3. Review of Literature

3.1. Burden of Tuberculosis

Zumla and Grange (1998) – Stated that the infectious fatal disease, tuberculosis (TB), is the leading cause of death worldwide from a single human pathogen, causes more deaths than diseases like acquired immunodeficiency syndrome (AIDS), malaria, diarrhoea, leprosy and all other combined tropical diseases.

Horne, (1996) - Stated that the tubercle bacillus, *Mycobacterium tuberculosis* (MT) is the organism usually responsible, discovered by Robert Koch in 1882. *M. bovis*, which usually infects cattle can also infect man and in West Africa, *M. africanum* also is a cause of TB. In addition, a number of normally non-pathogenic mycobacteria, particularly *M. avium*, *M. intracellulare* and *M. scrofulaceum*, may also be the causative agent and create opportunistic infections in AIDS patients.

WHO Global Tuberculosis Report (2015) - Reported that Tuberculosis (TB) is a big global health problem. It causes poor health among millions of people each year and close to the human immunodeficiency virus (HIV) as a major cause of death all over the world. It was estimated that in 2014, there were 9.6 million new TB cases: 5.4 million among men, 3.2 million among women and 1.0 million among children. Furthermore, there were 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people), of whom nearly 890,000 were men, 480,000 were women and 140,000 were children.

National Framework for Joint HIV/TB Collaborative Activities, Government of India, November (2013) - Says that the adult HIV prevalence in India is estimated to be 0.27% translating into 2.1 million people living with HIV/AIDS (PLHIV) in 2011. This is third highest number in the world. On the other hand, India is highest Tuberculosis (TB) burden country in the world with an estimated new TB cases occurring yearly are about 2.2 million.
Sharma S.K. et al. (2004) - Is of the opinion that Tuberculosis (TB) is primarily a disease of poor people, with 95 per cent of cases and 98 per cent of deaths occurring in developing countries of the world.

3.2. HIV –TB co infection
Rastogi N et al. (1998) – Stated that Tuberculosis is commonest opportunistic infection (OI) in Human Immuno-deficiency Virus (HIV) infected individuals. *M. tuberculosis*, *M. avium* and *M. kansasii* in the recent past have been reported as major opportunistic infections among patients with Acquired Immuno Deficiency Syndrome (AIDS).

National Framework for Joint HIV/TB Collaborative Activities, Government of India, November (2013) - Emphasizes that TB is commonest opportunistic infection (OI) in individuals infected with HIV, HIV infection is an major risk factor for acquiring TB infection and its further development into active TB. HIV/TB together is a killer combination with extremely high death rates (15 to 18%) reported among HIV-infected TB cases notified under Revised National TB Control Programme (RNTCP). Overall, TB is estimated to claim about 25% of all lives among PLHIV in India.

3.3. Drug Resistance
Devis et al. (1994) – Stated that due to indiscriminate and irrational use of Antimicrobial drugs, microorganisms have developed resistance to many drugs. This has created immense clinical problems to cure infectious diseases.

Punopas, K et al. (2002) is of the opinion that in the current scenario of emergence of antibiotic resistance in pathogenic organisms there is an urgent need to developed alternative antimicrobial drugs for treatment of infectious diseases.

Jiménez-Arellanes MA (2014) – Reported that present treatment for sensitive TB is based on a multi therapy consisting of the mixture of five first-line drugs INH, RIF, Ethambutol (EMB), Streptomycin (STR) and/or Pyrazinamide (PYR), a treatment lasting up to 6–8 months. However, currently, many cases
do not respond to the established therapies because of the presence and emergence of MDR strains, which are characterized by being resistant to RIF and INH (basic drugs for their treatment). MDR cases are treated with second-line drugs (Capreomycin, Kanamycin, Amikacin, Cyclocerin, Fluoroquinolones, Ciprofloxacin and Proteonamide, among others), but these agents have the disadvantage of not being specific, leading to severe side effects, poorly tolerated, expensive, easily inducing resistance, entailing a prolonged treatment duration (up to 30 months) and poor adherence to treatment.

A situation that even more threatening for the world is XDR-TB, as there is no other option for treating the disease and because these cases respond precariously to first- and second-line drugs. XDR-TB is caused by *M. tuberculosis*, which is resistant to INH and RIF; and also resistant to up to three injectable second-line drugs (capreomycin, kanamycin and amikacin), as well as to fluoroquinolones.

**WHO Global Tuberculosis Report (2015)** - Emphasizes that Tuberculosis becomes even more serious problem because, *Mycobacterium* developed resistance against both the first line and the second line drugs. Because of this, multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* have been emerged in the recent past, globally including India. All over the world, by 2015, an estimated 3.3% of new TB cases and 20% of previously treated cases have MDR- TB. Extensively Drug Resistant TB (XDR-TB) had been reported by 105 countries. It is estimated that 9.7% of people with MDR-TB have XDR-TB.

**Brigden G et al. (2014)** - Reposted that In 2005, extensively drug-resistant (XDR) cases of TB were found and identified as cases of patients who do not respond to any of the anti-tubercular drugs employed, they are resistant to at least Isoniazid (INH), Rifampicin (RIF), one fluoroquinolone and any of the second-line injectable drugs (amikacin, kanamycin, or capreomicin). The presence of XDR-TB is due to poor diagnosis, inadequate treatment and the abandonment of the patient’s treatment. Side effects also appear because the second-line drugs are more toxic and the treatment must be subjected to a
longer period (up to 36 months), it is a complex regime, ineffective, poorly tolerated and expensive.

3.4. Drug – drug reactions

Table No. 1


<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse effects</th>
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<tbody>
<tr>
<td>Isoniazid</td>
<td>Skin rash, hepatitis</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Abdominal pain, nausea, vomiting, hepatitis,</td>
</tr>
<tr>
<td></td>
<td>thrombocytopenic purpura</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Arthralgia, hepatitis</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Vestibular and auditory nerve damage, renal Damage</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Retrobulbar neuritis, ocular side effects</td>
</tr>
<tr>
<td>Thioacetazone</td>
<td>Skin rash, Exfoliative dermatitis</td>
</tr>
<tr>
<td>Para-aminosalicylic Acid</td>
<td>Anorexia, nausea, vomiting, hypersensitivity Reactions</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>Vertigo, auditory nerve damage, nephrotoxicity</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>Diarrhoea, abdominal pain, hepatotoxicity</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>Dizziness, headache, depression, psychosis, Convulsions</td>
</tr>
</tbody>
</table>

Punopas, K et al. – emphasized that antibiotics are associated with adverse effects on patients, which include elimination of beneficial gut/mucosal microorganisms (normal flora), immunosuppression and hypersensitivitv / allergic reaction.

Hasan Ali A et al.( 2013) and Singla R et al.(2010) – Are of the opinion that in addition to resistance development, Anti-TB drug induced hepatotoxicity, is a common serious adverse drug reaction of these drugs, is one of the most difficult clinical compliant and main reason of treatment interruption during TB treatment course, which causes hospitalization and life threatening conditions. Among the first line anti-TB drugs, pyrazinamide, isoniazid and rifampicin have all been associated with hepatotoxicity and the risk is increased when these drugs are used together in combination.
Van der Walt M et al. (2013) - Stated that different studies reported that 1–31% of TB patients experience drug related side effects like hepatotoxicity during and after TB treatment.

Mallolas J et al., (2007) – Studied that Rifampicin is an important drug for the treatment of TB. However, administration of Rifampicin in combination with antiretroviral therapy, mainly protease inhibitors, is problematic because of drug-drug interactions.

3.5. Anti-microbial activity of Medicinal Plants

Ahmad, Mehmood and Mohammad (1998) - Stated that World Health Organization (WHO) reported that 80% the world population depends chiefly on traditional medicine and a major part of the traditional therapies, which includes the use of the Plant Extracts or their active constituents.

A Handbook of Medicinal Plants: A complete source book, N. D. Prajapati, (2010 ) - Describes that Medicinal plants offer a great hope to fulfill these needs and have been used for treating diseases from many centuries. These have been used extensively as pure compounds or as a raw material. India is one of the few countries in the world which has unique wealth of medicinal plants and extensive traditional knowledge of use of herbal medicine to treat various diseases.

Taylor et al., (2001) - Emphasizes that traditionally used medicinal plants have recently dragged the attention of the clinicians, pharmaceutical and scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations.

Osbourne, (1996) - Reported that Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack.

Baris et al., (2006) – Are of the opinion that Plant-derived substances have recently become of great interest owing to their versatile applications.
Hammer et al., (1999) – Conclude that Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

Gibbons (2003) - Reported that a number of interesting outcomes have been obtained with the use of a mixture of natural products to treat diseases, most significantly the synergistic effects and polypharmacological application of plant extracts.

3.6. Plant Material

A Handbook of Medicinal Plants: A complete source book, N. D. Prajapati, 2010 - Describes that the plants growing in particular soils, with pattern of weather, sunlight, time of harvesting, method of preparation of medicine all are very important. The same species of plants growing in rain forest and city backyard may not have same medicinal value.

Pure and isolated plants constituents are of immense importance because they have given various world’s most useful drugs. For example, tubocurarine, the most power muscle relaxant in existence, is derived from curare (Chondrodendron tomentosum), and the strongest painkiller of all, morphine, is obtained from opium poppy (Papaver sominifarum). Many anesthetics are also derived from plants, for example cocaine, which is obtained from coca (Erythroxylem coca). It is difficult to think of a world deprived of the antimalarial properties of quinine; or heart remedy digoxin (from Digitalis) or the cough – reliving properties of ephedrine which is present in many prescriptions and over – the – counter cold remedies. These and many other conventional medicines are all obtained from isolated plant constituents. These are most effective of all traditional drugs.

Gautam R et al. (2007) - Stated that a few hundred plant species have been tested and have exhibited to have an antimycobacterial activity.

Santhosh RS et al. (2014) – Reported activity of 127 antimycobacterial plant compounds like alkaloids, flavonoids etc.
Gautam AH et al. (2012), Bernaitis L (2013) and Arya V et al. (2011) - Reported the anti-mycobacterial activity of *Aloe vera* in their studies.

Sharma P et al. (2013), Gautam AH et al. (2012), Kirimuhuzya C (2009) and Pradhan RR et al. (2012) - Stated that *Lantana camara* has the substances and potential to be an anti-mycobacterial agent.

Richard M Mariita et al. (2010) and Gautam AH et al. (2012) - In their studies reported various antibacterial activities of *Acacia Senegal*.

Sandra M. Newton et al. (2000), Nandagopal B et al. (2011), Arya V et al. (2011), Patrícia F. Leal et al, Jethva K et al. (2016), Reported *Ocimum sanctum* to have the anti-mycobacterial activity in different studies..

Gupta R et al. (2010) and Bernaitis L (2013) - Used fresh Juice of leaves of *Aloe vera* as extract in their study.

### Table No. 2

List of references who reported plant activity

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant Name</th>
<th>Activity</th>
<th>Reported / Studied by</th>
</tr>
</thead>
</table>
Kirimuhuzya C et al. (2009)- In his study, described that the leaves of plants were chosen by hand. Only mature plants included. Plants that appeared to have viral, bacterial or fungal infections were not included. All leaves were washed with distilled water to remove adherent dust. These washed leaves were then dried at room temperature away from direct sunlight. The completely dried leaves were pulverized to obtain a fine powder and stored at room temperature.

Fresh or dried plant material can be used to obtain secondary plant components. Although, majority of researchers are working on the chemistry of secondary components of plants have preferred to use dried plant material for many factors. Because differences and variation in water content can alter the solubility of subsequent separation by liquid-liquid extraction and the secondary metabolic plant components should be relatively stable, particularly if we are using it as an antimicrobial drug. Many plants are used in the dry form (or as an aqueous extract) by traditional healers. Plants are usually air dried (Dilika et al, 1996; Baris et al., 2006) to a constant weight but other researchers dry the plants in the oven at about 40°C for 72 hours (Salie et al., 1996). Also, plants will have difference in constituents depending on the geographic and environmental conditions where it is growing. The decision of plant material used in the extract preparation is generally decided by the traditional use of the plant and the ease of handling of the various plant parts like the leaves, stems etc.

3.7. Choice of Solvent & Extraction Method

Parekh et al. (2005) - Reported that plant extracts from organic solvents exhibited to give better consistent antimicrobial activity compared to water extracts. The most commonly used solvents for evaluation of antimicrobial activity in plants are methanol, ethanol, and water.

Fabricant and Farnsworth, (2001)- Are of the opinion that extraction is the important first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-
washing, drying of plant materials or freeze drying, grinding to grinding a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples.

**Cos et al. (2006)** – Stated that it is necessary to prepare the extract as it has been described by the traditional healer in order to imitate as near as possible the traditional ‘herbal’ drug. The selection of solvent system to a large extent depends on the specific nature of the targeted bioactive compound. Various solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll. Variations in extraction methods are generally the length of the extraction period, solvent used, pH, temperature, particle size and the solvent-to-sample ratio. The longer the contact between solvent and material the more is extracted until all possible materials have been extracted. The length of the extraction period can be cut short by grinding the plant material finely because this will enhance the surface area for extraction and in this way, increasing the rate of extraction. Shaking during extraction of the plant material-solvent mixture also increases the extraction rate.

A study by Eloff (1998b), concluded that 5 min extractions of very fine particles of diameter 10 µm gave higher quantities than values obtained after 24 hour in a shaking machine with less finely ground material.

Other researchers employ soxhlet extraction of dried plant material using organic solvents (Kianbakht and Jahaniani, 2003). In soxhlet extraction, the sample is continually exposed to fresh solvent, which improves the efficiency of the method. The method works well for compounds that can withstand the temperature of the boiling solvent, but can not be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (de Paira et al., 2004)
3.8. AST Method

(Lampinen, 2005) - Described that the antimicrobial susceptibility test (AST) is a technique necessary in most disciplines of medical science. It is used in pathology to evaluate resistance of microbial strains to antimicrobials, and in ethnopharmacology research, it is used to measure the effectively of novel antimicrobials against microorganisms, especially medical important ones. The test is the initial step for the development of new anti-microbial drug. There are different AST methods which are used by researchers and therefore, there is a possibility of variations in results obtained.

In research of ethnopharmacology the antimicrobial susceptibility test (AST) is employed to get the efficacy of potential antimicrobials from biological extracts against various microbial species. AST methods are used to screen plant extracts for antimicrobial activity and are widely used to determine the potentiality of an antimicrobial in combating infections by calculating its minimum inhibitory concentration (MIC). In vitro susceptibility tests are especially important in clinical research, when an organism is known or belongs to a species that has shown resistance to commonly used antimicrobial drugs. AST methods are also important in epidemiological studies of susceptibility and for comparisons of newer and existing microbial drugs. (EUCAST, 2003).

Lampinen, (2005) - Is of the opinion that successful discovery of novel natural antimicrobials has necessitated the discovery of new bioassay techniques that can be sensitive enough to find out even the smallest amounts of biologically active compounds.

Hammer et al. (1999) – Stated that although present standard methods, acknowledged by various bodies like the National Committee for Clinical Laboratory Science (NCCLS) [now known as Institute of Clinical Laboratory Standards (ICLS)], British Society for Antimicrobial Chemotherapy (BSAC) and the European Committee for Antimicrobial susceptibility testing (EUCAST), exist for guidelines of antimicrobial susceptibility testing of
conventional drugs, they may not exactly be used in the same manner applicable for plant extracts and modifications or improvisation need to be made.

**Satim and Washington (1991)** – Are of the opinion that screening plant extracts for antimycobacterial activity is generally carried out using *mycobacteria* cultured in various types of broth and agar based media. *M. tuberculosis* has the disadvantages of being slow growing so tests take many weeks and arrangement of containment facilities are also needed as it is an infectious pathogen. Many researchers have therefore used non-pathogenic species of mycobacteria such as *M. avium, M. intracellulare* and *M. kansaii*, which like *M. tuberculosis* are slow growing, and other species including *M. chelonei, M. fortuitum* and *M. smegmatis* which are faster growing so that the tests can be completed in a few days. Generally, the test methods used are the disc diffusion and the broth dilution methods. In the disc diffusion method, paper discs impregnated with the extract under test are placed on a semi-solid (agar based) medium which has been inoculated with mycobacteria. After incubation, zones of inhibition of bacterial growth around the discs are measured. The main disadvantages with this method are that non-polar compounds may not diffuse into the agar so that active compounds may be missed and that it is not possible to obtain reliable quantitative results for comparative purposes. In the broth dilution method, the minimum concentration required to inhibit bacterial growth (minimum inhibitory concentration, MIC) is determined using a series of tubes containing serial dilutions of the extract in inoculated broth; however solubilization of the extracts under test may be a problem.

**RNTCP Manual of SOPs (2009)** - **Suggests** that the proportion method using L J Medium is currently the method of choice, in the majority of laboratories in the world.

**Gupta R et al. (2010) & Bernaitis L et al. (2013)** - Described in their study that the plant extract was incorporated in the medium at concentration of 2 per cent v/v and 4 per cent and 6 per cent v/v (2 ml, 4 ml and 6 ml of 1% fresh plant
extract stock solution was dissolved into 100 ml of culture medium) prior to inspissation in inspissator at 85°C for 85 min.

**Gupta R et al. (2010)** - In their study stated that for comparison, extract free control slants were used. Blank slants were also incubated to check the sterility/quality of the medium. Susceptibility testing of this strain was also performed against standard drug isoniazid in the same batch of media for comparison of cfu on drug free controls.

**Bernaitis L et al. (2013)** - In their study reported that, mycobacterial suspension of 1 mg/ml, equivalent to MacFarland standard-1 was prepared. Ten-fold dilution of standard 1 mg/ml suspension was streaked on L-J medium for determining cfu in the presence and absence of plant extracts. A 0.01 ml of this suspension was inoculated on each L-J slant. Each test was done in duplicate. Percentage inhibition was calculated by mean reduction in number of colonies on extract containing as compared to extract free controls.

**Pfyffer GE (2012)** - Reported that the medium was set inoculated with the standard bacterial suspension incubated at 37°C for 21-42 days, reading was taken weekly.