemicals, vitamins and critical mineral. Their research demonstrated that plant-derived medicines are safer than synthetic alternatives while providing significant therapeutic benefits.

Devi *et al*. (2015) investigated *Ocimum tenuiflorum* antimicrobial activity and phytochemical analysis. They discovered that leaves extract had approximately identical antibacterial action against Gram-positive and Gram-negative bacteria and that they obtained positive results on phytochemical compounds.

Kumari *et al.* (2016) investigated the phytochemical characteristics and antibacterial activity of fenugreek. They discovered that fenugreek seeds showed medicinal and therapeutic components in their phytochemical and antibacterial bioprocesses

Nandagopalan *et al*. (2016) studied that the phytochemical analysis of 25 different medicinal plants. They used the standard procedures to screen the plants, which revealed the presence of bioactive compounds. They reported that flavonoids were found in 19 of the selected 25 plant species, whereas alkaloids were found in 16 plant species. They showed that the existence of these phytochemicals can be linked to a plant's therapeutic potential.

Chekuri *et al.* (2016) investigated the antimicrobial activity of *Acalypha indica* leaf extract using microbes such as *Bacillus sp., E. coli, Pseudomonas sp* and *Streptococcus sp* They discovered that the methanol extract had the highest zone of inhibition against *E. coli*. *Acalypha Indica* leaf extract showed essential phytochemicals, antimicrobial and antioxidant properties.

Simon *et al*. (2016) investigated the antimicrobial activity of dried leaf extracts of *Annona squamosa* (*L*) against two-gramme harmful bacterial strains and two clinical fungal pathogens. They discovered that the leaf extracts of *Annona squamosa* had higher antibacterial activity than antifungal activity.

Pradeepa *et al*. (2016) investigated the qualitative and quantitative phytoconstituents, as well as the bactericidal effect of medicinally important *Pelargonium graveolens* leaf extracts and found that the ethanolic extract of the *P. graveolens* leaves holds promise as a potential source of pharmaceutically essential phytochemicals, as well as having vigorous antibacterial activity against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeroginosa* and *Klebsiella pneumoniae.*

The phytochemical and antibacterial activities of extracts of *Psidium guajava* leaves against specific clinical bacterial isolates were investigated using water, ethanol, and methanol by Kenneth *et al.* (2017). Their research found that the leaves extract of *P. guajava* includes antibacterial and phytochemical compounds that can be used to help people live better and healthier lives.

The chemical compositions, antioxidant and antibacterial activity of *Artemisia diffusa* essential oil were investigated by Mohammadi *et al*. (2017). They discovered that *A. diffusa* has antibacterial properties, suggesting that it could be used in aromatherapy and pharmacy.

Malik *et al*. (2017) investigated the phytochemical content and antimicrobial activity of *Berberis aristata* aqueous and alcoholic extracts. A preliminary phytochemical study revealed the existence of carbohydrate, glycoside, alkaloid, and flavonoid, and their antimicrobial action was shown to be good with gram-positive bacteria.

Wolde *et al*. (2018) tested the antibacterial activity of crude garlic extracts against standard isolates of *S. aureus* and *E. coli*. They found that water had the highest production potential, followed by ethanol, chloroform and petroleum ether, in that order. They claimed that *E. coli* was more sensitive to the extracts than *S. aureus.* They identified that garlic might be employed as an efficient antibacterial agent against human pathogenic bacteria due to their research.

The phytochemical components and antibacterial activity of methanol extracts of big and small *Allium sativum* and *Allium cepa* were investigated by Yasmin *et al*. (2018). They discovered that the highest zone of inhibition against *K. pneumonia* in big garlic and tiny onion. They found garlic and onion were shown to be among the most effective therapeutic phytomedicines in their research.

Yin *et al*. (2018) investigated the antimicrobial activity of *Gentiana macrophylla* methanolic root extract against bacterial strains isolated from wound burns and discovered that the *G. macrophylla* extract was effective against all the tested bacterial strains. *According to their research*, *G. macrophylla* is a primary source of wound burn management.

Rafiee *et al*. (2019) assessed phytochemical analyses in *lemon verbena (Lippia citriodora*). At different temperatures, lemon verbena's phytochemical and biochemical patterns *(Lippia citriodora* H.B.K.) were evaluated. At 5oC, the content of carotenoids, lycopene, ßcarotene, and anthocyanin increased significantly.

Karthikeyan and Vidya (2019) investigated the phytochemicals found in pomegranate peel extracts (aqueous, acetone, ethanol and hexane). According to their findings, several phytochemicals such as phenols and flavonoids can be found in pomegranate peel extracts (aqueous, acetone, ethanol and hexane). They claim that phytoconstituents can be used to cure a variety of illnesses and that they have the potential to develop beneficial medications for human usage.

.Chakraborty *et al.* (2020) reported the phytochemical and FTIR. analysis on *Acacia auriculiformis* for dyeing and functional finishing for textiles. They observed that phytochemical screening shows that the aqueous extract of *Acacia auriculiformis* contains all active constituents except flavonoids. They discovered that coloured materials posses good antibacterial properties especially Staphylococcus *aureus* and *E. coli.*

Adarsh *et al*. (2020) investigated that *Cinnamon zeylanicum* plant for antimicrobial efficacy against *Escherichia coli* (gram-negative), *Enterococcus faecalis* (gram-positive), and *Salmonella typhi* (gram-positive) bacteria. In vitro studies, they discovered that cinnamon extracts in chloroform and methanol extracts were showed more efficient against Gram-positive bacteria.

Samira observed that *et al*. (2020) investigated the phytochemical analysis and antimicrobial antioxidant activity of *Cynodon dactylon* in various extracts and noticed that the methanolic extract of *C. dactylon* rhizomes could be used as an antibacterial agent and a source of antibiotics for the treatment of bacterial infections.

Greeshma *et al*. (2020) calculated antibiotic activity and minimum inhibitory concentration in Vallauris *Solanaceae* bacterium specimens. This study used agar, well diffusion technique, petroleum-ether, Chloroform, ethanol, methanol and aqueous specimens to investigate antimicrobial operation. Their findings show that all the extracts were effective against the pathogens tested when compared to standard drugs, with petroleum-ether and methanol extracts having a higher zone of inhibition against *S. typhi, E. coli,* and *E. faecalis,* and chloroform and ethanol extracts having a satisfactory zone of inhibition against all pathogens when compared to standard drugs.

Kalita *et al*. (2020) investigated the antibacterial properties of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), and *Vitex nugundo* (Pochotia) against oral bacteria. According to their findings, all three plants had antibacterial activity against isolated organisms, with the acetone group out performing the aqueous extract group.

MATERIALS AND METHODS

CHAPTER -III

MATERIALS AND METHODS

The present investigation was carried out to analyse the pharmacognostic and preliminary phytochemical analysis of *Acacia auriculiformis* A.Cunn.ex Benth

3.1 COLLECTION OF PLANT MATERIALS

Theplants of fresh leaves of *Acacia auriculiformis* were collected from Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore district, Tamilnadu**,** India.

3.2 PREPARATION OF PLANT MATERIAL

The plant materials were collected, washed with water. Then it was shade dried and it was powdered with the help of mortar and pestle. It was stored in an air tight container for further studies.

3.3 PHARMACOGNOSTIC STUDY

The pharmacognostic studies of the selected medicinal plants were evaluated using the following qualitative analysis.

- Organoleptic study
- Fluorescence analysis

3.4 ORGANOLEPTIC STUDY

Organoleptic evaluation can be performed using sense organs to specify some specific features of the material, which can be considered a first step toward determining identification and purity. The organoleptic studies (colour, odour and taste) were carried out.

3.5 FLOURESENCE STUDY

Fluorescence is a phenomenon that numerous chemical components in the selected plant material. The fluorescence of *Acacia auriculiformis* plant powder was examined under visible light / daytime and UV light when it was treated with various agents and solvents in the current study.

The development of different colours was revealed when the powder was exposed to visible and UV light. A little amount of dried and finely powdered sample was treated with fresh acids, alkaline solutions and various solvents. The solutions were examined under visible and UV light to determine their typical colour reaction.

3.6 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The leaf powder of *Acacia auriculiformis* was extracted using aqueous, ethanol and methanol. The plant extracts were analysed qualitatively for alkaloids, flavonoids, sterols, terpenoids, anthraquinones, anthocyanins, proteins, phenolic compounds, quinones, carbohydrates, tannins, saponins, phytates, cardiac glycosides, glycosides, lignins and coumarins, among other phytoconstituents.

3.6.1 Alkaloids

Mayer's Test: 1 ml of extract was treated with few drops of Mayer's reagent. Appearance of cream coloured precipitate indicates presence of alkaloids.

Dragendroff's Test: 1 ml of extract was treated with few drops of Dragendroff's reagent. Appearance of orange, red or reddish brown precipitate indicates presence of alkaloids.

3.6.2 Flavonoids

Alkaline test: 1 ml of extract was treated with 2% sodium hydroxide. Appearance of yellow colour which changes to colourless solution on addition of dilute hydrochloric acid indicates the presence of flavonoids.

Sulphuric acid test: 1 ml of extract was treated with dilute ammonia and then concentrated sulphuric acid is added. Appearance of yellow colour which later disappears shows the presence of flavonoids.

Lead acetate: 1 ml of extract was treated with 1 ml of lead acetate. Appearance of yellow coloured precipitate indicates the presence of flavonoids.

Shinoda test: To 2 ml of extract, concentration Hydrochloric acid is added in drops followed by addition of magnesium turnings. Appeared pink, orange, red or purple colour indicates the presence of flavonoids.

3.6.3 Sterols

Libermann test: To few drops of extract, chloroform, acetic anhydride and concentrated sulphuric acid were added. Appearance of green or greenish blue colour after few mins indicates the presence of sterols.

3.6.4 Terpenoids

Libermann test: To few drops of extract, chloroform, acetic anhydride and concentrated sulphuric acid were added. Appearance of dark green colour indicates the presence of terpenoids

3.6.5 Anthraquinones

Borntrager's test: To 1g of plant powder, 5 to 10 ml of dilute sulphuric acid is added and is boiled and filtered. The filtrate is treated with chloroform or benzene and then dilute nitric acid is added. Appearance of pink to red colour indicates the presence of anthraquinones.

3.6.6 Anthocyanins

HCL test: To 2 ml of plant extract, 2 ml of 2N hydrochloric acid is added. Appearance of pink to red colour which changes to purplish blue colour after addition of ammonia indicates the presence of anthocyanins.

3.6.7 Proteins

Ninhydrin test: To 1 ml of extract, 1 ml of 2 % Ninhydrin reagent is added. Appearance of deep blue or violet or purple colour indicates the presence of proteins.

Biuret test: To 1 ml of extract, 2 ml of sodium hydroxide, 5 to 6 drops of copper sulphate is added and shaken well. Appearance of bluish violet colour after 4 to 5 minutes indicates the presence of proteins.

Xanthoproteic test: To 1 ml of plant extract, 1 ml of concentrated nitric acid is added. Appearance of yellow colour indicates the presence of proteins.

3.6.8 Phenolic Compounds

Ferricchloride test : 1 ml of the plant extract is treated with 5 % neutral ferric chloride. Appearance of red, blue, green or purple colour indicates the presence of phenolic compounds.

Lead acetate test: 1ml of the plant extract is treated with 1 ml of lead acetate. Appearance of white precipitate indicates the presence of phenolic compounds.

3.6.9 Quinones

HCL test: A small amount of extract is treated with concentrated hydrochloric acid. Appearance of yellow precipitate indicates the presence of quinones.

KOH test: 1 ml of extract is treated with alcoholic potassium hydroxide. Change of colour from red to blue indicates the presence of quinones.

3.6.10 Carbohydrates

Molisch's test: To 1ml of extract 2 to 3 drops of Molisch's reagent is added followed by few drops of concentrated sulphuric acid along the sides of the test tube. Appearance of violet or purple or red ring indicates the presence of carbohydrates.

Fehling's test: To 2ml of extract, 1ml of Fehling's solution A and 1 ml of Fehling's solution B were added and boiled for 1 to 2 minutes in a water bath. Appearance of reddish brown precipitate indicates the presence of carbohydrates.

3.6.11 Tannins

Braymer's test: To 1ml of extract few drops of distilled water is added followed by 5% ferric chloride. Appearance of dirty green precipitate indicates the presence of tannins.

Gelatintest: To 1ml of extract 1% gelatin solution and 10% sodium chloride were added. Appearance of white precipitate indicates the presence of tannins.

NaOH test: To 2ml of extract,1ml of 10% sodium hydroxide is added. Formation of emulsion indicates the presence of tannins.

3.6.12 Saponins

When 1 ml of extract is shaken with water, formation of foam indicates presence of saponins.

3.6.13 Phytates

To 2 ml of extract, 1 ml of ammonium thiocyanate and 1 ml of ferric chloride were added. Appearance of brownish yellow colour indicates the presence of phytates.

3.6.14 Cardiac glycosides

Bromine water test: To 1 ml of extract, 1ml of bromine water solution is added. Appearance of yellow precipitate indicates the presence of cardiac glycosides.

Keller-killani test: To 2 to 3 ml of plant extract, 2ml of Glacial acetic acid and a drop of 2% ferric chloride were added and few drops of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring indicates the presence of cardiac glycosides.

3.6.15 Glycosides

Bontrager's test: To 1g of plant powder, 5 to 10 ml of dilute sulphuric acid is added and is boiled and filtered. The filtrate is treated with chloroform or benzene and then dilute nitric acid is added. Appearance of pink colour indicates the presence of Glycosides.

NaOH test: To 1 ml of extract, 1 ml of aqueous sodium hydroxide was added. Appearance of yellow colour solution indicates the presence of glycosides.

3.6.16 Lignins

To 1 ml of extract, 1 ml of gallic acid is added. Appearance of olive green colour indicates the presence of lignins.

3.6.17 Coumarins

To 1 ml of extract, 10% sodium hydroxide and chloroform were added. Appearance of yellow colour indicates the presence of coumarins.

ANTIBACTERIAL ASSAY

Preparation of Plant extracts

In a separate beaker, 25g of leaf powder soaked with 100 ml of aqueous, ethanol and methanol extracts for 24 hours. The extract was filtered with what man No. 1 filter paper before being placed in a rotatory evaporator at 37°C for 15 minutes under vacuum. For further investigation, the extract was scraped and placed in an effendorf tube.

Selection of microbes

The bacterial culture of *Escherichia coli* and *Staphylococcus aureus* were obtained from the IFGTB, Coimbatore.

Characterization of Bacteria

The selected microbes were characterized by the following tests.

1. Motility
2. Gram staining
3. Colony Morphology

The above selected microbes were pure cultured for the analysis of above parameters.

Motility

The pure culture was inoculated in the nutrient broth and it was incubated at 370C for 12 hr culture was used for testing the motility by hanging drop method

Gram Staining

The isolate of pure culture was smeared on the micro slide and it was fixed. The smear was covered with crystal violet for 1 minute and washed in running tap water. Again the smear was covered with Grams iodine for 1 min and washed in running tap water. Later it was decolorized with ethanol for 1 min and it was washed with running tap water. Later the smear was treated with safranin for 5 min and then washed. The slides were allowed to dry. Purple colour indicates the gram positive bacteria whereas the pink colour indicates the gram negative bacteria.

Colony morphology

The above selected microbes were grown in the petriplate by pour plate method and the plates were incubated at 370C for 48 hrs. The colony morphology was analysed by observing the size, shape, colour and surface by direct examination of culture plates using hand lens.

Biochemical test for selected microbes

The pure culture of selected microbes were subjected to analyse following bio chemical tests

.**IMVIC reaction**

(a) Indole production test

Peptone water broth

Peptone - 0.5 gm

Nacl - 1.4 gm

NaHCo3 - 0.02 gm

KCl - 0.04 gm

CaCl2 - 0.04 gm

KH2 PO4 - 0.024 gm

Na2 HPO42H2O - 0.88 gm

Distilled water - 1000ml

pH - 7.2

The isolate was inoculated in the peptone broth taken in the test tube under aseptic condition and incubated at 370C for 24hrs. A few drops of Kovac’s reagent were added. The formation of cherry red coloured ring in the surface of the medium indicates the positive result.

(b) Methyl Red test

The isolate was inoculated in the phosphate broth taken in the test tube under aseptic condition and methyl red indicator was added. The red colour formation indicated the positive result

c) Voges – proskauer test

Glucose Phosphate broth

Glucose - 5 gm

Peptone - 5 gm

KH2 PO4 - 5 gm

Distilled Water - 1000 ml

pH – 7

The isolate was inoculated in the glucose phosphate broth taken in the test tube under a septic condition and incubated at 370C for 48hrs. 1 ml of 40% potassium hydroxide and 3 ml of 50% α napthanol solution was added after 48hrs of incubation. The red colour formation indicated the positive result.

Simmon citrate agar medium

Mg SO4 - 0.2 gm

(NH4)2 HPO4 - 1.0 gm

K2HPO4 - 2.0 gm

NaCl - 5.0 gm

Agar - 1.5 gm

Bromothymol blue - 0.08 gm

Distilled water - 1000ml

The slant was prepared by using simmon's citrate agar medium. The bacteria was inoculated on the slant and incubated at 370C for 24 hr. The Positive result is indicated by the appearance of blue colour.

Litmus milk reaction

Litmus Milk Medium

Skimmed milk Powder -100 g

Litmus - 5 gm

Distilled Water - 1000 ml

pH - 6.05

The culture was inoculated in litmus milk media and it was incubated at 37oC for 24 hr. The colour change was noted by the appearance of red or blue. The red colour indicates the acid production and blue colour indicates the alkali.

Amylase Activity

Starch agar medium

Starch solution - 20ml

Peptone - 5 gm

Beef extract - 3 gm

Nacl - 5 gm

Agar - 5 gm

Distilled water - 1000 ml

pH -7

20 ml of 10% Starch solution was added to the 100 ml of molten nutrient agar medium.Nutrient starch agar plates were inoculated by a single streak of isolates in the

petriplate and it was incubated for 48 hr. The plates were flood with thinlayerlugol's iodine. A colour change in the medium surrounding the growth was considered as Positive result.

Casein hydrolysis test

Skimmed milk agar was prepared and plated in the petriplates. The Plates were inoculated with the bacterial isolate in a single line and incubated at 370C for 24 hr. The formation of clear zone around the colonies indicates positive result.

Carbohydrate digestion

Peptone broth (pH-7.2) was prepared and incorporated with 1% glucose solution. The medium was inoculated with loop full of culture and it was incubated at 370C. Phenol red is added as an indicator. The formation of red colour indicates the positive result and the yellow colour indicates the

negative result.

Oxidase test

A loop full of culture was inoculated in a sterilized nutrient agar medium in the petriplate and it was incubated at 370C for 24 hr. The plates were flooded with 1% tetra methyl P-Phenyl diaminedihydrochloride solution. The formation of Purple colour within a minute indicates positive result.

Catalase activity

The Cultures of isolates were incubated in a sterilized nutrient agar slant and incubated at 370Cfor 24 hr. The slants were flood with 3% H2O2. The formation of air bubbles indicates the presence of catalase activity.

Assessment of Antibacterial Activity

The antibacterial activity was assessed by the following method.

Nutrient Agar Medium was used to test the antibacterial activity in petriplates. Antibacterial activity was tested using the nutrient agar medium.

Inoculum Preparation

Growth Method

The procedure for growth is as follows:

1.From an agar plate culture, at least three to five well-isolated colonies of the same morphological type are chosen. The growth is transferred into a tube containing 4 to 5 ml of an appropriate broth medium, such as Muller-Hinton broth, by touching the top of each colony with a loop.

2.The broth culture is incubated at 35°C until the turbidity is reached or exceeded (usually 2 to 6 hours)

3. To achieve a turbidity, the turbidity of an actively growing broth culture is modified using sterile saline or broth. For *E.coli* and *Staphylococcus aureus*, this resulted in a suspension with about 1 to 2 × 108 CFU/ml.

Inoculation of Test Plates

1. A sterile cotton swab should be dipped into the corrected suspension within 15 minutes of modifying the turbidity of the inoculum suspension.

The swab should be spun several times and firmly placed against the tube's inside wall above the fluid level. This will clear the swab of any extra inoculum.

2. A swab is streaked across the whole sterile agar surface of a Müeller-Hinton agar plate to inoculate the dried surface. To ensure a uniform dispersion of inoculum, repeat the streaking method two more times, turning the plate 60 degrees each time. The rim of the agar is swabbed as a last step.

3. Before applying the medication impregnated discs, leave the lid ajar for 3 to 5 minutes, but no longer than 15 minutes, to allow any excess surface moisture to be absorbed.

4. The media was perforated by constructing a 6 mm diameter well and filling it with various concentrations of 10- 50 μl of a sample. After 24 hours of incubation at 37°C, the

petriplates were arranged inversely for complete diffusion and inhibition zones were measured by measuring the diameter (mm) created around the well. A standard (Hi-Media) scale was used to measure the zones.

RESULTS AND DISCUSSION

CHAPTER-IV

RESULTS AND DISCUSSION

The results of pharmacognostic efficacy, phytochemical analysis, and antibacterial activity of *Acacia auriculiformis* A. Cunn. ex Benth. are presented below.

The results were presented as follows:

1. Organoleptic study.
2. Fluorescence analysis.
3. Phytochemical screening.
4. Antibacterial activity

ORGANOLEPTIC STUDY

Table 1 and Plate 1 showed the results of an organoleptic analysis of the powder of *Acacia auriculiformis* on characteristics such as colour, odour, and taste.

Table-1 Organoleptic study of the leaf powder of *Acacia auriculiformis* A.Cunn ex Benth

S.No	Organoleptic studies	Results
1.	Colour	Darkgreen
2.	Odour	Pleasant
3.	Taste	Bitter

The colour of the dried leaf powder of *Acacia auriculiformis* was dark green in colour.
The odour was pleasant and bitter in taste.

Plate 2- Organoleptic studies -Dried leaf powder of *Acacia auriculiformis* A. Cunn. ex Benth.

Table 2: Fluorescence analysis of leaves of *Acacia auriculiformis*.

S.No	Treatment with chemical reagent	Visible /day light	Under uv light
1	powder	Green	Light green
2	Powder +distilled water	Light green	Dark green
3	Powder +NH_3 solution	Light brown	Brownish green
4	Powder+conc.H_2SO_4	Dark Brown	Light brown
5	powder+dil H_2SO_4	Light brown	Dark brown
6	Powder +conc,HNO_3	Light brown	Orange
7	Powder +dil.HNO_3	Light green	Yellowish green
8	Powder + conc.HCl	Light green	Dark green
9	Powder + dil.HCl	Green	Light green
10	Powder +NaOH	Brownish green	Light green
11	Powder +picric acid	Yellowish green	Moss green
12	Powder + ethyl acetate	Dark green	Blackish green
13	Powder +BR_2H_20	Green	Light green
14	Powder + glacial acetic acid	Brownish green	Dark green
15	Powder + $FECL_3$	Light green	Green
16	Powder +acetone	Yellowish green	Yellowish green
17	Powder + NH_4CL	Light green	Yellowish green

FLUORESCENCE ANALYSIS

Fluorescence is a phenomenon that numerous chemical components in plant material show. Using several chemical reagents, leaf powder of *Acacia auriculiformis* revealed variable coloration under visible and UV light. One of the most notable characteristics of fluorescence is that UV radiation causes fluorescence in many natural goods that would not be visible in natural daylight (Adham , 2015).

The present observation shows that all the treatments have some marked colour difference (powder as such).

Fluorescence characteristics were investigated under visible and UV light in this study. The powder was treated with several compounds during the fluorescence analysis. The fluorescence examination of *Acacia auriculiformis* leaf powder under UV and visible light was confirmed, as shown in above table 2. The powder fluoresced in various colours of green and brown, while UV light revealed varied shades of green, orange and brown.

The results is on par with Selvam and Bandyopadhyay .(2005)who identify behaviour in the powdered samples when treated with different reagents such as sky blue, pale green, light brown , yellowish brown , yellowish green , aqua blue in Roots of *Rauvolfia Serpentina.*

The findings are consistent with those of Viji et al. (2010), who found green, darkgreen, palegreen, yellowsish green, brownish yellow, yellow, blackish red, and dark yellow in the leaves of *Cardiospermum helicacabum* treated with various chemicals and determined under visible and ultraviolet light.

Similar results were observed by Adham *et al*.(2015)when testing the fluorescence analysis , the powdered samples leaves of *Mentha Piperita, Mentha longifolia* and *Osimum basilicum* showed different colouration under visible and UV light using different chemical reagents such as methanol 1% glacial acetic acid, 10% NaOH, dilute NH3, 1M H2SO4, 1M HCl, 10% Fecl3, Acetone + Methanol, 10% Iodine.

The current findings are consistent with those of Ranjith *et al*.(2018), who found light green, dark green, light blue, dark blue, brown, violet, yellow, greenish blue, pale yellow, and fluorescence yellow in the fluorescence analysis of *Curcuma longa* rhizome, *Murraya koenigii* leaves, and *Psidium guajava.*

The current discovery was linked to Pandiyan and Ilango (2022), who showed different colours when exposed to chemicals. They discovered that *Huberantha senjiana* has a range of colours, including dark green, light green, yellowish green, greenish yellow, and greenish black, when exposed to visible light, and green, light green when exposed to UV radiation.

PHYTOCHEMICAL ANALYSIS

The leaves of the chosen plants were cleaned and then air dried in the shade. It was afterwards pulverised and stored in sealed plastic containers with labels for further use. The powder was used in qualitative tests to determine secondary metabolites. Table 3 summarized the findings. Preliminary qualitative tests were conducted on the leaves of the selected plant *Acacia auriculiformis*, which were dried and pulverised separately. The ethanolic and methanolic extracts of both contained the highest phytochemical elements, followed by the aqueous extracts, as shown in the table above.

Table-3 Preliminary Phytochemical Analysis of *Acacia auriculiformis* A.Cunn. ex Benth

S. No.	Metabolite	Test performed	Ethanol	Methanol	Aqueous
1.	Alkaloids	Mayer’s test	_	+	_
		Dragendorff’stest	+	+	+
2.	Flavonoids	Alkaline test	+	+	+
		Sulphuric acid test	+	+	+
		lead acetate test	+	+	+

		Shinoda test	_	_	+
3.	Sterols	Libermann Test	+	+	+
4.	Terpenoids	Libermann Test	+	_	_
5.	Anthraquinone	Borntrager's Test	_	+	_
6.	Anthocyanin	HCl Test	_	_	_
7.	Proteins	Ninhydrin test	_	_	_
		Bieuret test	+	_	_
		Xanthoproteic test	+	_	_
8.	Phenolic compounds	Ferric chloride test	+	_	+
		Gelatin test	+	_	_
		Ellagic acid test	_	_	_
9	Quinones	HCL Test	+	_	_
		KOH test	_	_	_
10	Carbohydrates	Molisch's test	+	+	+
		Fehling's test	_	+	+
11	Tannins	Braymers test	+	_	+
		Gelatin test	+	_	_
		NaOH test	_	+	_

12	Saponin	Shaken with water	_	_	_
13	Phytates		+	_	_
14	Cardiac Glycosides	Balijet reagent	_	+	_
		Bromine water test	_	+	_
		Keller – killani test	+	+	+
15	Glycoside's test	Borntrager test	_	_	_
		NaOH Test	+	+	+
16	Lignin	Gallic acid test	+	_	_
17	Coumarin	Flourescence test	_	_	_
		NaOH +CHCL3	+	+	_

"+" showed Present

"-" showed Absent

The ethanolic and methanolic extracts of both contained the highest phytochemical elements, followed by the aqueous extracts, as shown in the table above.

The current findings are consistent with those of Vinoth et al. (2012), who detected steroids, triterpinoids, reducing sugars, alkaloids, phenolic compounds, flavonoids, and tannins in *Azadiracta indica.*

Similar result was observed by Deepti *et al*.(2012)who investigated the phytochemical screening test revealed that the leaf extracts contain a broad spectrum of secondary metabolites: Alkaloids, Phytosterols, Flavonoids, Phenols and Triterpenes in major proportion. Methanol extract was shown to be more effective against all the organisms followed by Ethylacetate, Chloroform and Hexane extracts

Deshpande *et al.* (2013) conducted the preliminary phytochemical screening of ethanol and petroleum ether extracts of *Acacia nilotica* stem bark and found similar results.

Both ethanol and ether extracts revealed the presence of alkaloids, polysaccharides, saponins, Tannins, Flavonoids, cardiacglycosides, and anthraquinone, whereas fixed oils and fats, proteins, and amino acids were lacking.

The results in support of Ehiowemwenguan *et al*.(2014) who studied the phytochemical anlaysis in banana fruit peels and found the ethanol to be a better solvent for the extraction of the bioactive agents in banana peels which include: glycosides, alkaloids, saponins, tannins, flavonoids and volatile oil.

The results is on par with Ibrahimet al.(2010)who evaluated the phytochemical screening on Commelina benghalensis and their result revealed the presence of phlobatannins, carbohydrates, tannins, glycosides, volatile oils, resins, balsams, flavonoids and saponins, while terpenes, sterols, anthorquinones and phenols were absent.

The results coincide with Bandiola et al.(2017)where their phytochemical screening showed that both the ethanolic and methanolic extracts contained most of the phytochemical constituents, followed by the ethyl acetate, hexane, and aqueous extracts, respectively. Among these phytochemicals present in the leaves of S. cumini are alkaloids; flavonoids; saponins; tannins; glycosides; phenols; proteins; triterpenoids; steroids; and fixed oils and fats. Proteins were the highest constituents in all types of solvent. However, carbohydrates were absent in all extracts of the leaves.

ANTIBACTERIAL ACTIVITY

The antimicrobial activity was carried out by using the microbes of *Escherichia coli* and *Staphylococcus aureus.* The above microbes were also characterized by the motility, staining, colony morphology and biochemical tests. The Escherichia coli, was characterized and the results are given in the (Table 4 and 5). The diameter of zone of inhibition produced by bacteria was measured and the diameter of more than 5 mm was considered as the inhibitory zone. In the aqueous,ethanol and methanol leaf extracts of *Acacia auriculiformis* the zone of inhibition produced by 2 different bacterial sps viz., *Escherichia coli* and *Staphylococcus aureus* by agar well diffusion method at 100 and 150µl concentration.

Table-4 Biochemical Characterization of *Escherichia coli*

S.No	Test	Results
A.	**Morphological analysis**	
	1.Colony morphology	**Circular, Smooth**
	2.Gram staining	**Negative**
	3.Motility	+
B.	**Biochemical test**	
	IMVIC Reaction	
	a.Indoleproduction test	-
	b.Methyl red test	-
	c.Voges – Proskauer test	-
	d.Citrate utilization test	-
	Litmus milk reaction	+
	Cetrimide agar medium test	-
	Amylase activity	-
	Casein hydrolysis test	+
	Carbohydrate digestion	+
	Catalase activity	+
	Oxidase test	-
	Nitrate reduction	+

Positive (+) **negative -)**

Table-5 Characterization of *Staphylococcus aureus*

S.No	Test	Results
A.	**Morphological analysis**	**Spherical**
	1. Colony morphology	
	2. gramstaining	**Positive**
	3. Motility	-
B.	**Biochemicaltest**	
	IMVICReaction	
	a. Indoleproduction test	-
	b. Methyl redtest	+
	c. Voges-Proskauertest	-
	d. Citrate utilization test	-
	Litmus milk reaction	-
	Cetrimide agar medium test	+
	Amylaseactivity	+
	Casein hydrolysis test	+
	Carbohydrate digestion	+
	Catalase activity	-
	Oxidase test	-
	Nitrate reduction	+

Positive (+) **Negative (-)**

Antibacterial activity was performed by well method using nutrient agar medium using three different solvents like aqueous, ethanol and methanol leaf extracts against two different bacteria like *Escherichia coli* and *Staphylococcus aureus*. The results were presented as follows.

The result of the zone of inhibition in aqueous leaf extracts of *Acacia auriculiformis* given in the table-6. The results of the zone of inhibitions were given below. As per the result, the zone of inhibition were 11mm and 10mm against *Escherichia coli* and *Staphylococcus aureus* respectively at 100µl concentration whereas the zone of inhibition at 150µl concentrations are 14mm and 13mm against *Escherichia coli* and *Staphylococcus aureus* respectively. The highest zone of inhibition 14mm was found against *Escherichia coli.*

The result of the zone of inhibition in ethanol leaf extracts of *Acacia auriculiformis* given in the table-7. The results of the zone of inhibitions were given below. As per the result, the zones of inhibition were 12mm and 11mm against *Escherichia coli* and *Staphylococcus aureus* respectively at 100µl concentration whereas the zone of inhibition at 150µl concentrations are 15mm and 14mm against *Escherichia coli* and *Staphylococcus aureus* respectively. The highest zone of inhibition 15mm was found against *Escherichia coli.*

The result of the zone of inhibition in methanol leaf extracts of *Acacia auriculiformis* given in the Table-8. The results of the zone of inhibitions were given below. As per the result, the zone of inhibition were 12mm and 13mm against *Escherichia coli* and *Staphylococcus aureus* respectively at 100µl concentration whereas the zone of inhibition at 150µl concentrations were 17mm and 16mm against *Escherichia coli* and *Staphylococcus aureus* respectively. The highest zone of inhibition 17mm was found against *Escherichia coli.*

Table-6 Antibacterial activity - Zone of inhibition against selected microbes in the aqueous extracts of *Acacia auriculiformis* A.Cunn .ex Benth at 100 µl and 150 µl concentration

S.NO	Pathogens used	Zone of inhibition in diameter (mm)	
		Concentrations of Extracts	
		100µl	150µl
1	*Escherichia coli*	11± 0.08	14± 0.04
2	*Staphylococcus aureus*	10± 0.02	13± 0.01

Values are mean ± SE of three samples

Figure -1 Aantibacterial activity -zone of inhibition against selected microbes in the aqueous extracts of *Acacia auriculoformis* at 100µl and 150µl concentrations

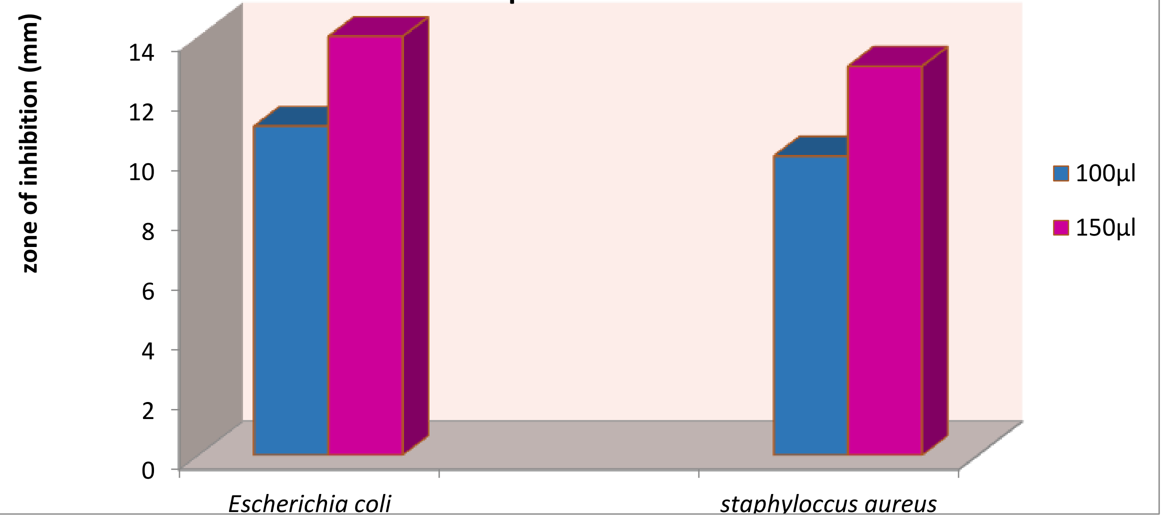


Table-7 Antibacterial activity - Zone of inhibition against selected microbes in the ethanol extracts of *Acacia auriculiformis* A.Cunn .ex Benth at 100 µl and 150 µl concentration

S.NO	Pathogens used	Zone of inhibition in diameter (mm)	
		Concentrations of Extracts	
		100µl	150µl
1	*Escherichia coli*	12± 0.08	15± 0.04
2	*Staphylococcus aureus*	11± 0.02	14± 0.01

Values are mean ± SE of three samples

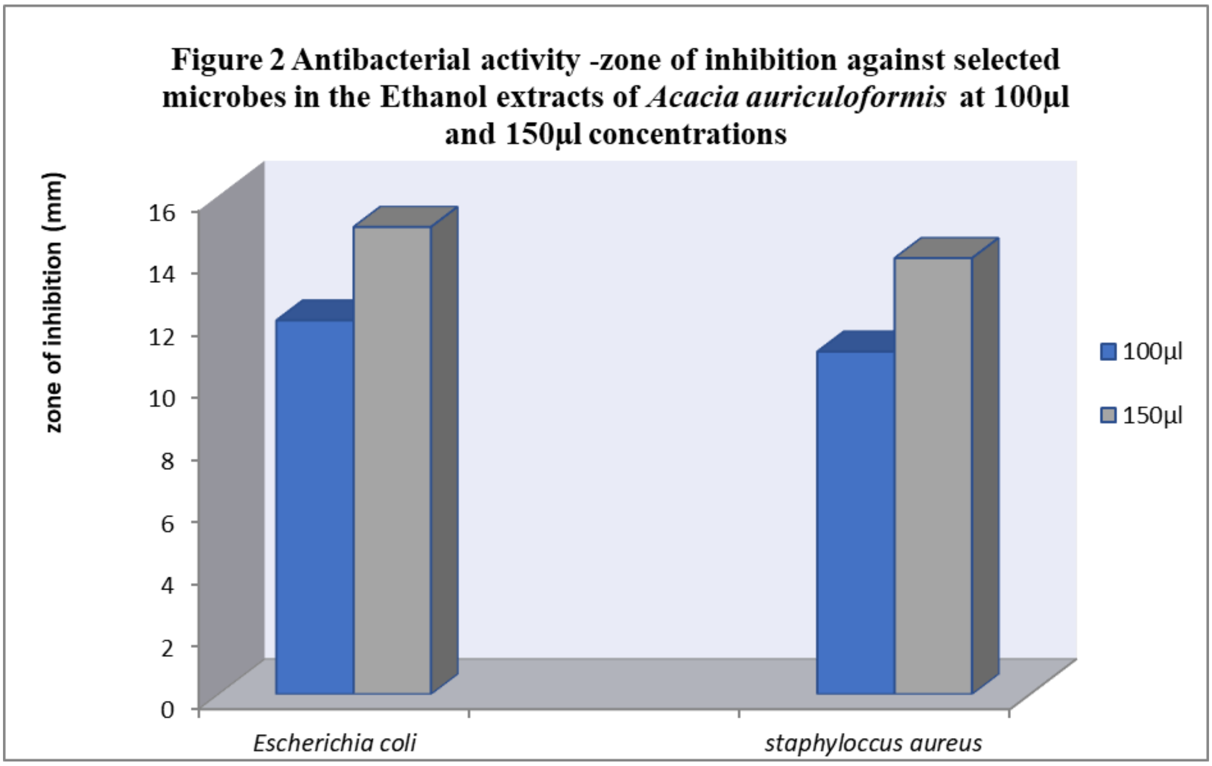
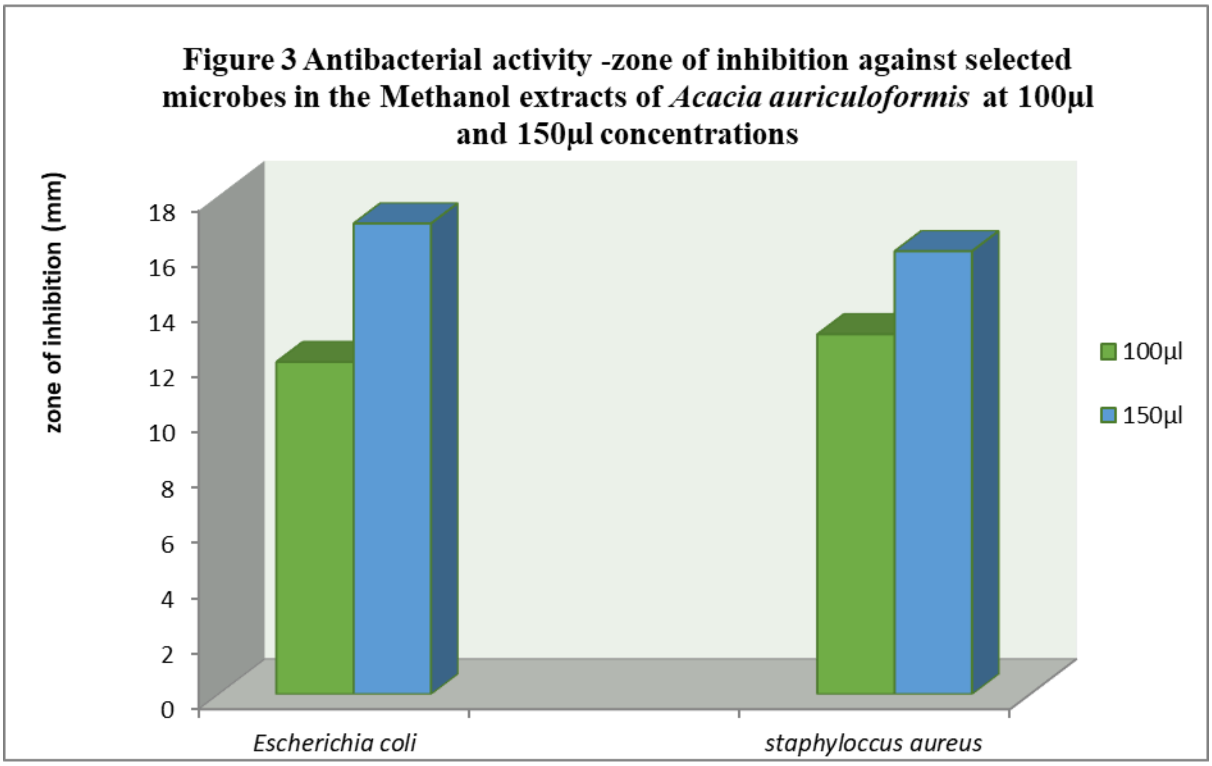


Table-8 Antibacterial activity- Zone of inhibition against selected microbes in the methanol extracts of *Acacia auriculiformis* at 100 µl and 150 µl concentration

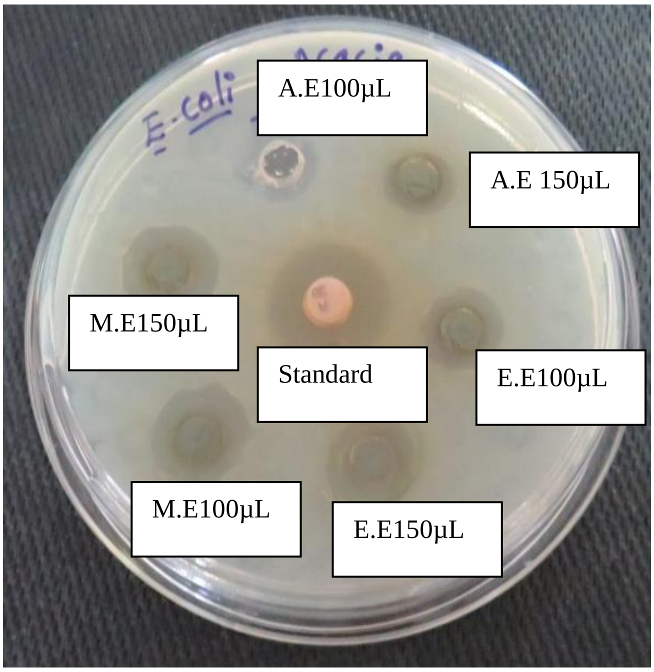
S.NO	Pathogens used	Zone of inhibition in diameter (mm)	
		Concentrations of Extracts	
		100µl	150µl
1	Escherchia coli	12± 0.08	17± 0.04
2	*Staphylococcus aureus*	13± 0.02	16± 0.01



Among the three different extracts, methanolic leaf extracts showed significant antibacterial activity, followed by ethanol and aqueous extracts respectively. This might be due to the solubility of the secondary metabolites were more in methanolic leaf extract when compared to the ethanol and aqueous leaf extracts. In all the extracts Gram negative bacteria (*Escherichia coli*) showed more inhibition zone as compared to the other extracts. The inhibitory zones were compared with standard antibiotic Teicoplanin (20mm) and Rifampicin (17mm) respectively.

PLATE 4

Antibacterial activity of Aqueous , Ethanol and Methanol leaf extracts of *Acacia auriculiformis* A.Cunn ex.Benth. against *Escherchia coli*



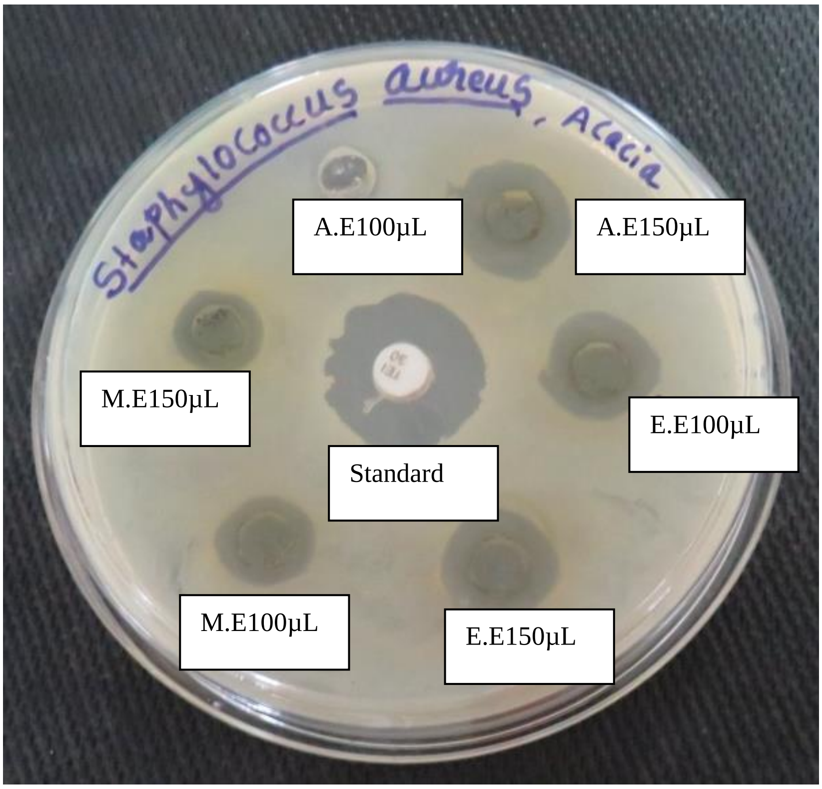
A.E- Aqueous leaf extract

E.E- Ethanol leaf extract

M.E -Methanol leaf extract

PLATE 5

Antibacterial activity of Aqueous , Ethanol and Methanol leaf extracts of *Acacia auriculiformis* A.Cunn ex.Benth. against *Staphylococcus aureus* .



A.E- Aqueous leaf extract

E.E- Ethanol leaf extract

M.E -Methanol leaf extract

The present findings are in confirmatory with the results of Adarsh *et al. (*2010) who reported that the chloroform extract of cinnamon showed notable antibacterial activity against gram-positive bacteria and gram-negative bacteria. It is well known that most spices are more active against gram-positive bacteria than gram-negative bacteria. This study showed that chloroform and methanol extracts of cinnamon were more effective against gram-positive bacteria *in vitro*.

The present study was positively correlated with the findings of Viji *et al. (*2010) who encouraging as the benzene, ethanolic and chloroform extracts have shown considerable antibacterial activity against the tested organism

The similar results were supported Amit pandey (2011) who evaluated the antimicrobial activity of *Citrus limon.* Their study revealed their antimicrobial susceptibility assay showed promising evidence for the antimicrobial effects of lemon fruit peels, seeds and juice against bacterial and fungal pathogens.

The results were positively correlated with Doss *et al.* (2011) who used the Successive solvent extract viz ., petroleum ether, chloroform, benzene, methanol, ethanol and water extracts of *Medicago sativa* was evaluated for antibacterial activity, against seven important bacterial strains by agar-well diffusion method. Methanol extract showed significant activity against all the tested bacteria followed by chloroform and ethanol extract. Benzene and Petroleum ether extracts did not show any significant activity

The similar results were observed by Vinoth *et al*. (2012). They observed the antibacterial activity of Methanol extract of *Azadirachta indica* showed maximum zone of inhibition (20mm) against *Salmonella typhi*, followed by *Pseudomonas aeruginosa* (17mm), *Escherichia Coli* (16mm) and *Staphylococcus aureus* (12mm).

The present investigation also coincides with Deepti *et al*. (2012). The methanol extract of *M. tinctoria* leaf exhibited potent antimicrobial activity towards all the microbes. The Zones of inhibition values was recorded in the leaf extract of *Morinda tinctoria* Roxb. B. Similar work was supported by Pradeepa *et al*. (2016) who detected the antibacterial activity *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in the leaf extracts of *pelargonium graveolens.*

. The present observation is in accordance with the findings of Kumari *et al.* (2016) their study shows that the methanol extract of *Trigonella foenum-graecum* L. revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formation.

Similar results were observed by Rangra *et al*. (2019) who stated that The highest antibacterial activity shown by the ethanolic extract was against the *P. aeruginosa* (19.54±0.40) mm while the highest antifungal activity was shown towards the *Aspergillus. niger* (20.62±0.17) mm at higher concentration of 100 mg/Ml.

The present observation is in accordance with the findings of Greeshma *et al*. (2020) who investigated that methanolic extract of *Vallaris solanacea* showed antibacterial activity against *S. typhi, E.coli,* and *E .faecalis.*

SUMMARY AND CONCLUSION

CHAPTER – V

SUMMARY AND CONCLUSION

With the globalisation of the world, opportunistic illnesses have progressed to a point where patient survival rates are rapidly declining. Microorganisms are the main cause of many disorders. Antimicrobial agents are becoming resistant to almost all bacteria. To solve the current challenge, more advanced prototype antibacterial drugs are required. Researchers all over the world are turning to the plant kingdom to discover alternative antibacterial drugs based on chemical compounds found in plants that can resist the growth of microorganisms in the human body. Many different vegetables, medicinal plants and spices have been utilised to treat a variety of diseases since ancient times.

The purpose of this work was to determine the phytochemical and antibacterial activity of several *Acacia auriculiformis* A. Cunn ex Benth leaf extracts. The investigation's findings are presented here. The powder of *Acacia auriculiformis* A.Cunn. ex Benth. was dark green in colour, with a pleasant odour and bitter taste.

In visible light, the fluorescence examination revealed colour shifts such as Green, Dark green, Greenish yellow, Dark Green, Brown, and Light green. Under UV light, green, light green, brownish green, light brown, pale yellow, olive green and dark green were observed. *Acacia auriculiformis* leaves contain a variety of plant components, including alkaloids, carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids.

Aqueous, ethanol and methanol leaf extracts of *Acacia auriculiformis* showed promising antibacterial efficacy against *Escherichia coli* followed by *Staphylococcus aureus* microns, respectively. With different pathogens and extracts, the zone of inhibition varied according to antibacterial activity. The methanolic extracts of *Acacia auriculiformis* have a very high zone of inhibition (17mm). This might be due to the solubility of the secondary metabolites were more in methanolic leaf extract when compared to the ethanol and aqueous leaf extracts. In all the extracts Gram negative bacteria (*Escherichia coli*) showed more inhibition zone as compared to the other extracts. Methanol>ethanol>aqueous leaf extracts were found to have the best antibacterial action against *Escherchia coli*.

The current work sheds information on the antibacterial activity of *Acacia auriculiformis* A.Cunn.ex Benth leaves and it provides a useful resource for the creation of antibacterial medication alternatives. Further more research and should be carried out this particular plant for more research output for our society in most economical feasible way.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adarsh, A., Chettiyar, B., Kanthesh, BM. and Raghu, N. (2020), Phytochemical Screening and Antimicrobial Activity of *Cinnamon zeylanicum.* **International Journal of Pharmaceutical Research and Innovation, 13,** 22-33.

Adesuyi, A.O., Elumm, I.K., Adaramola, F.B. and Nwokocha, A.G.M. (2012), Nutritional and Phytochemical Screening of *Garcinia kola*. **Advance Journal of Food Science and Technology 4(1)**, 9-14.

Adham, A.N. (2015), Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in *Mentha piperita, Mentha longifolia* and *Osimum basilicum*. **Journal of Pharmacognosy and Phytochemistry; 3(6),** 130-139.

Arekemase, M.O., Kayode, R.M.O., Ajiboye, A.E. (2011), Antimicrobial Activity and Phytochemical Analysis of *Jatropha Curcas* Plant against Some Selected Microorganisms. **International Journal of Biology, 3(3)**.

Arokiyaraj, S., Perinbam, K., Agastian, P., Kumar, R.M. (2009), Phytochemical analysis and antibacterial activity of *Vitex agnus-castus*. **International Journal of Green Pharmacy**.

Banu, K.S.S. and Cathrine, L. (2015), General techniques involved in phytochemical analysis. **International journal of Advanced research in Chemical science, 2**, 25-32.

Bhalodia, N.S. and Shukla, V.J. (2011), Antibacterial and antifungal activities from leaf extracts of *Cassia fistula*: An ethnomedicinal plant. **Free Radicals and Antioxidants, 1(1)**, 68-76.

Bhuvaneshwari, L., Arthy, E., Anitha, C., Dhanabalan, K., Meena, M. (2007), Phytochemical analysis & Antibacterial activity of *Nerium oleander.* **Ancient Science of Life, XXVI (4)**, 24-28.

Chakraborty, L., Pandit, P., Maulik, S.R. (2020), *Acacia auriculiformis* - A natural dye used for simultaneous coloration and functional finishing on textiles. **Journal of Cleaner Production, 245**, 1-7

Chekuri, S., Vankudothu, N., Panjala, S., Rao, N.B., Anupalli, R.R. (2016), Phytochemical analysis, anti-oxidant and anti-microbial activity of *Acalypha indica* leaf extracts in different organic solvents. **International Journal of Phytomedicine, 8(3),** 444-452.

Deepti, K., Umadevi, P., Vijayalakshmi, G., polarao, B.V. (2012), Antimicrobial Activity and Phytochemical Analysis of *Morinda tinctoria* Roxb. Leaf Extracts. **Asian Pacific Journal of Tropical Biomedicine,** S1440-S1442.

Deshpande, S.N. and Kadam, D.G. (2013), Phytochemical analysis & Antibacterial activity of *Acacia nilotica* against Streptococcus mutans. **International Journal of Pharmacy and Pharmaceutical Sciences, 5(1)**, 236-238.

Desingh, M., Jesudas, J.M., Balasubramaniam, P., Mayakrishnan, K., Ganesan, B., and Mohan, R. (2014), Phytochemical Analysis and Anti-microbial Activity of *Eupatorium glandulosum*. **Interntional Journal of Current Microbiology and Applied Sciences, 3(7)**, 882-885.

Doss, A., Parivuguna, V., Vijayasanthi, M., and Surendran, S. (2011), Antibacterial evaluation and phytochemical analysis of *Medicago sativa* L. against some microbial pathogens. **International Journal of Science and Technology, 4(5),** 550-552.

Ehiowemwenguan, G., Emoghene, A.O., and Inetianbor, J.E. (2014), Antibacterial and phytochemical analysis of Banana fruit peel. **IOSR Journal Of Pharmacy, 4(8),** 18-25.

Ganesh, P.S., Gajalakshmi, S., Abbasi, A.S. (2009), Vermicomposting of the leaf litter of *acacia* (*Acacia auriculiformis*): Possible roles of reactor geometry, polyphenols, and lignin. **Bioresource Technology, 100**, 1819–1827

Ganesh, P., Kumar, R.S., and Saranraj, P. (2014), Phytochemical analysis and antibacterial activity of *Pepper (Piper nigrum* L.) against some human pathogens. **Central European Journal of Experimental Biology, 3(2)**, 36-41.

Greeshma, N., Hande, U.N., Kanthesh, B.M., and Nataraj, R. (2020), Phytochemical Analysis and Antimicrobial Activity of *Vallaris solanacea* Leaves Extract. **International Journal of Innovations in Biological and Chemical Sciences, 13**, 10-18

Gulfrazi, M., Sadiqi, A., Tariq, H., Imran, M., Gureshi, R., and Zeenat, A. (2011), Phytochemical analysis and antibacterial activity of Eruca sativa seed. **Pakistan Journal of Botany, 43(2),** 1351-1359,.

Jemilat, I., Chioma, A.V., Omoregie, E.H. (2010), Pharmacognostic and Phytochemical Analysis of *Commelina benghalensis* L. **Ethnobotanical Leaflets, 14**, 610-15.

Kalita, C., Raja, D., Saikia, A., and Saikia, A.K. (2020), Antibacterial property of *Azadirachta indica*, *Ocimum sanctum*, and *Vitex negundo* against oral microbes. **Journal of conservative dentistry, 22(6)**, 602–606.

Karthikeyan, G., and Vidya, A.K. (2019), Phytochemical anlysis, antioxidant and antibacterial activity of pomegranate peel. **Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences, 5(1)**, 218-231.

Kavitha, R., Kamalakannan, P., Deepa, T., Elamathi, R., Sridhar, S., Suresh Kumar, J. (2011), In vitro Antimicrobial Activity and Phytochemical Analysis of Indian Medicinal Plant *Couroupitaguianensis* Aubl. **Journal of Chemical and Pharmaceutical Research, 3(6),** 115-121

Kenneth, E., Paul, T., Istifanus, N., Uba, U., Rejoice, A., Victor, O., and Mohammed, S. (2017), Phytochemical analysis and antibacterial activity of *Psidium guajava* L. leaf extracts. **GSC Biological and Pharmaceutical Sciences, 01(02)**, 013–019.

Khodjaa, N.K., Makhlouf, L.B., and Madani, K. (2014), Phytochemical screening of antioxidant and antibacterial activities of methanolic extract of some Lamiaceae. **Industrial Crops and Products, 61**, 41-48.

Kumari, O.S., Rao, N.B., and Gajula, R.G. (2016), Phytochemical analysis and anti-microbial activity of *Trigonella foenumgracum* (Methi seeds). **Journal of Medicinal Plants Studies, 4(4)**, 278-281

Malik, Z.H., Jain, K., Ravindra, K.C., Sathiyaraj, G. (2017), In vitro antimicrobial activity and preliminary phytochemical analysis of *Berberis aristate*. **International Journal of Ethnobiology & Ethnomedicine, 4(1)**, 1-6.

Manjula, P., Mohan, C.H., Sreekanth, D., Keerthi, B., and Devi, P. (2013), Phytochemical anlysis of *Clitoria ternate* LINN, a valuable medicinal plant. **Journal of Indian botanical Society, 92 (3&4)**, 173-178.

Mohammadi, A., Arianfar, A., and Noori, M. (2017), Chemical Composition, Antioxidant and Antibacterial Activity of *Artemisia diffusa* Essential Oil. **Journal of essential oil bearing plants, 20(5)**.

Naik, L.S., Shyam, P., Marx, K.P., Baskari, S., Devi, V.R. (2015), Antimicrobial Activity and Phytochemical Analysis of *Ocimum tenuiflorum* Leaf Extract. **International Journal of PharmTech Research, 8(1)**, 88-95.

Nandagopalan, V. Doss, A., and Marimuthu, C. (2016), Phytochemical analysis of some traditional medicinal plants. **Bioscience Discovery, 7(1),** 17-20.

Nascimento, G.G.F., Locatelli, J., Freitas, P.C., Silva, G.L. (2000), Antibacterial activity of plants extracts and phytochemicals on antibiotic resistant bacteria. **Brazilian Journal of Microbiology, 31**, 247-256

Okereke, C.N., Iroka, F.C., Chukwuma, M.O. (2015), Phytochemical analysis and medicinal uses of *Hibiscus sabdariffa*. **International Journal of Herbal Medicine, 2(6),** 16-19.

Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., Bansal, Y.K., (2015), Secondary metabolities of plants and their role :Overview. **Current Trends in Biotechnology and Pharmacy, 9(3),** 293-304.

Pandey, A., Kaushik, A., Tiwari, S.K. (2011), Evaluation of antimicrobial activity and phytochemical analysis of *Citrus limon.* **Journal of Pharmaceutical and Biomedical Sciences, 13(13),** 1-7.

Pandiyan, R. and Ilango, K. (2022), Pharmacognostical, physicochemical and phytochemical evaluation of *Huberantha senjiana* (Annonaceae) leaf: An endemic tree of Gingee Hills Tamil Nadu India. **Journal of Pharmacy & Pharmacognosy Research, 10(1),** 158-172.

Parekh, J., Karathia, N., and Chanda, S. (2006), Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. **African Journal of Biomedical Research, 9**, 53 – 56

Patel, P., Patel, N., Patel, D., Desai, S., Meshram, D. (2014), Phytochemical analysis and antifungal activity of *Moringa olifera*. **International Journal of Pharmacy and Pharmaceutical Sciences, 6(5)**, 144-147.

Pradeepa, M., Kalidas, V., Geetha, N., (2016), Qualitative and quantitative phytochemical analysis and bactericidal activity of *Pelargonium graveolens* L'Her. **International Journal of Applied Pharmaceutics, 8(3),** 7-11.

Pramila, D.M., Xavier, R., Marimuthu, K., Kathiresan, S., Khoo, M.L., Senthilkumar, M., Sathya, K., and Sreeramanan, S. (2012), Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Mentha piperita*: Lamiaceae). **Journal of Medicinal Plants Research. 6(2)**, 331-335

Raffie, H., Mehrafarin, A., Omidi, H., Naghdi, H., Farahnaz, B., Sigaroodi, K. (2019), Evaluation of Phytochemical and Biochemical Patterns of *Lemon Verbena* (*Lippia citriodora* H.B.K.) at Different Temperatures. **Research Journal of Pharmacognosy**.

Rangra, N.K., Samanta, S., Pradhan, K.K. (2019), A comprehensive review on phytopharmacological investigations of *Acacia auriculiformis* A.Cunn. ex Benth. **Asian Pacific Journal of Tropical Biomedicine, 9(1)**, 1-11.

Ranjith, D. (2018), Fluorescence analysis and extractive values of herbal formulations used for wound healing activity in animals. **Journal of Medicinal Plants Studies, 6(2)**, 189-192.

Roy, S., Rao, K., Bhuvaneswari, Ch., Giri, A., Mangamoori, L.N. (2010), Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. **World Journal of Microbiology and Biotechnology, 26**, 85–91.

Saranya, S.R., Krishna, P.J., Singh, R.K., Gaanapriya, M., Dhivya, E., and Rajasekar, S. (2013), Screening and phytochemical analysis of pharmacologically active compounds from *Abutilon Indicum* and *Phyllanthus Niruri* and assessing their In vitro Antimicrobial activity against pathogens. **International Journal of Advanced Biotechnology and Research, 4(4),** 496-504.

Savadi, S., Vazifedoost, M., Didar, Z., Nematshahi, M.M., and Jahed, E. (2020), Phytochemical Analysis and Antimicrobial/Antioxidant Activity of *Cynodon dactylon* (L.) Pers. Rhizome Methanolic Extract. **Journal of Food Quality, 2020**, 1-10.

Selvam, A.B.D., and Bandyopadhyay, S. (2005), Fluorescence Analysis on the Roots of *Rauvolfia Serpentina* (L.) Benth. Ex Kurz Under UV Radiation. **Ancient Science of Life, XXIV(4)**, 164 – 167.

Senthilkumar, A and Venkatesalu, V. (2009), Phytochemical analysis and antibacterial activity of the essential oil of *Clausena anisata* (Willd.) Hook. f. ex Benth. **International Journal of Integrative Biology, 5(2)**, 116- 120.

Simon, N.K., Santhoshkumar, R., and Kumar, N.S. (2016), Phytochemical analysis and antimicrobial activities of *Annona squamosa* (L) leaf extracts. **Journal of Pharmacognosy and Phytochemistry, 5(4),** 128-131.

Sridhar, S., Kamalakannan, P., Elamathi, R., Deepa, T., Kavitha, R. (2011), Studies on antimicrobial activity, physio chemical and phytochemical analysis of *Wrightia tinctoria* R.BR. **International Journal of Pharmaceutical Research and Development, 3(8)**, 139 - 144.

Sumathy, V., Lachumy, S.J., Zakaria, Z., and Sasidharan, S. (2011), In Vitro Bioactivity and Phytochemical Screening of *Musa Acuminata* Flower. **Pharmacologyonline, 2,** 118-127.

Velu, S., and Baskaran, C. (2012), Phytochemical analysis and in-vitro antimicrobial activity of *Withania somnifera* (Ashwagandha). **Journal of Natural Product and Plant Resource, 2(6)**, 711-716

Viji, M., Sathiya, M., Murugesan, S. (2010), Phytochemical analysis and antibacterial activity of medicinal plant *Cardiospermum helicacabum* Linn. **Pharmacologyonline, 2**, 445-456.

Vinoth, B., Manivasagaperumal, R., and Rajaravindran, M. (2012), Phytochemical analysis and antibacterial activity of *Azadirachta indica* Juss. **International Journal of Research in Plant Science, 2(3)**, 50-55.

Wolde, T., Kuma, H., Trueha, K., and Yabeker, A., (2018), Anti-Bacterial Activity of Garlic Extract against Human Pathogenic Bacteria. **Journal of Pharmacovigilance, 6(1),** 1-5.

Yadav, R.N.S., and Agarwal, M. (2011), Phytochemical anlaysis of some medicinal plants. **Journal of phytology, 3(12),** 10-14.

Yasmin, H., Anbumalarmathi, J., Sharmili, S., Aruna, (2018), Phytochemical analysis and antimicrobial activity of garlic (*Allium sativum* L.) and onion (*Allium cepa* L.). **Research on Crops, 19(2)**, 245-248.

Yin, C., Xie, L., Guo, Y. (2018), Phytochemical analysis and antibacterial activity of Gentiana macrophylla extract against bacteria isolated from burn wound infections. **Microbial pathogenesis, 114**, 25-28.

Yisa, J. (2009). Phytochemical Analysis and Antimicrobial Activity Of *Scoparia dulcis* and *Nymphaea Lotus.* **Australian Journal of Basic and Applied Sciences, 3(4)**, 3975-3979.