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ANTIMICROBIAL POTENTIAL OF HERBAL MEDICINES

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ABSTRACT

Herbal medicines are widely used since time immemorial indicating that herbs are a growing part of modern, high-tech medicine.India has an ancient heritage of traditional herbal medicine. The World Health Organization estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. The use of herbal drugs for treating various diseases predates human history forms the origin of much of the modern medicine.The medicinal plants are used for various diseases because of their safety and effectiveness. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to control the use of antibiotic, to develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop either synthetic or natural new drugs. Numerous studies have been done on herbals confirming their potential antimicrobial property against microorganisms. One of the strategies towards attaining this objective is the rational localization of bioactive phytoconstituents. This review provides the important methods for evaluating the antibacterial potency of selected plants and also the mechanism of action responsible for antimicrobial potency.

INTRODUCTION: "An apple a day keeps the doctor away"- Traditional American rhyme.

The idea of obtaining healing properties from plants and plant products dates back centuries ago. Plants provide a vast collection of natural chemicals that can be used as medications. Plants have an extraordinary ability to synthesize aromatic substances which are usually phenols or their oxygen-substituted derivatives. The medicinally active plant compounds are usually their secondary metabolites like terpenoids, quinones, flavonoids, tannins etc that are responsible for protecting the plants from microorganisms, insects and other natural pests. The classified treatment of diseases with the help of antibacterial substances dates back to 1500's starting from the cinchona bark against malaria and ipecacuanha for dysentery. Nowadays antibacterials are one of the most widely used drugs and since the effective lifespan of any antibiotic is limited the importance of plant derived antibiotics is gaining more importance. It is estimated that only 10% of the total stock of plant metabolites have been isolated for medicinal purposes which again shows the vastness of therapeutic agents in the plant kingdom [1]. One of the major advantages of phytopharmaceuticals over synthetic ones is the decreased side effects even on extended use and their increased biocompatibility.

Stanway in 1980 reported the presence of secondary enhancing side effect eliminating substances in plants and his statement was exemplified by digitalis [2]. Due to the natural phenomenon of bacterial adaptation to antibiotics, the plant products are purified and only the active chemical constituents are isolated and used as opposed to the use of crude drugs in ancient times. This ensures the efficacy of the medications and reduction in the side effects. The quality and efficacy of phytopharmaceuticals also depends on the methods of cultivation, collection, storage and processing.

Mechanism of Antibacterial Action: To understand the mechanism of action of antibacterial drugs it is necessary to have a clear understanding of the structural organization of the bacteria. Microorganisms especially bacteria's can be broadly classified into gram-positive organisms and gram-negative organisms based on the staining with grams stain. The cell wall of gram positive bacteria's is relatively simple and consists mainly of peptidoglycan, acidic polymers, proteins and polysaccharides. The cell wall of gram negative bacteria is much more complex and consists of an inner and an outer membrane of a bilipid layer. The complex polysaccharides constitute the endotoxins that may cause fever and other inflammatory responses. The effectiveness of any antibacterial drug depends on its ability and extent of penetration into the complex outer layer [3].Some basic mechanisms of action of antibacterial drugs are;

Interferation with the synthesis or action of folates: These compounds usually disrupt the metabolic pathways of bacteria and are often called antimetabolites. They act by blocking the functioning of metabolic pathways by completely inhibiting the use of metabolites by key enzymes. Folate is an essential component required for the synthesis of DNA and RNA precursors both in mammals and microbes. But unlike mammals who obtain folate through nutrition bacteria's have to synthesis their own folate[4]. Compounds like sulphonamides compete with the PABA (p-aminobenzoic acid) for the enzyme dehydropteroate synthetase. Sulphonamides belong to the class of bacteriostatics (inhibit bacterial growth) rather than bactericidal.

Interferation of the bacterial cell wall peptidoglycan synthesis: This type of action is usually exhibited by β-lactam antibiotics. They act by attaching to the penicillin binding sites on the bacteria's and inhibiting the transpeptidation enzyme that links the peptide chains that are attached to the backbone of peptidoglycan. The lysis of the bacterium occurs due to the inactivation of an autolytic enzyme inhibitor. Penicillin and all its derivatives belong to this group of antibacterial.

Disrupting Bacterial Protein Synthesis: Certain compounds act by inhibiting the bacterial protein synthesis upon intake into susceptible organisms by active transport. They usually act by binding with the prokaryotic ribosome, either with the 50s subunit or the 30s subunit. The binding of the compound with the ribosomes will cause a misreading of the m-RNA and this in turn will affect the process of protein synthesis. They destroy the organism by affecting any of the following steps: aminoacyl t-RNA binding, peptide bond formation, mRNA reading, and translocation [4].

Effecting Topoisomerase- 2: Certain compounds affect the replication and transcription process of bacteria. They exert their action by inhibiting topoisomerase-2 which is basically a DNA gyrase. This enzyme is very crucial especially in single cellular organisms like bacteria as it is responsible for carrying out the processes of replication and transcription.

Antimicrobial Susceptibility Test:

Microdilution method: Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by Zgoda and Porter, with some modifications. A dilution series of the extract, ranging from 10 to 0.5 mg/mL, were prepared and then transferred to the broth in 96–well microtiter plates. The final concentrations were in the range 1000 to 50 µg/mL in the medium.Before inoculation of the test organisms, the bacterial and yeast strains were adjusted to0.5 McFarland and diluted 1:1000 in MuellerHinton Broth (Oxoid) and Malt Extract Broth (Oxoid), respectively. The plates were incubated at 35°C for 18 - 24 h for bacteria and 30°C for 48 h for the yeast cultures. Allthe tests were performed in broth and repeated twice. While the MIC

values of the extracts were defined as the lowest concentration that showed no growth, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by plating samples from clear wells onto Mueller Hinton Agar and Malt extract Agar, respectively. MBC and MFC were defined as the lowest concentration yielding negative subculture. Ampicillin and streptomycin were used as the standard antibacterial agents, while nystatin was used as the standard antifungal agent. Their dilutions ranged from 128.0 to 0.25µg/mL concentrations in microtiter plates.

General methods for Anti Microbial Screening: The commonly used test methods may be classified based on whether or not they require sterile samples. Sterilization by membrane filtration is excluded for aqueous dispersion or emulsions and can result in loss of antimicrobial activity. Sterilization by gamma radiation is effective, inexpensive but rather time consuming.

Cylinder Plate Method (Cup Plate): This type of assay method is based on measuring the area of microbial growth inhibition, around the sterile one centimeter stainless steel cylinder containing various concentrations of the test solution. The cylinders are placed on the surface of the solid nutrient media, preferably agar that is previously inoculated with a culture of the suitable microorganism. The area of inhibition is measured as the diameter of the region and the inhibition produced by the test compound is compared with that produced by the known concentration of the reference standard [5].

Diffusion Method: Here a reservoir with the plant extract to be tested is brought into contact with an inoculated medium and after incubation the diameter of the zone of inhibition around the reservoir is measured. The inoculated system is kept at a low temperature to decrease the detection limit. This process favors the diffusion over microbial growth and thus increases the inhibition. Different types of reservoirs include filter paper discs, porcelain or stainless steel cylinders placed on the surface of the medium and holes punched through it.

Dilution Method: In this method the samples being tested are mixed with a suitable medium that has been previously inoculated with the test organism. The minimum inhibitory concentration (MIC) is measured for the extract that produces complete inhibition of growth of microorganism. Various concentrations of the crude extracts is prepared using suitable solvent (DMF, DMSO) and added to a series of test tubes containing nutrient broth medium with microorganism. It is then incubated for a specified period of time. After the incubatory period the microbial growth can be estimated by direct visual or turbidimetric comparison of the test culture with a control culture which was not inoculated.

Disc Diffusion Method: It is the only suitable diffusion technique for testing aqueous suspensions of plant extracts. Here the presence of suspended particles in the sample being tested is much less likely to interfere with the diffusion of the antimicrobial substance into the agar than in the filter paper disc and cylinder plate methods. Here, Muller-Hinton Agar (MHA) medium is sterilized and poured into the plate; the agar is allowed to settle and stored at 4 degree Celsius. The surface of the MHA plate is then inoculated with the swab of the micro organism. The discs containing anti-microbial agent, various extracts are arranged on the surface of the inoculated plates in such a way so as to be at least 20mm away from one another and incubated at 35-37°C for 18-25 hours and the results are observed. The plates are then examined for the presence of inhibitory zones and the diameter of the inhibition zone is measured [65].

Bioautographic Methods: It is the method to localize antibacterial activity of organisms using a chromatogram, has found widespread application in the search for new antibiotics from microorganisms. Here the antimicrobial agent is transferred from the thin layer or paper chromatogram to an inoculated agar plate through a diffusion process. Then the zones of inhibition are visualized by appropriate vital stains. Although bioautographic methods are suitable for testing highly active antibiotics (MIC values of at least 10µg /ml). They did not prove very promising for testing plant extract, which often contain much less potent antimicrobial agents than the currently available antibiotics.

PLANT NAME	PARTS USED	METHOD OF SCREENING	MECHANISM OF ACTION
Arctium lappa-L[5]	Roots	Agar disc diffusion and well diffusion	Disrupting protein synthesis
Astragalus membranaceus[6]	Root	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Artemisia annua-L[7]	Leaves	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Beriberis chitria[9]	Rhizomes	Cup plate method	Disrupting protein and cell wall synthesis
Bupleurum chinense[10]	Root	MIC	Disrupting protein and cell wall synthesis
Camellia japonica- L[11]	Petals	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Cinicifuga foetida[12]	Roots	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Coccinia grandis[13]	Leaves	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Cornus chinensis[14]	Fruits	Cup plate method	Disrupting protein and cell wall synthesis
Cornus oblonga[15]	Fruits	Agar dilution method	Inhibiting folate synthesis
Cornus officianale[16]	Fruits	Cup plate method	Disrupting protein and cell wall synthesis
Corydalis solida[17]	Tubers	Agar dilution method	Disrupting protein and cell wall synthesis
Crataegus pinnatifida[18]	Fruits	Agar disc diffusion method	Inhibiting folate synthesis
Citrus limon[19]	Fruits	Cup plate method	Effecting Topoisomerase 2
Cimicifuga heracleifolia[20]	Root	MIC	Disrupting protein and cell wall synthesis
Cnidium officianale[21]	Roots	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Cuminum cyminum[22]	Essential oils	Agar dilution method	Disrupting protein and cell wall synthesis
Diospyros peregrine[23]	Fruits	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Dryopteris filix- mas[24]	Roots	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Eclipta Prostata[25]	Whole plant	Agar disc diffusion method	Inhibiting folate synthesis
Elsholtzia ciliata[26]	Whole plant	MIC	Disrupting protein and cell wall synthesis
Eriobotrya japonica[27]	Leaves	Cup plate method	Disrupting protein and cell wall synthesis
Eucalyptus globules[28]	Leaves	Agar well diffusion method	Disrupting protein and cell wall synthesis
Gentiana triflora[29]	Root	Agar disc diffusion method	Effecting Topoisomerase -2
Inula Britannica[30]	Flowers	MIC	Disrupting protein and cell wall synthesis
Lavandula latifolia[31]	Flowering stems	Agar dilution method	Effecting Topoisomerase -2
Lepidium sativum[32]	Roots, Leaves and seeds	Agar disc diffusion method	Inhibiting folate synthesis
Lonicera japonica[33]	Stem and flower	Cup plate method	Disrupting protein and cell wall synthesis
Lycium barbarum[34]	Root bark	Cup plate method	Inhibiting folate synthesis
Melaleuca alternifolia[35]	Leaves	Agar dilution method	Inhibiting folate synthesis
Mentha arvenis[36]	Whole plant	Agar disc diffusion method	Effecting Topoisomerase -2
Mahonia repens[37]	Root and Root bark	MIC	Disrupting protein and cell wall synthesis

Mahonia swaseyi[38]	Rhizomes	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Morus alba[39]	Leaves	MIC	Inhibiting folate synthesis
Paeonia japonica[40]	Root	Cup plate method	Disrupting protein and cell wall synthesis
Plantago lanceolata[41]	Leaves	Agar well diffusion method	Disrupting protein and cell wall synthesis
Prunella vulgari[42]	Whole plant	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Prunus mume[43]	Fruits	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Pulsatilla koreana[44]	Root	Agar disc diffusion method	Effecting topoisomerase-2
Psoralea corylifolia[45]	Seed	Agar disc diffusion method and Agar well diffusion method	Disrupting protein and cell wall synthesis
			Inhibiting folate synthesis
Perilla frutescens nankinensis[46]	Leaves, stems and leaves	Cup plate method	Disrupting protein and cell wall synthesis
Panax pseudoginseng[47]	Roots and flowers	MIC	Disrupting protein and cell wall synthesis
Phellodendron amurescence[48]	Bark	Cup plate method	Disrupting protein and cell wall synthesis
Picralima nitida[49]	Seeds and stem bark	Agar dilution method	Disrupting protein and cell wall synthesis
Pinus koraiensis[50]	Seeds	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Plantago asiatica[51]	Leaves and seeds	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Raphanus alba[52]	Whole plant	Agar disc diffusion method	Disrupting protein and cell wall synthesis
Rossa gallica[53]	Petals	Agar disc diffusion method	Inhibiting folate synthesis
Rubia cordifolia[54]	Root and stem	Agar dilution method	Disrupting protein and cell wall synthesis
Satureja thymbra[55]	Leaves	Agar dilution method	Disrupting protein and cell wall synthesis
Saxifraga stolonifera[56]	Whole plant	MIC	Disrupting protein and cell wall synthesis
Solanum indicum[57]	Fruits	Agar disc diffusion method	Inhibiting folate synthesis
Sinalis alba[58]	Seeds	Cup plate method	Inhibiting folate synthesis
Swietenia macrophylla[59]	Barks	Cup plate method	Disrupting protein and cell wall synthesis
Thlaspi arvense[60]	Whole plant	Agar well diffusion method and agar disc diffusion method	Inhibiting folate synthesis
Thuja orientalis[61]	Leaves	Cup plate method	Inhibiting folate synthesis
Verbena officinalis[62]	Leaves and flowering stems	MIC	Disrupting protein and cell wall synthesis
Vitex negundo[63]	Whole plant	Agar disc diffusion method	Disrupting protein and cell wall synthesis

CONCLUSION: Scientists and researchers across the world are eying plants as a future source of unlimited antimicrobial agents and are in a desperate need of isolating compounds to keep in pace with the resistance of microorganisms. Thousands of compounds of plant origin with inhibitory effects on bacteria have been isolated and are in the different stages of development. A standardized method of

extraction and in vitro testing is bound to facilitate and speed up the process of drug development and also interpretation of results will be made easy. The flora of earth is dwindling day by day and with it the vast collection of therapeutic constituents that may hold the key to heal many 'incurable' diseases of today and many other diseases that are yet to unleash itself on mankind. Emphasizing more on phytopharmaceuticals and renewed investment in research and development in this field will trigger a revolution in chemotherapeutic treatment using plant derived constituents.

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