

ANTIMICROBIAL ACTIVITIES OF MEDICINAL *CATHARANTHUS ROSEUS* PLANT EXTRACT

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Microbiology

By

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of **INDHUMATHI.M (38300006)** and **ARCHANA DEVI.K (38300004)** who carried out the project entitled **"ANTIMICROBIAL ACTIVITIES OF MEDICINAL CATHARANTHUS ROSEUS PLANT EXTRACT"** under our supervision from October 2020 to April 2021.

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ABSTRACT

Ayurveda is the Indian traditional system of medicine which focuses on the medical potential plant. *Catharanthus roseus* is one plant recognized well in Ayurveda. It is known for its anti-tumour, anti-diabetic, anti-microbial, anti-oxidant and anti-mutagenic effects. It is an evergreen plant first originated from island madagascar. The flowers may vary in colour from pink to purple and leaves are arranged in opposite pairs. It produces nearly 130 alkaloids mainly ajmalcine, vinceine, resperine, vincristine, vinblastine and raubasin. Vincristine and vinblastin are used for the treatment of various type of cancer such as Hodgkin's disease, Breast cancer, skin cancer, and lymphoblastic leukemia. It is an endangered species and need to be conserved using techniques like Micropropagation. It has high medicinal values which need to be explored extensively.

Catharanthus roseus_L. plant leaf extracts were prepared by using solvents such as acetone, ethanol, and chloroform and tested against pathogenic Microorganisms to determine their antimicrobial potential. *Catharanthus roseus* leaf extract showed maximum antibacterial activity against all the pathogenic microorganisms. Among the solvents tested, ethanol showed maximum antibacterial activity of plant extracts viz., *catharanthus roseus* leaf Streptococcus sp were found to be more susceptible against *catharanthus roseus* leaf extract tested followed by E.coli . The aim of this study is to identify the Antimicrobial activity of *Catharanthus roseus* leaf extract by using the agar well diffusion method. The results of the present study clearly suggested the importance of *catharanthus roseus* in treatement against infection disease causing pathogenic microorganisms. Moreover, the therapeutic potential of the plant should also be checked when used in combination with other herbal drugs.

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LIST OF ABBREVIATIONS AND SYMBOLS:

gms	-	Grams
ml	-	Millilitre
min	-	Minute
w / v	-	Weight by volume
nm	-	Nanometres
mg	-	Milligrams
mg/ml	-	Milligram per millilitre
mM	-	Millimolar
ug/ml	-	Microgram per millilitre
°C	-	Degree celcius
cm	-	Centimetre
%	-	Percentage
HCl	-	Hydrochloric acid
H ₂ SO ₄	-	Sulphuric acid
GAE	-	Gallic acid equivalents
PBS	-	Phosphate buffer solution
Na ₂ SO ₃	-	Sodium sulfite
NaOH	-	Sodium hydroxide
UV	-	Ultra Violet
TLC	-	Thin layer chromatography
GC-MS	-	Gas chromatography Mass spectrophotometry

CHAPTER 1: INTRODUCTION

1.1 Catharanthus roseus

The *Catharanthus roseus* L. plant leaf extracts will be prepared by using solvents such as acetone, ethanol, and chloroform and tested against pathogenic microorganisms to determine their antimicrobial potential. *Catharanthus roseus* leaf extracts showed maximum antibacterial activity against all the pathogenic microorganisms. Among the solvents tested, ethanol showed maximum antibacterial activity of plant extracts viz., *Catharanthus roseus* leaf when compared to acetone and chloroform extracts viz., *Catharanthus roseus* leaf *Staphylococcus sp* were found to be more susceptible against *Catharanthus roseus* leaf extracts tested followed by *E.coli*. The results of the present study clearly suggested the importance of *Catharanthus roseus* in treatment against infection disease causing pathogenic microorganisms. Moreover, the therapeutic potential of the plant should also be checked when used in combination with other herbal drugs.

Catharanthus roseus (L.) which is an important medicinal plant of the family Apocynaceae is used to treat many of the fatal diseases. *C. roseus* also possess good antioxidant potential. There are about two common cultivars of *C. roseus* which is named on the basis of their flower color that is the pink flowered 'Rosea' and the white flowered 'Alba'. *C. roseus* is extensively cultivated in northern India in order to meet their commercial and the ever increasing demand in the indigenous systems of the medicine also their need to the pharmaceutical industry. However, certain factors like the soil salinity hampers the cultivation of this plant very severely. *Catharanthus roseus* which is proudly known as the Madagascar periwinkle is found to be a species of *Catharanthus* native and also endemic to Madagascar. The synonyms of the plant name include *Vincarosea*, *Ammocallisrosea* and *Lochnerarosea*. Other English names occasionally used for the plant include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle and "Old maid". A traditionally used medicinal plant *Catharanthus roseus* is an erected procumbent herb or under the shrub containing latex. It grows widely to about 1m tall at the subtropical area. The leaves are found to be of oval to oblong, 2.5 – 9.0cm long and 1.0-3.5 cm broad, glossy green, hairless,

with a pale midrib and a short petiole of about 1.0-1.8 cm long and they are arranged in the opposite pairs. The flowers looks white to dark pink with a darker red centre and a basal tube of 2.5 – 3.0 cm long with a corolla of about 2.0 – 5.0 cm diameter with five petals like lobes. The fruits are found to be a pair of follicles of about 2.0-4.0 cm long and 3 mm broad. This plant is found to be rich in their pharmacological action that includes antibacterial, antifungal, antioxidant, anticancer , and antiviral activates. **(Gajalakshmi ,et al, 2013).**

Plants have been used in the preparations of traditional medicine for a long time and most of these folk medicines were prepared from locally grown wild plants. Knowledge about the uses of plants was compiled by trial and error and passed down from one generation to another orally. Nowadays, world markets are turning to plants as the sources of ingredients in healthcare products. It offer alternative remedies with tremendous opportunities to generate income, employment and foreign exchange for developing countries. Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure several human diseases. Herbal drugs constitute a major part in all traditional system of medicines. It is a triumph of popular therapeutic diversity.

In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care. Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics. Bacterial resistance to antibiotics increases mortality, likelihood of hospitalization and the length of stay in the hospitals. Therefore, the research and reviews for new and effective anti-microbial agents with broad-spectrum of activity from natural sources is increasing day by day**(MonokeshKhmerSen.et al, 2013).**

Natural products including plants, animals and inerals have been the basis of treatment of human diseases. Use of plants as a source of medicine has been inherited and is an important com- ponent of the health care system. **(Nayaret al., 1999).** In recent years, many drugs have been isolated from natural source as the modern medicine system treats the symptoms and suppresses the disease but does little to ascertain the real cause. Toxic drugs which may suppress or relive some aliments

usually have harmful side-effects. Drugs usually hinder the self-healing efforts of the body and make recovery more difficult. There, the current scenario is to isolate the active constituents present in the plant material to develop medicinally drugs which are having rare chances of adverse effects **(Mohammed et al., 2011)**.

Medicinal plants grow naturally around us. Over centuries, cultures around the world have learned how to use plants to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable health care regime and are an important source of livelihood for indigenous and rural population **(Rajalakshmi et al 2013)**.

The plant *Vincarosea* Linn (periwinkle) is an apocynaceous, ever-blooming, pubescent herb or sub- shrub which has been shown to be a source of many alkaloids. It has enjoyed a popular reputation in indigenous medicine in various parts of the world. Its alkaloids are Hypotensive , sedative and have tranquilising properties and are anti cancerous. It helps in relieving muscle pain, depression of central nervous system and wasps stings. It is used in case of nose bleed, bleeding gums, mouth ulcers and sore throats. It is also used internally for loss of memory, hypertension, cystitis, gastritis and enteritis, diarrhea and raised blood sugar levels **(Hemamalini Balaji, 2014)**.

Catharanthus roseus (Madagascar periwinkle) is a species of *Catharanthus* native and endemic to Madagascar. Synonyms include *Vincarosea* (the basionym), *Ammocallisrosea*, and *Lochnerarosea*; other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and "Old-maid". In India it is known as **"Nithyakalyani"** .

Studies in the 1950s revealed that *Catharanthus roseus* contained 70 alkaloids, many of which are biologically active. While initial studies for its use in diabetes mellitus were disappointing, the discovery that it caused myelosuppression (decreased activity of the bone marrow) led to its study in mice with leukemia, whose life span was prolonged by the use of a *Vinca* preparation. The other scientific name for "*Vincarosea*" is *Catharanthus roseus*. Vincristine is Vinca Alkaloids from *Catharanthus roseus*. It is a mitotic inhibitor and used in cancer chemotherapy. The leaf juice of *C. roseus*

produced dose-dependent reduction in blood glucose of both normal and diabetic, rabbit and comparable with that of the standard drug, glibenclamide .

The results indicate a prolonged action in reduction of blood glucose by *C. roseus* and the mode of action of the active compound(s) of *C. roseus* is probably mediated through enhance secretion of insulin from the β -cells of Langerhans or through extra pancreatic mechanism. The species has long been cultivated for herbal medicine and as an ornamental plant. In traditional Chinese medicine, extracts from it have been used to treat numerous diseases, including diabetes, malaria, and Hodgkin's disease. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. This conflict between historical indigenous use, and recent patents on *Catharanthus roseus* derived drugs by western pharmaceutical companies, without compensation, has led to accusations of biopiracy. It can be dangerous if consumed orally. It can be hallucinogenic, and is cited (under its synonym *Vincarosea*) in Louisiana State Act 159. **(Parameswari.P,2015).**

Vincarosea has a variety of medicinal properties such as antibacterial **(Carew et al., 1970)**, antifungal **(Jaleel et al., 2007)**, antiviral **(Farnsworth et al., 1968)**, anticancer **(Ram et al, 2001)**. *Calotropis gigantea* has been reported to possess a number of medicinal properties and is used in toothache, earache, sprain, anxiety, pain, epilepsy, mental disorder and also it possesses anti- diarrheal, analgesic and CNS activity **(Pathak & Argal, 2007).**

An extensive literature survey indicates antioxidant and antimicrobial activity in *Calotropis gigantea* and *Vincarosea* . But only scanty information is available on such potential regarding the individual plant parts concerned (root, stem, leaf, flower & seed). In the present research study, the experiment was carried out in triplicate so as to compare the antioxidant and antimicrobial activities of the different parts of the two selected Indian medicinal plants and the values were compared with that of the previous reports. The concentration dependency of the antioxidant and antimicrobial activities was also investigated **(Jayakumar, et al,2010)**. *Catharanthus roseus* possesses known antibacterial, antifungal, antidiabetic , anticancer and antiviral

activities. The extracts have demonstrated significant anticancer activity against numerous cell types **(EL Sayed and Cordell, 1981)**.

The plant shows the presence of various alkaloids, viz. Vincristine, which binds to tubulin dimmers , inhibiting assembly of microtubule structures. Disruption of the microtubules arrests mitosis in metaphase. The vinca alkaloids therefore affect all rapidly dividing cell types including cancer cells, but also intestinal epithelium and bone marrow **(Graf et al., 1996)**.

Vinblastine is an anti micro tubule drug used to treat certain kinds of cancer **(Starling, 1976)**. Yohimbine (Procomil) is an alkaloid with stimulant and aphrodisiac effects found naturally in Pausinystalia yohimbine **(Millonet al., 2002)**. *C. roseus* also shows the presence of this compound along with another flavonoid hirsutidin **(Piovan and Fillipini, 2007)**. The different parts of *C. roseus* (leaf, stem, flower and root) were used and extracts were subjected to antibacterial assay. The extracts of *C. roseus* did not exhibit antibacterial activity against *Staphylococcus aureus*. Moreover, leaf, stem and flower extracts were also ineffective against *Pseudomonas aeruginosa* . The leaf extract did not exhibit activity against *Corynebacterium diphtheriae*; similarly, the crude extract of stem did not shown activity against *Shigella boydii* . The most effective was the root extract, which exhibited broad-spectrum antibacterial activity against *Salmonella typhimurium* and *S. boydii*. The flower extract showed activity against *C. diphtheria* **(Perez et al., 1990)**. The present investigation is focused on screening of leaf extracts of the plant for its antibacterial potential adopting the routine antibacterial assay techniques **(Prajakta .J, et al, 2010)**.

The research for new therapeutic treatments for various disease conditions is expanding. In many poor countries, plants have been looked at as a very promising source of new lead compounds for drug discovery and development **(Kong et al., 2003)**.

Catharanthus roseus is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities **(Jaleel et al., 2008)**.

Worldwide attention has been shifted towards finding new herbal chemicals for the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery **(Nisbet and Moore, 1997)**. Several research groups have shown that *Catharanthus roseus* has a high potential for many varieties of medicinal properties, such as antibacterial **(Carew and Patterson 1970)**, antifungal **(Jaleel et al. 2007)** and antiviral **(Farnsworth et al. 1968)**.

Plant derived medicines are still the mainstay of about 80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and fewer side effects **(De Smet, 1997; Ullah and Khan, 2008)**.

The quest for plants with antimicrobial properties continue to receive attention due to development of multiple drug resistance, an increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and adverse effects of synthetic products on host such as hypersensitivity, immune suppression and allergic reactions. The relatively lower incidence of adverse reaction to the plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is also encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs **(Itokawa et al., 2008)**.

These are mostly the secondary metabolites. Plant based antimicrobial represents a vast untapped source of medicines and needed to be explored further. They have enormous therapeutic potential and are effective in treatment of infectious disease **(Harborne et al., 1998)**. In this context, “Medicinal plants are rightly said to be Tradition of yesterday and drugs of tomorrow”**(Shanmugaraju et al, 2016)**.

1.2 TAXONOMY OF *C.roseus* L.:

Medicinal plants are classified according to the part used, habit, habitat, therapeutic value etc. Besides the usual botanical classification. But the botanical classification is

the most comprehensive and scientific classification. The botanical classification of *C.roseus*L.is as following:

Table 1.2.1 Scientific Classification

Family	Apocynaceae
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Genus	Catharanthus
Species	Catharanthus roseus
Binomial Name	Catharanthus roseus L.
Vernacular Name	Madagascar periwinkle, Rose perwinkle , vinca
Trade Name	Madagascar periwinkle,vincea
Part used	Leaves and Roots
Status	Mostly Cultivated

Botanical name: *Catharanthus roseus*

Common name: Rosy/Pink/Madagascar peiwinkle (English) Chichirica (Tagalog)

Table 1.2.2 Different Alkaloids Produced By *Catharanthus roseus*

Produced by	Alkaloids	Properties
Roots	Ajmalicine	Cardio-Vascular Diseases And High Blood Pressure
	Catharathine	Anti-Diabetic Properties
	Raubasin	Pain Relieving Properties
	Reserpine	Tranquilizers
	Serpentine	Cardio-Vascular Diseases And High Blood Pressure
Aerial parts like leaves	Vinblastine	Anti-Tumour Properties
	Vincristine	Anti-Tumour Properties
	Vindoline	Anti-Diabetic Properties

1.3 MEDICINAL PROPERTIES OF *C.roseus*

Anti-Cancerous Effect

C. roseus produces vinblastine and vincristine which are well known for their anti-cancerous properties (El Merzabani *et al.*, 1979) injected ethanol (70 %) extract of leaves intra peritoneally to female mice. As a result the alkaloids showed great responses against different types of cancers especially those that are multi-drug resistant. The nineteen patients suffering from plantar, genital, flat or *Verruca vulgaris* warts were cured. When the chloroform extract was injected to the patients suffering from Leuk-P3887 then only one was resistant to the treatment, five showed

50 % response and six were completely treated. Both the malignant and non malignant, platelet and platelet-associated disorders are effectively treated using the *C. roseus* alkaloids. In recent studies it has been found that growth of blood vessels that enhance tumour growth is inhibited by alkaloids secreted by *C. roseus*. These alkaloids, vincristine and vinblastine are used as injectable anticancer drugs such as vinblastine as Velban and vincristine as Oncovin. The division of cancer cells is interfered by these drugs along with their derivatives such as vinflunine. Vincristine is used in the treatment of leukemia in children and vinblastine for choriocarcinoma and Hodgkin's disease.

Anti Microbial Effect

A number of anti-microbial agents have been used to prevent the adverse effects caused by various microbes. It is very important to discover new antimicrobial agents because the rate of antibiotic production is much lower than the rate at which the microbes are becoming resistant to them. The plant extracts are useful in attaining the long term health response and also reducing the ill effects of various chemotherapeutic agents. Plants like *C.roseus* provide a broad spectrum of resistance against microbial agents and can be used as a prophylactic agent in the treatment of number of diseases. It has proved to possess efficient anti-viral, anti-bacterial and antifungal compound. Tobacco Mosaic Virus (TMV) was inhibited by water extract of callus culture of micro propagated plant. *Bacillus megatarium* and *Staphylococcus albusi* did not grow on agar plates with 70 % ethanol extract of dried leaves but *B. cereus* and *S. aureus* were resistant to same extract. *Proteus*, *Staphylococcus*, *Shigella* and *Pseudomonas* species are inhibited by the benzene extract of dried flowers (50 %) whereas the same concentration of benzene extract of dried leaves inhibited *Salmonella* species in addition to the above mentioned bacterial species. Antifungal effect was shown using different plant part extracts and each of them was active against different fungi. *Trichophyton mentagrophytes* was inhibited by hot water extract of both leaves and stem. There was no effect on *Neurospora crossa* by the acetone and water extract

(70 %) of aerial parts of plant. A study by Khalil, 2012 has shown the antimicrobial activity of *C. roseus* against bacteria (*S. aureus* and *E. coli*) and fungus (*Candida albicans*). The antibacterial activity of *C. roseus* was also shown by Govindasamy and Srinivasan in 2012 in which they studied the effects of extracts from leaf, stem, root, and flower against a number of bacteria. The maximum antibacterial activity was shown against *S. typhi* and minimum against *S. aureus* and *E. coli*. The antimicrobial activity of *C. roseus* was studied against *B. fusiformis*, *C. albicans*, *E. coli* and *Aspergillus fumigatus* using agar well disc diffusion assay and paper disc diffusion assay. The methanolic extract of stem was active against *B. fusiformis*, leaves against *A. fumigates* and flowers against *B. fusiformis*. The crude extract from different parts of this plant was used to study the antibacterial activity against *Salmonella typhimurium* (NCIM2501), *S. aureus* (NCIM5021) and *Pseudomonas aeruginosa* (NCIM2036). The results showed that it was a potent antibacterial agent. Stem extracts of *C. roseus* were used to save rubber trees (*Hevea brasiliensis*) from White rot disease caused by *Rigidoporus microporus* named fungus. It was found that the extract proved to be an efficient healing agent for the infected trees.

Anti-Mutagenic and Anti-Mitotic Effect

The anti-mitotic effect was shown by administering the female mice with ethanol (70 %) extract of dried leaves which showed positive results on CA-Fhrlich ascites vs. induction of metaphase arrest in ascites cells. In a study conducted by Sharma *et al.*, 1982 it was found that when the red blood cells (RBCs) were subjected to hot water extract of dried leaves the number of micro-nucleated polychromatic RBCs decreased which proved the anti mutagenic effect of *C. roseus*. The mutagenic effect of vincristine, an alkaloid secreted by *C. roseus* was studied using sex linked recessive lethal (SLRL) test system in *Drosophilla melanogaster*. The results thus obtained showed that vincristine produced many chromosomal effects, arrest cells at metaphase with highly contracted chromosomes and inhibition of tubulin polymerization. When the root tips of *C. roseus* were treated with ethylmethane sulphonate (EMS) then a number of chromosomal anomalies were observed such as

persistent nucleolus, condensation, fragmentation, lagged, bridge, cleft and binucleolated cells.

Anti-Oxidant Effect

Tannins, phenolics and flavonoids are the anti-oxidants produced by medicinal plants which are more potent than the anti-oxidants secreted by dietary plants. Reactive oxygen species (ROS) are the harmful compounds that our body generates during normal aerobic respiration and anti-oxidants are helpful in eradicating these compounds. *C. roseus* is known to produce many different alkaloids such as flavonol glycosides, caffeoylquinic acids, etc. Phenolic compounds are the products of secondary metabolism of plants and act as anti-oxidants, anti-inflammatory, anti-microbial, cardio protective and anti-allergic agents. Their anti-oxidant potential is because they are efficient hydrogen donors, reducing agents, metal chelators or singlet oxygen quencher. When a number of medicinal plants were screened for their oxygen radical absorbance capacity (ORAC) it was found that *C. roseus* has the highest ORAC value i.e. 22.30 μmol Trolox equivalent (TE)/g of fresh weight. *C. roseus* has also been shown to scavenge the nitric oxide, superoxide and DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals. In 2011 Rasool and his colleagues demonstrated through their research that *C. roseus* is a viable source of natural antioxidants which can be exploited for food and nutraceutical applications. The anti-oxidant activity of *C. roseus* was studied in different regions of Rajasthan and it was found that plants of Bikaner region had highest phenolic content followed by Kota and Jaipur, India. *C. roseus* is free of side effects and cost effective and efficient means to get rid from oxidative stress mediated diseases. The antioxidant effect of *C. roseus* (pink flowers) and *C. alba* (white flowers) was checked and it was found that *C. roseus* has higher antioxidant activity than *C. alba*.

Anti-Diabetic Effect

Diabetes, malaria, dementia, etc. can also be treated by using the different extracts of this plant. *Vinca rosea* has the ability to improve the blood supply to brain which results in increased supply of oxygen and glucose to brain and also preventing the abnormal coagulation of blood. *V. rosea* also helps in increasing the levels of serotonin whose deficiency may cause diseases like migraine, bulimia, phobias and schizophrenia. This plant helps in increasing the insulin production and utilization of sugar from food which helps in curing diabetes. An alkaloid named alstonine which is mainly present in its root bark helps in regulating the blood pressure. In a case study in Malaysia the diabetes patients who took decoctions of this plant in combination with regular medications showed better response as compared to those who undergone only oral medications and insulin. Maximum of the reported work about anti-diabetic potential of this plant is being conducted using the crude extract rather than the pure bioactive compounds. In streptozotocin induced diabetic rat this plant induces hypoglycemic effect. From this plant four alkaloids: vindoline I, II, III and IV were extracted which enabled high glucose uptake in pancreatic β -TC6 or myoblast C2C12 cells and elicited their use against type 2 diabetes. The alkaloid vincamine helps in the treatment of vascular dementia, a disease caused by the plaque development of arteries carrying blood to brain. Vincamine also causes blood thinning and helps to enhance the memory properties. *C. roseus* lowers the blood sugar levels in a dose dependent manner. The anti-diabetic potential of *C. roseus* was evaluated by studying the protein content in its different parts with highest content in half mature leaves. **(Punia Sandeep et,al 2014)**

1.4 FOLKLORIC USES

- In India- The juice of leaves is used as application to bee sting/ wasp sting.

- In Philippines- Decoction of leaves is used in diabetes and decoction of young leaves is used in stomach cramps, root decoction is used for intestinal parasitism. Infusion of leaves is used for treating menorrhagia. Crude leaf extracts and root has anti cancer activity. Roots used for dysentery.
- In Madagascar- The bitter and astringent leaves are used as vomitive, roots used as purgative, vermifugl, depurative, hemostatic and toothache remedies.
- In Mauritius- The juice of leaves is used for indigestion and dyspepsia.
- In West Indies and Nigeria- The plant is used in diabetes.
- In Cuba and Jamaica- Flower extract is used for eye wash in infants.
- In Bahamas- Decoction of flower is used in asthma, tuberculosis and flautlence.
- In Malaysia- The plant is used in diabetes, hypertension, insomnia and cancer.
- In Hawai- Extract of boiled plant is used to arrest bleeding.
- In America- Gargle of plant is used to ease soarthroats, chest ailments and laryngitis.
- In Africa- Leaves are used for menorrhagia and rheumatism.

CHAPTER 2: REVIEW OF THE LITERTURE

Nowadays, the demand for natural products and plant based medicines is growing throughout the world. MP is a remarkable herb owing to its broad spectrum of

applications. Apart from its natural supply of anticancer compounds for medicine on the international market, it ranks highly as a popular ornamental plant in the horticultural industry and as a model plant for studies in phytopathology as well as biotechnology. The ultimate goal of current research is to produce Antimicrobial activities cultivars with a high content of antitumor alkaloids. Several cultivars have been introduced primarily as ornamental plants with their alkaloid composition, agronomic performance, and genetic affiliations unknown . As such, agronomic and genetic elucidation of new cultivars with exploration of their physiology and secondary metabolism are required for achieving a high quantity and quality output of TIAs. It is unfortunate that MP anticancer drugs have several adverse side effects. This renders further exploration of the clinical pharmacokinetics of MP alkaloids and drug-drug interactions indispensable.(Naghmeh Nejat et,al 2014)

Origin and Distribution :

The origin of *Catharanthus roseus* is from islands of Madagascar (Mishra et al, 2017; Aruna et al, 2015).It is widely distributed in different subtropical and tropical area of the world along United States (Aruna et al, 2015). It is present in every continent except Antartica or some islands. Therefore it is available throughout the year except winters and hence in temperate regions because their growth is inhibited in low temperature. It is also cultivated in gardens and widely distributed in the wasteland (Qureshi et al, 2008).

Nutritional Composition :

From past few decades, *Catharanthus roseus* has been reported to be a perfect blend of ornamental and medicinal plants which have witnessed its utilization in the traditional medicinal systems. Currently, *Catharanthus roseus* has been found to have immense potential in food industry also. To know the nutritional significance of any edible plant, the proximate and nutritional composition analysis is important. The plant and its different parts i.e. root, shoot, leaves and flower comprises of wide range of physicochemical and phytochemicals and the same has been presented in table.

Therapeutic importance of *C. roseus*:

Traditionally people utilize this crop because of its numerous health benefits such as antidiabetic, anticancer, antimicrobial, antioxidant, antihelmintic, antidiarrheal, antiulcer, antihypertensive, phytoremediation, antimutagenic, memory enhancement activity, wound healing property, blood cleanser, anti hyper cholesterolemic , disinfectants, tranquilizing and sedative actions throughout the world (Ramesh and Subramani, 2015; Kumar 2014), (Balaji et al, 2014; Kumar 2014; Vaidya and Devasagayam, 2007, Kumar et al, 2014).

The different parts of plant have been reported to have wide application in various parts of the world, where its extract is being used for curing rheumatism, menorrhagia, indigestion, dyspsia, diabetes, eye problems, cancer, asthma, tuberculosis, sleeping disorders, hypertension, chest ailments, sore throat etc.

Folkloric Uses

- In Mauritius- The juice of leaves is used for indigestion and dyspepsia.
- In West Indies and Nigeria- The plant is used in diabetes.
- In Cuba and Jamaica- Flower extract is used for eye wash in infants.
- In Bahamas- Decoction of flower is used in asthma, tuberculosis and flautlence.
- In Malaysia- The plant is used in diabetes, hypertension, insomnia and cancer.
- In Hawai- Extract of boiled plant is used to arrest bleeding.
- In America- Gargle of plant is used to ease soarthroats, chest ailments and laryngitis.
- In Africa- Leaves are used for menorrhagia and rheumatism.

Flower :

Flowers of *Catharanthus roseus* contain high amount of tannins, triterpenoids and alkaloids which possesses numerous biological activity such as antidiabetic and

antibacterial properties (Barik et al, 2016). The flower of *Catharanthus roseus* shows high antioxidant activities 97.44% at 800µg, which is higher than standard L- ascorbic acid that shows 94% in the same concentration (Barik et al, 2016). This prevents the formation of free radicals due to the presence of volatile phenolic compounds such as glycosides, flavanols and caffeoylquinic acids (Rao et al, 2013). Anthocyanin pigment i.e. rosidin is present in this flower (Mishra et al, 2017; Das and Sharangi, 2017). *Catharanthus roseus* contains indole alkaloids in all its plant parts.

Bark:

Bark of this flower contains alkaloid Alstonin that helps in regulating blood pressure (Barik et al, 2016). Bark of the root of *Catharanthus roseus* contains vincristine and vinblastine which is used as sedative (Barik et al, 2016).

Root :

In root tissues catharanthine is present which possesses antidiabetic, menorrhagia and antihypertensive properties (Barik et al, 2016). Ajmalicine is a monomeric alkaloid that cures circulatory diseases and maintains normal cerebral blood flow by increasing the blood flow in the brain and peripheral parts of the body (Barik et al, 2016). Roots also contain quinones which are antibacterial in function (Barik et al, 2016). Flavonoids, triterpenoid, tannins, saponin, coumarin, quinones and phenolic compounds are also present which possess antioxidant activity which prevents enzymatic and non-enzymatic oxidation (Barik et al, 2016). *Catharanthus roseus* has the highest oxygen radical absorbance capacity (ORAC) (Barik et al, 2016). Roots contains antihypersensitive alkaloids such as serpentine, ajmalicine, reserpine (Rao et al, 2013).

Leaves :

Leaves contain good amount of alkaloids and polyphenols. Polyphenols exhibit antioxidant properties. *C.roseus* contains 130 alkaloids of indole group from which 25 are dimeric in nature and leaves produce two important commercially cytotoxic dimeric alkaloids which is vinblastine and vincristine (Barik et al, 2016). Vincristine and

vinblastine are helpful in the treatment of leukemia and lymphoma. The ethanolic extract of *Catharanthus roseus* leaves, estimated by HPLC (High pressure liquid chromatography) method shows the presence of natural antioxidants such as quercetin and rutin (Rao et al, 2013). The leaves of *Catharanthus roseus* helps in reducing the blood glucose level and hence in curing diabetes. (Barik et al, 2016; Sen et al, 2008)

Bioactive compounds present in different parts of *Catharanthus roseus* :

Bioactive compounds, produced from the plant secondary metabolism, are the non-nutritional constituents of plants, and which have been found to have significant positive health effects on humans. Recently compounds like flavonoids, alkaloids and tannins, anthocyanin have been reported in various parts of *Catharanthus roseus* which are responsible for the numerous biological activities. For example, *Catharanthus roseus* contains vinca alkaloids, which is the second most and oldest plant alkaloids groups used as drug for cancer treatment (Moudi et al, 2013). Among all the bioactive compounds, alkaloid is the dominant compound present in the Vinca rosea and is responsible for numerous health benefits. Vinca alkaloids such as vincristine, vinblastine, vinorelbine were also use in the western medicines (Pan et al, 2010). It contains more than 300 alkaloids which is having antineoplastic agents to treat leukemia, hodgkine's disease (Punia et al, 2014; Rosario et al, 2015), malignant lymphomas, neuroblastoma, wilm's tumour and other cancers, also have indole alkaloid such as lochnerine, tetrahydroalstonine and vindolidine which possesses hypoglycemic effect (Barik et al, 2016). Mostly the alkaloids present are ajmalicine, vinceine, reserpine, vincristine, raubasine and vinblastine (Mishra et al, 2017). Anticancer property of *Catharanthus roseus* is due to vinblastine and vincristine compounds (Mishra et al, 2017; Khanuja, 2012; Gupta and Raina, 1998). Vinblastine compound prevents germ cells, renal cancers, breast cancer and lymphoma cancers by stopping mitosis in metaphase and prohibiting microtubule formation (Mishra et al, 2017). *Catharanthus roseus* is having the phenolic compounds which includes C6C1 compounds such as 2, 3-dihydroxybenzoic acid and phenylpropanoids such as derivatives of cinnamic acid, flavonoids and anthocyanin (Barik et al, 2016).

Percentage of alkaloids on dry weight basis in different parts of the *Catharanthus roseus* is root; 0.12-9.00, stem; 0.07-0.46, leaf; 0.10-1.16, fruit; 0.40, seed; 0.18, flower; 0.005, pericarp; 0.14 (Barik et al, 2016).

Catharanthus roseus is one of the 21000 important medicinal plants found. It is used for the cure of a number of diseases such as diabetics ,sore mouth,mouth ulcers,and leukemia.It produces about 130 alkaloids such as reserpine , vinceine, raubasin and ajmalcine. Anti-leukemic activity is shown by vinblastine and vincristine. Different parts of this plant produce different amounts of alkaloids ,out of which root bark produce the maximum i.e. nearly 1.79% . There are a number of reports supporting its antimicrobial activity against E.coli, Pseudomonas, shigella, Streptococcus etc. Its anti- oxidant and anti-mutagenic . (Punia Sandeep et,al 2014)

Since all the tested of *Catharanthus roseus* were highly effective against the tested pathogenic microorganisms. The antimicrobial activity can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drug from *Catharanthus roseus* should be emphasized for the control of pathogenic microorganisms. The present results of *Catharanthus roseus* have provided scientific evidence for the development of antibacterial products and the treatment of bacterial infection in the future.(Shanmugaraju et,al 2016)

Current progress in pharmacological studies and traditional used of *C. roseus* have proved the significant medicinal properties of the plant, which continuously being utilized to cure various diseases. Enormous amount of phytochemicals especially alkaloids have been identified with their medicinal properties such as anti-cancer, anti-diabetic, anti-helminthic, anti-malarial, antimicrobial and so on. The commercial production of alkaloids containing in this plant need to be urged since most monomers were successfully identified in

the culture media with intense yield. A thorough research need to be done to optimized the cultural conditions at various levels and consider the synthesis of the novel life-saving drug with a powerful combination of existing bioactive compounds from natural resources. (Sara E Gomaa et,al 2019)

CHAPTER 3: AIM AND OBJECTIVES

3.1 AIM

To study the Antimicrobial activity of medicinal *Catharanthus roseus* leaf extract against some human pathogen and determine minimal inhibitory concentration (MIC) by Disk diffusion method.

3.2 OBJECTIVES

The main objectives of the present investigation are as follows:-

1. To extract the phytoactive compound present in the *Catharanthus roseus* leaf using solvent such as ethanol, chloroform and acetone.
2. To study the in vitro antimicrobial activity of different solvent extract against some human pathogens by disc diffusion method.
3. To determine the minimal inhibitory concentration (MIC) of solvent extracts by disc diffusion method.

CHAPTER 4: MATERIALS AND METHODOLOGY

4.1 MATERIALS:

- Petri dish
- Nutrient agar
- Ethanol
- Micro pippet
- Well cutter
- Antibiotic disk
- Marker
- Conical flask
- Distilled Water
- Eppendorf
- Cotton swab
- Measuring cylinder
- Extract (*Catharanthus roseus* leaf extract)

4.2 EQUIPMENT USED:

- Autoclave
- Centrifuge
- Laminar air flow
- Incubator
- Weighing Balance

4.3 METHODOLOGY

4.3.1 COLLECTION OF PATHOGENIC MICRO- ORGANISAM

The pathogenic microorganisms chosen for the present study were obtained from The Green Life Lab , Ramanathapuram .

The pathogenic microorganisms obtained are,

a) *E. coli*,

b) *Strptococcus sp*

The clinical isolates were confirmed by using inoculating them on selective media. The microorganisms were maintained on nutrient agar.

4.3.2 COLLECTION OF PLANT SAMPLE

Healthy *Catharanthus roseus* Leaves were collected from Mandapam, Ramanathapuram district, Tamil Nadu, India.

The plant material like leaf were washed thorough with tap water and then with sterile distilled water for the removal of dust and sand particles. The leaf was shade dried and powered by Mechanical blender. The powdered samples were hermetically sealed in air tight bottles until the time of the extraction. This was used as the raw material for the extraction of antimicrobial compounds against the pathogenic microorganisms used.

Figure4.3.2 *Catharanthus roseus*(Rosea) plant



Figure4.3.3 *Catharanthus roseus* (Rosea) Leaves are allowed to dry



Figure4.3.4 Grinded *Catharanthus roseus* (Rosea)leaf



4.3.3 CULTURE MEDIUM

Nutrient agar medium (for bacteria) were used as growth media for these microorganisms in this study.

4.4 EXTRACTION OF PLANT

4.4.1 SOLVENT EXTRACTION

The shade, dried, powdered leaf were used for the solvent extraction procedure. About 10 g of this powder was soaked in 100 ml of ethanol for 24 Hrs. The contents were then filtered through filter paper. This dried extract was then dissolved in ethanol for testing its antimicrobial potential. Extracts were stored at room temperature until further use.

Figure4.4.1 The dried extract dissolved in ethanol for antimicrobial testing



4.4.2 ANTIMICROBIAL ACTIVITY

The antimicrobial activity studies were carried out by disc diffusion technique (Newall *et al.*, 1996). The mixing of 2.60g of Nutrient agar powder and 100ml of distilled water to prepare Nutrient agar media. The nutrient agar media was sterilized at 121°C under 15 lbs pressures for 30 minutes. Petri plates were prepared by pouring 20 ml of Nutrient Agar medium. The plates kept at room temperature for solidification and stored at 4°C until using. After, The Plates were solidified and 20 µl of bacterial culture *E. coli*,

Streptococcus sp was poured and uniformly spread over the nutrient agar plates by using separate sterile Cotton swab. The excess inoculum was drained away and the inoculum was allowed to dry for 5 minutes. Agar well of 5 mm in diameter were prepared with the help of a sterilized stainless cork borer. The wells were labelled appropriately and to each well were loaded with 10 µl, 20 µl and 30 µl plant extract using a Micro pipette, place disk along with for identification. Standard reference antibiotic Amikacin (methanol) (25 mcg/disc) was used as controls for the tested bacteria.

The plates were incubated for 24 hours at 37°C along with negative controls. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period. At the end of the incubation period the zones of inhibitions were measured to the nearest millimeter. (Andrews et al., 2001). The inhibition zone is the area surrounded the hole and there is no growth of inoculated microorganism. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition against the tested bacteria.

Figure 4.4.2 Nutrient agar 20ml prepared for autoclaving



Figure 4.4.3 Nutrient agar plates are for streaking



Figure 4.4.4 Nutrient agar plates before Incubation with the organism *Streptococcus Sp*

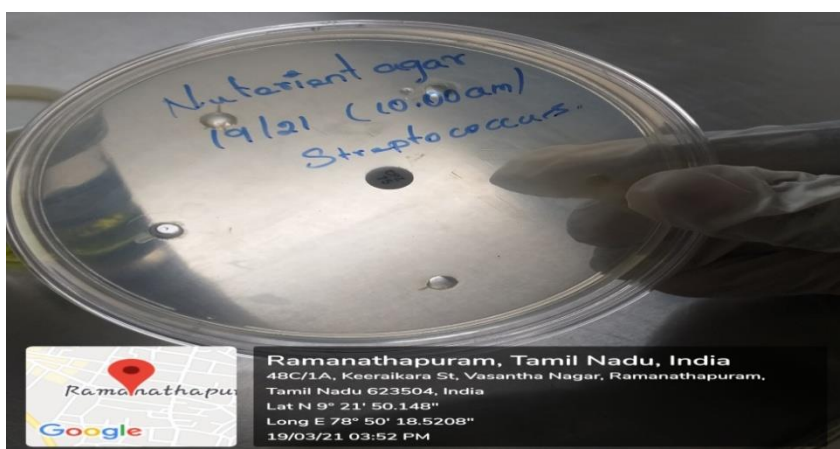


Figure4.4.5 Nutrient agar plates before Incubation with the organism *E.Coli*

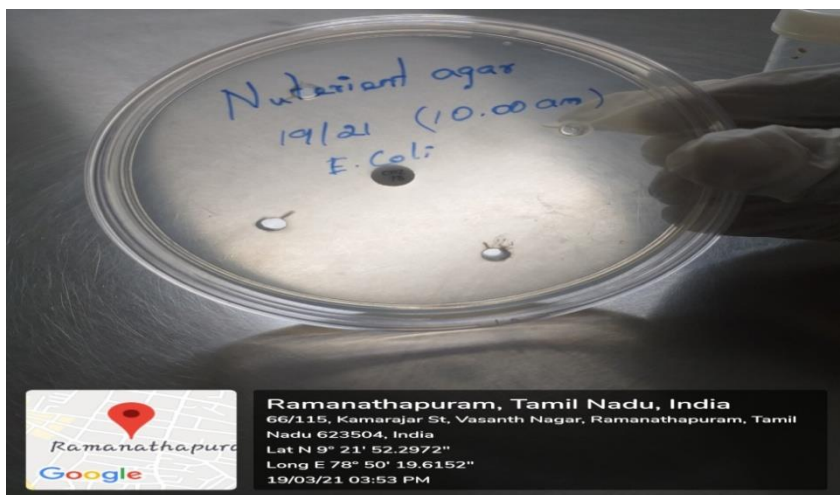


Figure4.4.6 Plates are allowed for Incubation



CHAPTER 5: RESULT AND DISCUSSION

5.1 CONFIRMATION OF THE PATHOGENIC MICRO ORGANISMS

The pathogenic microorganism obtained from Green life lab, Ramanathapuram were confirmed as *E.coli*, *Streptococcus sp* on the basis of Gram staining and biochemical tests .

5.2 IN VITRO ANTIMICROBIAL ASSAY BY DISC DIFFUSION METHOD

The *in vitro* antibacterial activity of ethanol extracts *Catharanthus roseus* L. leaf against two pathogenic microorganisms was carried out by disc diffusion method .It was found that the plant extract showed good inhibitory activity on almost all the pathogenic microbes tested. The results of antibacterial assay of ethanol extracts are described as follows.

5.3 ANTIMICROBIAL ACTIVITY OF CATHARANTHUS ROSEUS LEAF EXTRACTS

The anti-microbial activity of *Catharanthus roseus* L. leaf extract against pathogenic microorganisms viz., *E.coli* and *Streptococcus sp* was studied under *in vitro* condition with ethanol solvent extracts viz., It was observed that inhibitory activity of ethanol solvent extracts with the tested pathogenic microorganisms . Ethanol extract were intermediary in their activity. The maximum anti-bacterial activity . The maximum antibacterial activity was observed against *Streptococcus sp* (5mm) by ethanol extracts.

5.4 INTERPRETATION OF RESULT OF MINIMAL INHIBITORY CONCENTRATION (MIC)

Minimal inhibitory concentration of *Catharanthus roseus* L. leaf extracts were tested against two pathogenic micro organisms viz., *Streptococcus sp* and *E. Coli* on the basis of results obtained by disc diffusion method. The two isolates were susceptible to the solvent extracts when compared to other pathogenic microorganisms. *Staphylococcus sp* were found to be more susceptible to the plant extracts viz., *Catharanthus roseus* leaf extracts, since their growth was inhibited at relatively lower Concentration *E. coli*. The plant extract *Catharanthus roseus* leaf extracts inhibited the tested pathogenic microorganisms viz., *streptococcus sp* and *e.coli* with lowest concentration to a maximum level than other extracts. The *Catharanthus roseus* leaf extracts found to possess least MIC values and leaf extracts were found to be intermediary in their action. The ethanol extracts inhibited pathogenic micro- organisms with lower concentration than other extracts.

5.5 ANTIMICROBIAL STUDIES:

Antimicrobial activity was carried out for ethanol extracts of *Catharanthus roseus* leaves with test organism *E.coli* and *Streptococcus sp* were swabbed on Nutrient agar plate. Plant extract 10µl , 20µl and 30µl and commercial anti bacterial disk Amikacin (25 mcg) with plant extract and the disk was placed in nutrient agar plant. After 24 hours incubation, it was found to be that antimicrobial activities in the prepared plates.

Table 5.5.1Antimicrobial Activity of *Catharanthus roseus* leaf extract against *E.Coli* and *Streptococcus sp*

Microorganism Used	Concentration of Extract mg/ml(100)	Average of Inhibition Zone (mm)
<i>E.coli</i>	10µl	1mm
	20µl	1mm
	30µl	1mm
	40µl	2mm
Streptococcus	10µl	1mm
	20µl	1mm
	30µl	2mm
	40µl	3mm

Figure 5.5.1 Antimicrobial activity of Catharanthus roseus leaf Extract at Ethanol with E. Coli

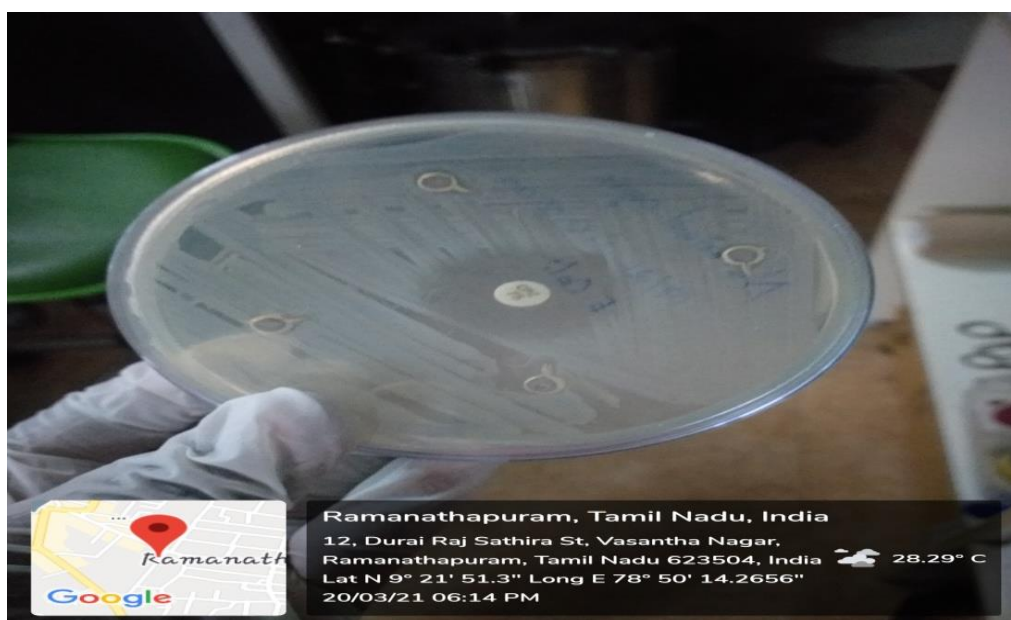


Figure 5.5.2 Antimicrobial Activity of *Catharanthus roseus* leaf Extract at Ethanol with *Streptococcus sp*



CHAPTER 6: SUMMARY AND CONCLUSION

Medicinal plants are very important and widely available resource for primary healthcare and complementary healthcare systems. The literature in this area of research showed that plant kingdom contain many species of plants harboring substances of medicinal value that have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects (Pankaj *et al.* 2008).

These plants show that they are very rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary. The present study reveals the antimicrobial activity of ethanol leaf extracts of *Catharanthus roseus*. The antimicrobial activity of *Catharanthus roseus* leaf extract was tested against two pathogenic bacteria, *Streptococcus sp* and *E.Coli*. The tested extract showed very strong antimicrobial activity against these pathogenic microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition. The strongest inhibition activity of the leaf extract was observed against *Streptococcus sp* (5 mm zone) at 100 mg/ml of leaf extract followed by *E.Coli* which showed 2mm inhibition zone at 100 mg/ml leaf extract. The results of this work are in agreement with other studies that the leaf extracts of this plant was has significant antibacterial activity against *Xanthomonas campestris* (Satish *et al.* 1999).

This study illustrate that a Gram-positive bacteria were more susceptible to this extract as compared to Gram negative bacteria species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms (Pankaj *et al.* 2008). The extraction of antimicrobial substances by organic solvents is better extracts (Thongson *et al.* 2004).

In the present study, the in vitro antibacterial activity of *Catharanthus roseus* showed the inhibitory action against the tested microorganisms. All the plant extracts inhibited the microorganisms to a significant level. When comparing among extracts, the leaf extracts showed maximum antibacterial activity. In general, the leaf extracts of *Catharanthus roseus* was found to possess less antibacterial activity when compared

to other plant extracts. All the plant extracts showed antibacterial activity against both Gram positive and Gramnegative organisms and this was conformity with earlier findings (Pratheeba *et al.*, 2013., Abbas *et al.*,Mahadev *et al.*, 2014).

Micro-organisms, including Gram positive and Gram negative bacteria, have been recognized as the main causative agent of various human infections. Though effective antimicrobias have been developed over the years, there has been increased development of antimicrobial drug resistance to presently available antimicrobials(Chopra, 2007). The fight against bacterial infection has resulted in the development of a wide variety of antibiotic, after years of misuse and overuse of antibiotics, bacteria are becoming antibiotic resistant, leading to a potential global health crisis. Acceptance of medicines from plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes (Koura *et al.* 2006). Some of the phytochemical compounds e.g. glycosides, saponin, tannin, flavonoids, terpenoid, alkaloid have variously been reported to have anti-microbial activity (Okeke *et al.*, 2001); Rahman *et al.*, 2010).

Acceptance of medicines from plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes (Koura *et al.* 2006). Some of the phytochemical compounds e.g. glycosides, saponin, tannin, flavonoids, terpenoid, alkaloid have variously been reported to have anti-microbial activity (Okeke *et al.*, 2001); Rahman *et al.*, 2010).

The aim of this study is to identify the Antimicrobial activity of *Catharanthus roseus* leaf extract by using the agar well diffusion method. The results of the present study clearly suggested the importance of *catharanthus roseus* in treatment against infection disease causing pathogenic microorganisms. Moreover, the therapeutic potential of the plant should also be checked when used in combination with other herbal drugs. The present study shows that the acetone, ethanol and chloroform extracts have inhibitory activity against most of the pathogenic microorganisms. The inhibition of the growth of these organisms *in vitro* by the pathogenic microorganisms.

The inhibition of the growth of these organisms in vitro by the extracts may be due to the presence of some active constituents in the extracts. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The medicinal uses of these plants to heal diseases including infection one has been extensively applied by people. The problem of microbial resistance is growing and the outlook for use of antimicrobial drug in the future is still uncertain. Therefore, action must be taken to develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drug, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

In the present study it was generally observed that *Catharanthus roseus* have brought about the possibility of utilization of plant extract, which has provided scientific evidence for the development of antibacterial products and treatment of bacterial infection in the future. (**R.Bakayaraj et,al 2016**)

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