6.1. INTRODUCTION

Ayurveda remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurveda drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. It is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field. In India,
many forms of substitute medicines are available for those who do not want conventional medicine or who cannot be helped by conventional medicine. Ayurveda and Kabiraji (herbal medicine) are two important forms of alternative medicine that is widely available in India.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant.

More than two decades ago, desert adapted landscape trees began gaining in popularity in south-western landscape designs. Promoted primarily as an alternative to higher water demanding trees, landscape architects soon realized that desert trees could be used in both naturalistic re-creations of desert scenes and highly formal, more traditional landscape designs.

Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. (Colombo et.al., 1996; Okunji et.al., 1999). With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential antimicrobial activity. Many herbs contain dozens of active constituents that combine to give the plant its therapeutic value.

Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from Medicinal plants (Cox et.al., 1990; Cox, et.al., 1994). Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. Research to find out scientific evidence for claims of plants used for Indian Ayurveda system of medicine has been intensified. Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases (Dev et al. 1997).
Summary

In absent of modern medicinal remedies people relied on herbal remedies derived from herbs and spices. There are many medicinal herbs and spices, which find place in day-to-day uses, many of these, are used as herbal remedies. Many cooked foods contain spices. Some minor ailments like common cold, cough, etc. may be cured by herbal remedies with use of medicinal properties of spices. Herbal remedies can be taken in many forms. Infusions are steeping herbs or spices, with parts like leaves and flowers with boiling water for some time. Filtered or unfiltered use this water extracts of spices as herbal remedies.

Decoction is boiling roots, bark and hard parts of herbs and spices with water for a long time. Infusion and decoction both are known as herbal teas. Sometimes essential oil of herbs and spices are also used as herbal remedies. Action of herbal remedies may vary from human to human and care should be observed in using it. Always inform your healthcare professional while taking any of the herbal remedies or consuming large quantity of medicinal herbs or spices as medicinal product.

Medicinal plants have a rich source of antimicrobial agents. Plants are used medicinally in different countries and these are the source of many effective drugs. (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. Hundreds of plants species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. (Bollinger et al., 1985). Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents. Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs.

Although medicinal plants produce slow recovery, the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms. Antimicrobial activities of many plants have been reported by the researchers.
Summary

The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids etc. that are present in these plants. Green plants produce and maintain a kind of biochemical merchandise, many of them are capable of being extracted and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolizable substances of plant are commercially very important and find utility in a number of compounds used in medical treatment. However, a fix supply of the source material often becomes difficult due to the various factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant repository and over utilization by pharmaceutical industry.

Taking into consideration the enormous potentiality of plants as sources for antimicrobial drugs with direction to antibacterial and antifungal agents, a systematic examination was undertaken to screen the confined plants for antibacterial and antifungal activity from two Indian medicinal plants, *Wrightia tinctoria* (Roxb) R.Br. (Leaf, stem, and fruit) and *Dyerophytum rubrum* (Gibs Ex Wt.) (Leaf, stem, root).

6.2. REVIEW OF LITERATURE

The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contribution to human health and well being, on the other hand, there is an increment use of herbal products all over the world. In USA, it reached 380% between 1990 and 1997. Many studies indicates that in same plants there are many substances such as peptides, unsaturated long chain aldehyde, alkaloid constituents, some essential oils, phenols, and water, ethanol, chloroform, methanol and butanol, soluble compounds, these
plants then emerged as compounds, these plants then emerged as compounds with, potentially significant therapeutic application against human pathogens, including bacteria, fungi, or virus. Therefore, in the present work plan all the extracts were performed for bioactivity guided fractionation and were reaching to pure bio actives with antibiotic potentials.

Medicinal plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo et al., 2008). The use of medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996).

Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala et al., 2005).

Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Cowman, 1999; Adesokan et al., 2008).

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000). Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host.
So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important. The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them (Cowman et al., 1999; Adesokan et al., 2008).

The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges (Nautiyal et al., 2002).

Therefore, they are more prone to extinction (Jablonski et al., 2004). Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes (Kala et al., 2000).

Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector (Kala et al., 2006).

In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action. However a large number of tropical plants have not been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostical characterization including DNA sequencing etc. An attempt is also made to recapitulate the current state of knowledge of relatively indeterminate herbal products, since clinicians in this country will come across their use among patients.

6.3. MATERIAL AND METHODS
6.3.1. Collection of the plant materials:

Plant sample of two medicinal plants *Wrightia tinctoria* (Roxb) R.Br. (LMP 0059) (pale indigo plant) (leaf, stem, and fruit) and *Dyerophytum rubrum* (Gibs Ex Wt.) (LMP 00169) (Leaf, stem, and root) were procured from different areas in ethnic pockets of Mt. Abu; it is arid zone area of Rajasthan, in the month of July, 2010. These plants were used by these regions in their daily lives to heal different diseases.

6.3.2. Identification of the plant materials:

All the samples which were selected for experiments were validated and the samples were given Recognition number. The recognition was as follows:

These samples were validated and submitted in Ethno medicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

6.3.3. Processing of plant materials:

Throughout the way of the study before use the sample, each sample was screened for its foreign matter and milled.

6.3.4. Experimental information’s:

Present studies were performed on two medicinal plants for the following studies—.

1. Extraction of plant materials
2. Phytochemical test of plant extracts.
3. TLC of the plant extracts
5. MIC of the successive extracts of the plants
6. Antioxidant activity of Methanolic extracts of plants.
7. Anti-cancerous activity successive extracts of plants.
8. Protein isolation and SDS PAGE for its characterization.
9. DNA isolation and Gel Electrophoresis for its DNA Fingerprinting.
10. NMR spectrum of isolation pure compounds.
11. Statistical analysis of the data
6.3.4.1. Extraction of the plant materials:

In the process of extraction of the plants, selected species were dried and powdered (gm.) and for the six hours duration the samples were soxhelt extracted in petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water. Then extracts were filtered, dried and weighed. These extracts were used for phytochemical, TLC and antimicrobial antioxidant, anti tumour screening of plant active compound.

6.3.4.2. Phytochemical screening of the plant materials:

Phytochemical screening was performed using standard procedure:
1. Test for reducing sugars (Fehlings test)
2. Test for terpenoids (Salkowski test)
3. Test for flavonoides (HCL test)
4. Test for tannins (Ferric chloride test)
5. Test for saponins (Olive oil test)

6.3.4.3. TLC (Thin Layer Chromatography) of the plants extracts:

In the practice of thin layer chromatography of the plant extracts concentrated extracts were used for separation and categorization of compounds. Crystallization of the plant extracts were done by the different solvent systems Hexane: Acetone (3:1) Chloroform: methanol (1:2), Benzene: Ethyl acetate (2:1) Toluene: Ethyl acetate (2:1). Crystallization of the plant extracts was performed to split the pure bio actives which were checked by thin layer chromatography and high pressure liquid chromatography (Harborne, 1973).

6.3.4.4. Antimicrobial efficacy of successive plant extracts:

For the examination of antimicrobial efficacy of two successive plants extracts disc diffusion method (Gould and Bowie, 1952).were employed. In this method the discs of different concentrations were prepared and the discs were imposed on respective media previously impregnated with selective microorganism.

6.3.4.5. MIC value (Minimum inhibitory concentration) of plant extracts:
In the investigation of minimum inhibitory concentration of the plant extracts the antimicrobial activity of selected microorganisms were observed by replicate disc diffusion assays. The selected microorganisms are *Enterobacter aerogenes*, (ATCC No.111) *Staphylococcus aureus* (7403), *Klebsiella pneumonia* (109),*Chryseobacterium gleum* (1916), *Shigella flexneri* (1457), *Proteus vulgaris* (744), *Bacillus subtilis* (441) and fungi *Candida albicans*, (MTCC Code - 3017), *Aspergillus niger* (MTCC Code - 1344), *Aspergillus flavus*, etc.and statistically MIC values were determined through replicate serial dilution assays. The MIC value was used for presenting antimicrobial activity against test microorganisms. After the incubation of plates the zone of inhibition were measured and the average of the zone was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values (Esimone *et al.*, 1998; Osadebe and Ukwueze, 2004).

6.3.4.6. Antioxidant activity of Methanolic extracts of plants:

In the screening of antioxidant activity of *Dyerophytum rubrum* (Gibs Ex Wt.) *Wrightia tinctoria* (Roxb R.Br.) DPPH (2, 2'-DIPHENYL-1-PICRYLHYDRAZL) assay was used (Takao *et al.*, 1994) for qualitative and quantitative estimation. These potentials can be used as to cure various ailments in human.

6.3.4.7. Anti-cancerous activity successive extracts of plants:

The potato disc assay (Ferigni *et al.*, 1982; McLaughlin, 1991; McLaughlin and Rogers, 1998; Coker et.al., 2003) was used to evaluate the antitumor activity of the extracts of the *Dyerophytum rubrum* (Gibs Ex Wt.) and *Wrightia tinctoria* (Roxb R.Br.) plants. *Agro bacterium tumefaction* was used to initiate tumor growth in fresh, disease-free and red skinned potato discs. Dimethyl sulfoxide (DMSO) was used to prepare test extracts, and appropriate volumes of test solutions were dispensed to achieve 50, 100, and 200 μg/disc.
6.3.4.8. Protein isolation and SDS PAGE for its characterization:

For isolation of total proteins of *wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, and Fruit) and *Dyerophytum rubrum* (Gibs Ex Wt.) (leaf, stem) 0.1 M Tris-borate pH 8.5 buffer was used and for leaf, stem, and root proteins, leaf stem, and root protein extraction buffer containing urea and 2-mercaptoethanol as a major compound was used. Total soluble proteins from *Wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, and fruit) and *Dyerophytum rubrum* Gibs. Ex Wt.(leaf, stem) were extracted by using 0.1 M Tris-borate buffer (pH = 8.5) and then total amount of proteins were estimated using Bradford Method.

6.3.4.9. DNA isolation and Gel Electrophoresis for its DNA Fingerprinting:

For DNA fingerprinting ,to score and preserve banding pattern a photograph of gel was taken by digital photographic system, under UV Tran’s illuminator. RAPD bands were designated on the basis of their molecular sizes (length of polynucleotide amplified). λ DNA EcoRI/Hind III double digest was loaded with each primer products to estimate the molecular size. The distance run by amplified fragments from the well was translated to molecular sizes with reference to molecular weight marker. The presence of each band was scored as ‘1’ and its absence as ‘0’. Faintly visible bands were not scored but a major band corresponding to faint band was considered for scoring. To confirm the presence of bands and determine reproducibility all the primers were replicated twice and if necessary thrice.

6.3.4.10. NMR spectrum of isolation pure compounds:

In the process of NMR spectrum of isolation pure compounds the compound was subjected to NMR analysis (model Brukur-DPX-300 MHz, using CDCL₃ and DMSO- d₆ as an internal reference) along with the standard reference compound.

6.3.4.11. Statistical examination of data:
All the data was analyzed by standard statistical analysis methods. The results were evaluated by www.physics.csbsju.edu.

6.4. RESULTS AND DISCUSSION

6.4.1. Extraction of the plants

In the process of extraction of the plants selected species were dried and powered (gm.) and for the six hours duration the samples were soxhelt extracted in petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water. Then extracts were filtered, dried and weighed. These extracts were used for phytochemical, TLC and antimicrobial antioxidant, anti tumour screening of plant active compound.

6.4.2. Phytochemical examination of successive plant extracts:

In the screening of phytochemical examination of successive plant extracts, two Indian medicinal plants *Wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, and fruit) and *Dyerophytum rubrum* (Gibs Ex Wt.) (leaf, stem, and root) were selected for the evaluation of various bio-active compounds. Likewise reducing sugar, saponins, tannins, flavonoids and terpenoids etc.

6.4.3. Thin Layer Chromatography (TLC) of the plants:

Thin layer chromatography technique used for separation, identification and semi-quantification of a wide variety of substances by scanning chromo-strips with or without detecting reagents, under normal or UV light. It is very simple, fast and low priced method.

The resulting differential chromatographic fingerprints used as markers in the standardization of each extract in a particular solvent system separating the compounds at specific $R_f$ value, which will differ to other plant extracts. These $R_f$ values are easy, reproducible and consistent marker to confirm the purity of the crude drugs. In view of this, TLC examinations of different plants were carried out. TLC fingerprints were generated from petroleum ether extracts of *Wrightia tinctoria* (Roxb R.Br.) Pet.ether extract (leaf, stem, and fruit), *Wrightia tinctoria* (Roxb R.Br.) Chloroform extract (leaf,fruit), *Wrightia tinctoria* (Roxb R.Br.) Methanol extract (leaf), *Dyerophytum rubrum* (Gibs Ex Wt.) Ethyl acetate extract (stem), *Dyerophytum rubrum* (Gibs Ex Wt.)
Methanol extract (stem), *Dyerophytum rubrum* (Gibs Ex Wt.) Pet. Ether extract (leaf, stem) using solvent system Hexane: Acetone (3:1). Chloroform: methanol (1:2), Benzene: Ethyl acetate (2:1) Toluene: Ethyl acetate (2:1) by these fingerprints, the quality control of an authentic drug in various quarantines can be achieved.

6.4.4. **Antimicrobial activity of successive extracts:**

Plants have a rich source of antimicrobial agents. Plants are used medicinally in different countries and these are the source of many effective drugs (Srivastava *et. al.*, 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. Hundreds of plants species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. (Bollinger *et. al.*, 1985).

In the present work a two selected medicinal plants were screened for potential antimicrobial activity by disc diffusion method and found significant results. *Wrightia tinctoria* (Roxb R.Br.) fruit petroleum ether extracts showed the most excellent activity against *Enterobacter aerogenes* (13.5mm) at different concentration then other extracts and leaf and stem also show the effective antimicrobial agent against selected microorganisms. *Wrightia tinctoria* (Roxb R.Br.) Leaf Benzene extract also showed the highest inhibition zone against *Shigella flexneri, Bacillus subtilis* (15mm), *Wrightia tinctoria* (Roxb R.Br.) Stem Benzene extract were screened as antimicrobial agent and fruit Benzene extract showed the maximum efficacy against *Shigella flexneri* (15.5mm) and *Wrightia tinctoria* (Roxb R.Br.) Leaf, stem and Fruit ethyl acetate extracts showed the excellent efficacy. The highest activity showed by *Wrightia tinctoria* (Roxb R.Br.) leaf ethyl acetate extract against *Klebsiella pneumonia* (26mm), *Wrightia tinctoria* (Roxb R.Br.) leaf Methanol extract showed the maximum inhibition against *Staphylococcus auras* (21 mm), *Wrightia tinctoria* (Roxb R.Br.) plant extracts also showed the effective anti fungal activity. The maximum efficacy was showed by *Wrightia tinctoria* (Roxb R.Br.) leaf Methanol extract against *Aspergillus fumigates* (20mm), it showed that the *Dyerophytum rubrum* (Gibs Ex Wt.) Plant was the highly effective against fungal infection and Bacterial infection and also inhibits the growth of other microbial infections. *Dyerophytum rubrum* (Gibs Ex Wt.) Plant leaf, stem, root was also screened
for antimicrobial efficacy against selected test microorganisms. *Dyerophytum rubrum* (Gibs Ex Wt.) Root Pet. ether showed the maximum inhibition zone against Klebsiella pneumoniae (11mm), *Dyerophytum rubrum* (Gibs Ex Wt.) leaf Benzene extract showed maximum inhibition zone against *Bacillus subtilis* and *Staphylococcus aureus* (18mm) and stem extract against Klebsiella pneumonia (16mm). *Dyerophytum rubrum* (Gibs Ex Wt.) leaf chloroform extract was highly effective against *Bacillus subtilis* (20.33mm) stem and root also showed the moderate activity against selected microorganisms. *Dyerophytum rubrum* (Gibs Ex Wt.) Stem and root also showed the effective inhibition zone against *Staphylococcus aureus* (16mm). *Dyerophytum rubrum* (Gibs Ex Wt.) Stem Methanol extract showed the effective activity against *Bacillus subtilis* (20mm). *Dyerophytum rubrum* (Gibs Ex Wt.) leaf showed the effective antifungal activity against *Aspergillus fumigates* (12mm). In all cases concentration play an important amount of variation in the quantity of bacteria and fungi killed as measured using the statistical analysis and graphs shows a graphical example of the statistical relationships.

**6.4.5. MIC (minimum inhibitory concentration) of the plant extracts:**

In the determination of MIC value, the lowest MIC’s were obtained from plant extracts, signifying that the microbial strains were more sensitive to the extracts. No connection was distinguished between the antibiotic susceptibility of the strains and their receptiveness to the plants, as the plants efficiently inhibited antibiotic resistant strains, while some antibiotic sensitive strains appeared to show resistance to the plants.

**6.4.6. NMR spectrum of isolation pure compounds:**

The NMR spectroscopy and its spectral outline are really reactive and have the ability to distinguish not just the compound but also the extracts of different plant materials of common individuality. In the NMR spectroscopy Monograph finished from the important findings of the current study. Besides this, the resultant conclusions are of practical nature.
6.4.7. Statistical analysis of the data:

In the statistical analysis of the data, Diameter of bacterial and fungal growth was measured and expressed as means of percentage growth inhibition of three replicates. Significant differences within the means of treatments and controls were calculated using statistical test.

The MIC value is defined as the lowest possible concentration of antibiotic or extract at which there is no visible growth. The agar plates without extract or standard antibiotic (the negative control) and the plates containing standard antibiotics, tetracycline (the positive control) were also streaked with the micro organisms. The agar plates were incubated at 37°C for 24 h (for the bacteria) and at 25°C for 48 h (for the fungus). The inhibition zone diameter, the measure of activity, was consequently determined by plotting the square of the inhibition zone diameter (IZD²) against the log concentration of the extract and the MIC calculated from the intercept on the log concentration axis.

6.4.8. Antioxidant activity of Methanolic extracts of plants:

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Some natural antioxidants are already commercially exploited as antioxidant additives or as nutritional supplements. The major constituents of biological membranes are lipids and proteins.

In the exploration antioxidant activity of Dyerophytum rubrum (Gibs Ex Wt.) showed good activity against the DPPH assay method, where the regression line showed the effectiveness of it as it have potentials which are comparable to ascorbic acid. It appeared that all tested samples showed dose dependent inhibitory potency and the effective dose (ED₅₀) was 1.2065µg/ml. The plant Wrightia tinctoria (Roxb R.Br.) showed appreciable activity against the DPPH assay method, where the regression line clear cut showed the effectiveness of it as it have potentials which are comparable to ascorbic acid. It appeared that all tested samples showed dose dependent inhibitory potency and the effective dose (ED₅₀) was 0.209µg/ml.
6.4.9. Anti-cancerous activity successive extracts of plants:
(C.G. =crown galls)

The arid zone plants *Wrightia tinctoria* (Roxb R.Br.) and *Dyerophytum rubrum* (Gibs Ex Wt.) showed the effective anti-tumour activity against *Agerobacterium tumefaciens*. The *Wrightia tinctoria* (Roxb R.Br.) Leaf methanol extract showed the significant inhibition against *Agerobacterium tumefaciens* (Bacteria). The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-7). The *Wrightia tinctoria* (Roxb R.Br.) leaf Ethyl acetate extract showed the significant inhibition against *Agerobacterium tumefaciens*. The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-8). The *Wrightia tinctoria* (Roxb R.Br.) leaf Benzene extract showed the significant inhibition against *Agerobacterium tumefaciens*. The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-7). The *Wrightia tinctoria* (Roxb R.Br.) leaf water extract showed the significant inhibition against *Agerobacterium tumefaciens* (Bacteria). The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-7). When analysis of antitumor action of *Dyerophytum rubrum* (Gibs Ex Wt.) leaf methanol extract) was study in opposition to *Agerobacterium tumefaciens*. The *Dyerophytum rubrum* (Gibs Ex Wt.) leaf methanol extract showed the significant inhibition against *Agerobacterium tumefaciens*. The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-5). The *Dyerophytum rubrum* (Gibs Ex Wt.) leaf water extract showed the significant inhibition against *Agerobacterium tumefaciens* (Bacteria). The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-7). The *Dyerophytum rubrum* (Gibs Ex Wt.) leaf Ethyl acetate extract showed the significant inhibition against *Agerobacterium tumefaciens*. The maximum Crown gall was showed by the A3 (200 μg/disc concentration) was against *Agerobacterium tumefaciens* (C.G.-7).

6.4.10. Protein isolation and SDS PAGE for its characterization
The present investigation included *Wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, and fruit) for the analysis of proteins for characterization. SDS-PAGE technique and leaf stem, and root proteins was used for identify and characterization of the plant in the present study. The concentration of proteins in estimated sample was found to be from 3.8 to 7.9 mg/120 mg of *Wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, and fruit). Finally the equal amount of extracted and estimated proteins subjected to SDS-PAGE to generate banding pattern with a protein molecular weight marker (PMW-M from Banglore genei). After electrophoretic separation of seed storage proteins, 15 band positions were observed and finally scored for analysis Among 15 band positions, 6 were found to be polymorphic with 40 % polymorphism. In the case of *Dyerophytum rubrum* (Gibs Ex Wt.) electrophoretic separation of seed storage proteins, 15 band positions were observed and finally scored for analysis among fifteen band positions; three were found to be polymorphic with 20 % polymorphism.

### 6.4.11. DNA isolation and Gel Electrophoresis for its DNA Fingerprinting

In the DNA Fingerprinting of *Dyerophytum rubrum* (Gibs Ex Wt.) plant eight band positions were observed and finally scored for analysis, Among 8 band positions, and three were found to be polymorphic with 37% polymorphism. And in the case of *Wrightia tinctoria* (Roxb R.Br.) plant DNA fingerprinting eight band positions were observed and finally scored for analysis, among eight band positions, two were found to be polymorphic with 25% polymorphism.

### 6.5. CONCLUSION

Many medicinal plants have been found effective in the cure of bacterial disease. Petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water extracts of certain Indian Medicinal Plants *Wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, Fruit) and *Dyerophytum rubrum* (Gibs Ex Wt.) (leaf, stem, and root) were examined for their anti-microbial, potentials against selected bacteria and fungi. The purpose of screening is to justify, authenticate and validate the use of Indian Medicinal Plants in ethno-medicinal or folklore as traditional treasure to cure various ailments and disease caused by environmental pollution. The various extracts from traditional medicinal plants with folklore reputation have been examined to identify the source of therapeutic drugs,
were tested against selected test bacteria and fungi as antimicrobial assay through disc diffusion assay where standard tetracycline was used. Indian Medicinal Plants have a traditional background that they have potentials to use as antimicrobial agents. The results showed that all the extracts possess good antimicrobial activity against selected test bacteria and intermediate against fungus, therefore offer a scientific basis for traditional use of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water extracts of *Wrightia tinctoria* (Roxb R.Br.) (Leaf, stem, Fruit) and *Dyerophytum rubrum* (Gibs Ex Wt.) (leaf, stem, root) it justify their use in our traditional system of medicine to cure various diseases. And in future references’ these plants can be used as effective antioxidant agents due to the presence of anti oxidant activity and these plants exhibited the excellent anti tumour activity against selected microorganisms these plants can be implanted in the anti tumours drugs to cure the cancer.

Various bioactive compounds which were isolated from progressive research of various extracts have vast antibiotic activity and in future these plant extract can be employed in drug recovery against incurable diseases and protein isolated from these plants can be used for curing of ailments.