Review Article

Redressal of Antibiotic Resistance using Plant Extracts

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Abstract | With the passage of time antibiotic resistance is prevailing in microbes and current drugs are not effective enough to cure infectious diseases caused by these resistant pathogens. In this battle, there is a continuous need to develop new drugs or compounds that can cure these infections or to reverse the antibiotic resistance in these pathogens. In recent years different experimental approaches highlighted the importance of phytochemicals present in plants and its ability to kill these pathogens and microbial inability to develop resistance against these pathogens. Therefore, the trend has been shifting from using synthetic or semi-synthetic antibiotics to the use of medicinal plants which are the part and parcel of traditional medicines. Thus, this review article explains the different mechanisms adopted by microbial life to develop resistance against antibiotics. This article also explains how the resistance can be reversed as well as the bio-medicinal importance of plants and their effectiveness against many pathogens.

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1. Introduction

From the earlier times, people have been using different natural substances as medicines to treat various infections and diseases. Plants and herbs were the biggest source of natural substances or phytochemicals that have proven beneficial. Plant sources are claimed to contain several biomedical active ingredients known as phytochemicals that also show importance in combating age related diseases particularly caused by oxidative stress (Lewis and Ausubel, 2006). In recent practices, there has been a recommendation to treat cancer with diet rich in fruits and vegetables having a great quantity of phenolics and flavonoids (Azwadina, 2015). Later on, the trend of using plants for medicinal purposes shifted towards semi-synthetic (produced by modifying the structure of natural substances) and synthetic medicines. With the passage of time microorganisms adopt different mechanisms to develop resistance against antibiotics (Ibezim, 2005). Usually the genes responsible for effective resistance development are present on plasmids which are transferred to nearby bacterial cells and due to smaller generation time of these bacterial cells, a large number of bacterial populations become resistant to the antibiotics in a short time period (Satesh *et al*., 1997).

Several factors contribute for the development of resistance including excessive or unnecessary use of antibiotics, structural or genetic modifications occurred in bacteria or production of enzymes to combat the antibiotic affectivity. Mechanism of resistance developed is different for different antibiotics depending on their mode of action and their targets on bacterial cells (Biswas *et al*., 2002).

Resistance against several generations of antibiotics has led the scientific and pharmaceutical industry to investigate more and more medicinal plants for the production of more effective medicines (Reddy *et al.*, 2013).

1.1 Mechanisms of action of antibiotics

Antibiotics are the substances that are used to combat the infections and diseases caused by pathogenic microorganisms either by killing or by hampering their growth. Mechanism of action of these antibiotics depend upon the difference of prokaryotic (microbial) and eukaryotic (host) cell (Kapoor *et al.*, 2017). Targeting these differences, antibiotics either kill the bacterial cell or inhibit the growth of new cells. Effectiveness of these antibiotics depends on their target specificity against bacterial cells. These antibiotics either target the formation of bacterial structures including cell wall, cell membrane, proteins or nucleic acids or hamper the metabolic activities of microbial cell (Kahne *et al.*, 2005). Table 1 summarizes the mechanism of action of antibiotics.

1.2 Inhibition of cell wall synthesis

Cell wall is composed of a polymer called peptidoglycan which is formed by the joining of Amino acids chains (Kahne *et al.*, 2005). Inter-joining of peptidoglycan chains is catalyzed by an enzyme called DD transpeptidase also known as penicillin binding protein (PBP). Beta-lactams targets the PBPs which are attached to the outer surface of cytoplasmic space. Beta lactam ring of penicillins bond with DD transpeptidase and block its activity as shown in Figure 1. Polymeric structure formation of peptidoglycan stops and bacterial cell losses its integrity (Wright, 2005). Cephalosporins also target the synthesis of bacterial cell wall by inhibiting peptidoglycan formation due to which cell losses its integrity (Yotsuji *et al.*, 1988). Monobactams, due to the presence of beta lactam ring, also target bacterial cell wall preventing the cross linking of peptidoglycan chains. Carbapenems also have the same mechanism of action against bacteria as other beta lactam antibiotics (Bozdogan and Appelbaum, 2004).

1.3 Inhibition of protein synthesis

Inhibition of protein synthesis by antibiotics can be done at several steps by; inhibiting the assembly formation of 30S subunit of amino acid with messenger RNA and formyl-methionyl-transfer RNA, inhibiting the complex formation of 30S and 70S ribosomal subunits or inhibiting the elongation of peptide chains by blocking exit site or attachment site of tRNA at ribosomal assembly (Johnston *et al.*, 2002). Aminoglycosides target the A site of 16S ribosomal RNA of 30S ribosomal subunit resulting in the altered physiology of A-site which does not allow the proper placement of transfer RNA. Incorrect placement of amino acids forms the degraded polypeptide chains that are unable to perform the proper function and disturb the cell function. Disoriented protein formation hinders the cells proper functioning and cell dies (Blondeau, 2004). Macrolides halt the protein synthesis by binding with the 50S ribosomal subunit at the exit tunnel as shown in Figure 2 and stops elongation of polypeptide chain (Yoneyama and Katsumata, 2006).

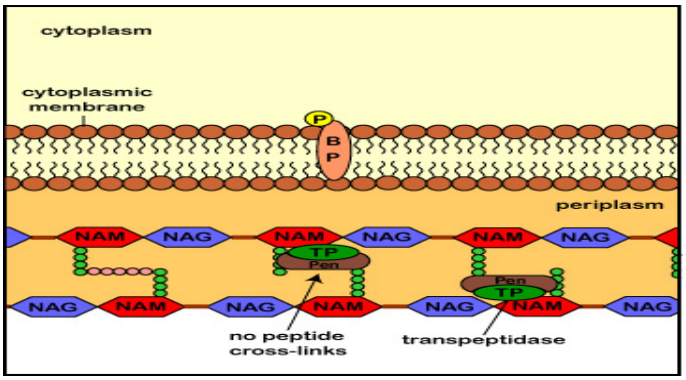

Figure 1: Mechanism of action of beta-lactamases (Michael, 2016).

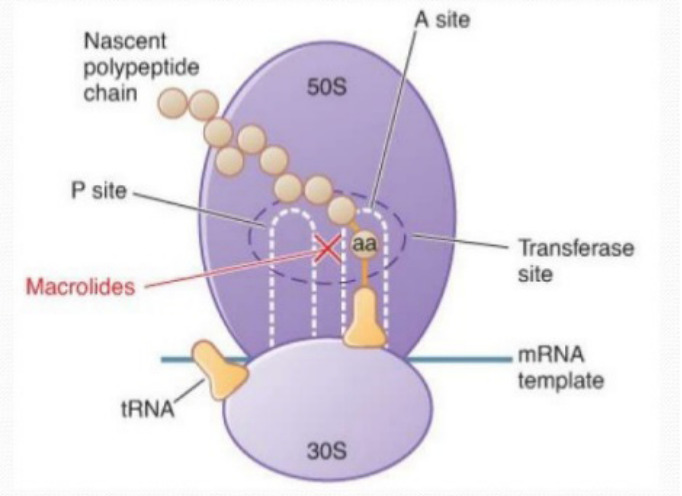

Figure 2: Mechanism of Action of Protein synthesis inhibitor (Macrolides) (Blondeau, 2004).

1.4 Alteration of cell membrane

Antibiotics target cell membrane because of structural difference between prokaryotic and eukaryotic cell membrane. Anionic lipids are the target sites for cell membrane alternating antibiotics as these anionic

lipids are exposed at the outer side of cell membrane in prokaryotes while in eukaryotic membrane these anionic lipids are sequestered to the inside of cell membrane facing inner cellular sap (Yoneyama and Katsumata, 2006). Polymyxins were the first antibiotics that altered the cell membrane for its action by attaching themselves with the anionic lipids and inserted into the cell membrane disrupting its integrity. Loss of cellular membrane made cellular sap leakage and eventually cell dies (Hossain *et al.*, 2013).

1.5 Inhibition of nucleic acid synthesis

Fluoroquinolones inhibit the activity of bacterial enzymes involved in replication including DNA Gyrase and Topoisomerases IV which are not present in human cells but are required by bacterial cells for division making them target specific. DNA topoisomerases separate the parental strand of duplex DNA and add another strand and separate the originally duplicated strand from each other (Appelbaum and Hunter, 2000). Topoisomerases initiate decatenation which is essential for the separation of daughter chromosomes from each other. Inhibition of topoisomerases does not allow the proper separation of strands, leading to damage cell formation. Daughter cells degenerated due to improper distribution of chromosomes (Blondeau, 2004). On the other hand, DNA gyrase is responsible for creating negative supercoils in DNA double helix which is an important step for the attachment of replication proteins. Fluoroquinolones inhibit the DNA gyrase activity thus stops the replication process of bacterial cells. In absence of replication process, bacterial cells eradicated from certain locality (Higgins *et al.*, 2003).

1.6 Inhibition of metabolic pathway

These antibiotics work by blocking the metabolic pathway of bacterial cells acting as competitive inhibitors for the metabolic enzymes of bacterial cells. They bind with bacterial enzymes at the place of substrates and block the metabolic activity due to which bacterial cell dies. Sulfonamides are the drugs which are the competitive inhibitors in the synthesis of para-aminobenzoic acid which is an early intermediate in bacterial folic acid synthesis (Klančnik *et al.*, 2010).

1.7 Antibiotic resistance

Antibiotic resistance is a term used to define mechanisms that bacteria develop to mitigate the static or cidal effects of antibiotics upon their existence, which were earlier enough to kill them or stop their growth (Reddy *et al.*, 2013). From the advent of antibiotics, bacteria adopt different strategies to develop resistance against antibiotics for the purpose of their survival. Development of resistance is a scientific problem since the discovery of penicillin. Infections which were easily treated by antibiotics persist these days and become incurable due to the development of resistance that makes pathogens survive for longer time period and enable them to mitigate the negative impacts of antibiotics upon their survival. The mechanism of resistance appeared alongside the use of these antibiotics and with every passing decade, number of antibiotic resistant microorganisms starts to increase and previously using antibiotics become ineffective (Kawami *et al.*, 2017). Events in the history of antibiotics are illustrated in Figure 3. Table 2 summarizes the mechanisms of antibiotic resistance.

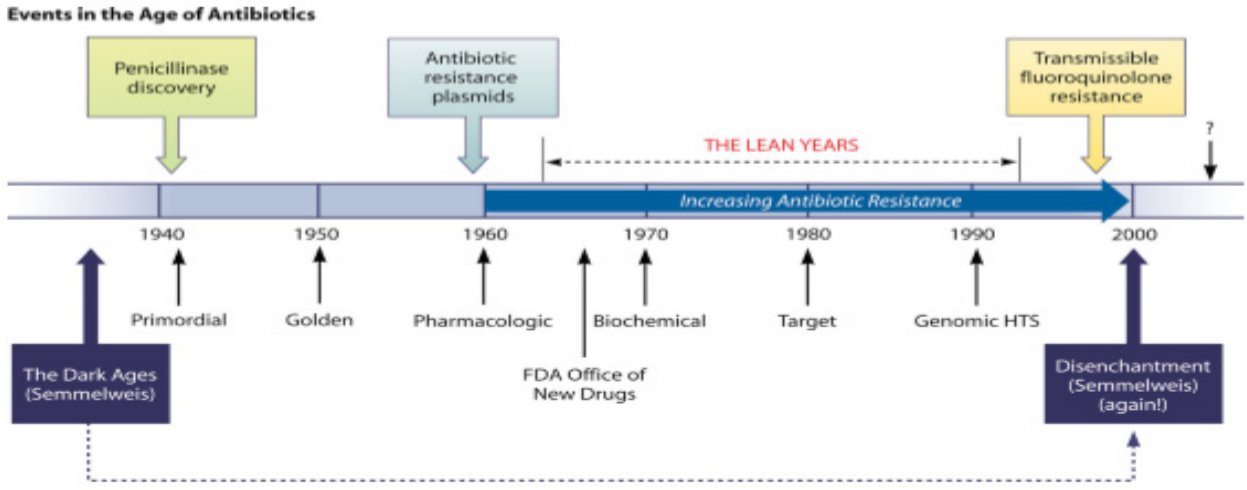

Figure 3: Events in the history of antibiotics (Davies and Davies, 2010).

Table 1: Mechanism of action of antibiotics.

Sr. no	Mechanism of action	Groups of antibiotics	Examples	References
1	Inhibition of Cell wall synthesis	Beta- Lactamases, Cephalosporins, Glycopeptides, Carbapenems	Penicillins, cephalosporins, vancomycins	Kahne *et al.*, 2005
2	Inhibition of Protein synthesis	Tetracyclins, Lincomycins, Macrolides, Aminoglycosides	Amikacin, gentamycin	Wright, 2005
3	Alteration of cell membrane	Polymixins	Polymixin B and E (colistins)	Lederberg, 1956
4	Inhibition of nucleic acid synthesis	Quinolones	Norfloxacin, ciprofloxacin	Yotsuji *et al.*, 1988
5	Inhibition of Metabolic Pathway	Sulfonamides	Sulfamethoxazole, sulfasalazine	Klančnik *et al.*, 2010

Table 2: Mechanisms of antibiotic resistance

Sr. no	Type of resistance	Mechanism of resistance	Examples	Reference
1	Enzymatic	Inhibition of bacterial structures including cell wall, proteins etc.	Hydrolysis, Group Transfer	Wright, 2005
2	Genetic	Inhibition of DNA synthesis or degradation of DNA	Plasmid transfer, Addition or deletion of genome.	Forgea and Schachtb, 2000
3	Biochemical	Limiting uptake of drug	LPS layer, porin channels, biofilm formation	Blair *et al.*, 2014
		Modification of drug target	Changes in structure or number of PBPs, inhibition of cell wall synthesis, depolarization of cell membrane, modification of enzymes, mutation in genes, methylation of ribosomal subunit.	Beceiro *et al.*, 2013
		Inactivation of drug	Transfer of chemical groups or degradation of drug.	Blair *et al.*, 2015
		Drug efflux	Extrusion of drugs	Blair *et al.*, 2014

1.8 Mechanism of resistance development

There are two types of resistance i.e. Active: which is developed specifically towards an antibiotic or a class of antibiotics through the evolutionary process between several generations of bacteria. Passive: which includes all the structural capabilities of bacteria that work against antibiotic action. This include all the extracellular membranous structures present in gram negative bacteria. Different strategies are being used by bacteria to protect themselves from the effects of antibiotics including prevention of interaction of drug with target by modifying key elements like ribosomal RNA, efflux of the antibiotic from the cell by several pumping proteins present inside membranes and direct destruction or modification of the compound (Wright, 2005).

1.9 Enzymatic mechanisms

There are certain bacteria that can resist the antibiotics by modifying chemical mechanism or by adapting new chemical pathways that include hydrolysis and group transfer. Hydrolysis: Many antibiotics have hydrolytically susceptible bonds i.e. esters and amides whose integrity is majorly required for their action against bacteria. Hydrolytic enzymes produced by several bacteria can easily break these bonds and disintegrate the chemical structure of antibiotics before they reach the bacterial cell wall. Most of these enzymes require only water as the co-factor, which is easily available for their action. So many enzymes are involved in hydrolysis process like amidases, beta-lactamases and macrolide esterases (Wright, 2005). Group transfer: Structural modifications are brought by the largest family of resistance enzymes that are group transferases. They change structure of antibiotics by changing side chains or the groups attached to them as a result antibiotics loss their strength and become ineffective to treat infections. Acyltransferases covalently modify vulnerable hydroxyl or amide group and thus antibiotic lose its ability to bind with target cells and deactivate them (Gajalakshmi and Abbasi, 2004).

1.10 Genetic mechanisms

Genetically resistance develops either through mutation or through transfer of plasmid containing resistance gene. Mutation is a random process and any change in the genetic makeup of bacteria can cause the development of resistance. Insertion of foreign DNA to the bacterial genome can also leads

to the development of antibiotic resistance. Transfer of plasmid having resistant genes among bacterial cells is also a major source of antibiotic resistance development (Forgea and Schachtb, 2000).

1.11 Biochemical mechanisms

Antibiotic resistance has four main mechanisms comprising (1) limiting uptake of drug; (2) modification of drug target; (3) inactivation of a drug; (4) increased efflux. Due to the differences in structure, etc. Gram positive bacteria generally do not limit the uptake of a drug as they don't possess an LPS outer membrane also they don't have the tendency for particular types of drug efflux mechanisms (Chancey *et al.*, 2012). While, Gram negative bacteria use all four mechanisms for the development of resistance (Mahon *et al.*, 2014).

1.12 Limiting uptake of drug

The ability to uptake the drug is different in gram positive bacteria as compared to gram negative bacteria. The LPS layer in the membrane of gram positive bacteria is structurally and functionally different and act as a barrier for these types of molecules thus providing innate resistance against particular groups of antibiotics (Blair *et al.*, 2014). Bacteria possess larger outer membranes that have porin channels through which molecules enter the cell. In gram negative bacteria, these porin channels give access to hydrophilic substances. These porin channels limit the uptake of drug by two ways i.e. by mutating the selectivity of porins or by decreasing the number of porins (Kumar *et al.*, 2005). Drug uptake can also be limited by the formation of thick and sticky biofilm by a bacterial colony thus making it difficult for antibiotic molecules to reach the bacterial cell. Moreover, biofilm have sessile bacterial cells and much higher antibiotic concentration is required to be effective upon these slow growing cells. Resistant genes can be easily transferred in bacterial community forming biofilm as horizontal gene transfer is aided by bacterial proximity (Mah, 2012).

1.13 Modification of drug target

Modification of structure or alteration in number of PBPs (penicillin-binding proteins) is one of the mechanisms used by gram positive bacteria against β-lactam antibiotics. An alteration in the structure or change in the number of PBPs can decrease the drug binding ability or completely inhibit the binding of drug (Beceiro *et al.*, 2013). Inhibition of cell wall synthesis, depolarization of cell membrane, mutation in genes, methylation of ribosomal subunits and modification in enzymes used in metabolic pathways or in synthesis of DNA also cause intrinsic resistance to develop by inhibiting the binding of antibiotics (Kumar *et al.*, 2013).

1.14 Inactivation of drug

Bacteria can inactivate the drug either by transfer of chemical group or by actual degradation of the drug molecule. Most common chemical groups that are transferred are phosphoryl, acetyl or adenyl groups. Acetylation, adenylation and phosphorylation are usually done against fluroquinolones, chloramphenicols and aminoglycosides (Blair *et al.*, 2015).

1.15 Drug efflux

Chromosomally encoded genes for the efflux pump are present in bacterial cells. Efflux pumps extrude toxic substances from cell which describes the inherent resistance to certain antibiotics. Efflux pumps can be substrate specific (getting rid of only one class of antibiotics) or they can be non-specific and can transport several compounds or classes of antibiotics and these efflux pumps are known as MDR efflux pumps (Blair *et al.*, 2014).

1.16 Redressal of antibiotic resistance

One of the strategies to reverse the antibiotic resistance is to avoid the use of antibiotics against which bacteria have developed resistance especially in the decreased use of third-generation cephalosporins (particularly ceftriaxone), imipenem, and vancomycin and increased use of extended-spectrum penicillins and combination therapy with aminoglycosides. In absence of these antibiotics the production of resistance enzymes like in case of penicillins the production of penicillinases is diminished (Paterson and Bonomo, 2005). Most of genes transferred through conjugation reside over bacterial plasmids. Due to continuous conjugation process, the resistance developing genes persists longer in bacterial genome. To overcome this strategy, use of combination of conjugation inhibitors is applied. Moreover, to destroy conjugation related resistance genes, plasmids are being removed from bacterial cell to reverse the antibiotic resistance (Pagès *et al.*, 2011). Another strategy to reverse the antibiotic resistance is the editing of bacterial genome. Through CRISPR/Cas9 genome editing technology, bacterial genome having

resistance gene locality is removed (Baym *et al.*, 2016). To reverse the resistance developed by the use of efflux pump mechanism, specified molecules have been developed that decrease or inhibit the activity of relevant efflux pump (Stavri *et al.*, 2007).

1.17 Phytochemicals

With the emergence of resistance against antibiotics and other synthetic and semi-synthetic medicines, society is back gearing towards natural or organic products and chemicals products are being replaced by organic or plant based natural alternates (Azwanida, 2015). Plants of different species and areas are being searched down to discover phytochemicals effective as therapeutic drugs. High content of flavonoids and phenolic compounds have antioxidant properties that prevent onset of age-related diseases (Biswas *et al.*, 2002). Plants produce a significant number of secondary metabolites having adversely complex structures and provide defense mechanism to the plant from microbes and herbivores by altering their immune system.

The biomolecules possessing antimicrobial properties includes alkaloids which are organic heterocyclic nitrogen compounds (derived from amino acids) that are basic-forming water-soluble salts. Due to structural compatibility they intercalate with DNA and interfere with the bacterial DNA replication and mitosis. Phenolics and polyphenolics provide defense to plants (Chandra *et al.*, 2017). Flavonoids interact with proteins present in bacterial membranes. They have prominent anti-inflammatory and anti-tumor properties. Quinones have major role in many biological activities and due to their complex structures, it is not easy for microbes to develop resistance against these biomolecules. Tannins possess antibacterial and antifungal properties and they inactivate the production of microbial adhesins so they are effective for eradication of bacterial biofilms. Coumarins are the aromatic compounds mostly produced in plants in response to antifungal infections, thus exhibit remarkable antifungal properties. Terpenes are the largest naturally occurring biomolecules components known to have 30,000 structures. Their exact mechanism of action is not known but they believed to interfere with the bacterial cell membrane. Lactins and polypeptides form ion channels in microbial membrane and saponins are the main ingredient in many Chinese medicines (Reddy *et al.*, 2013). Some important plants are listed in Table 3 with their mechanisms to reverse the resistance.

1.18 Clove

Clove is a widely used food preservative and found to have antimicrobial properties against many microbial species. Clove extracts are obtained from buds and leaves of clove tree. Clove biomolecules react with bacterial cell membrane, inhibit their respiratory mechanisms and interact with the replication of their DNA. Interaction of clove with microbial cells damages the integrity of bacterial cellular membrane and breaks down essential biomolecules including proteins, DNA and ATP. The broken constituents of these molecules release out from cellular sap and bacterial cell dies (Wright, 2005). Application of clove oil hinders the production of enzymes isocitrate dehydrogenase, citrate synthase and α-ketoglutarate dehydrogenase. Clove oil also happens to change the bacterial DNA integrity causing its deformation (Nazzaro *et al.*, 2013). Main bioactive constituent of clove is eugenol which is responsible for antibacterial and antifungal properties of clove. Experimental studies showed 99% effectiveness of clove against *E. coli* and *S. aureus* with incubation time of 9 hours. It is also very effective against fungal species of *Candida, Aspergillus* and other genra (Hu *et al.*, 2018).

Table 3: Some important plats with their chemical constituents and mechanisms of resistance reversal.

Sr. No	Name of Plant	Part used	Chemical constituent	Mechanisms of Resistance Reversal	Reference
1	Clove	Bud and Leaves	Eugenol	Alter bacterial cell membrane, inhibit bacterial DNA synthesis.	Blondeau, 2004
2	Cinnamon	Bark	Cinnamaldehyde	Changes bacterial cell membrane permeability	Mau *et al.*, 2001
3	Oregano, thyme and basil	Leaves and flower buds	Carvacrol, thymol and phenolics	Alters fungal cell membrane, interferes ergosterol synthesis	Blondeau, 2004
4	Nutmeg	Seed	Sabinene, beta-pinene and alpha-pinene	Targets bacterial cell membrane and DNA synthesis	Forgea and Schachtb, 2000
5	Neem	Leaves,	Azadirachtin	Bacterial cell wall breakdown	Biswas *et al.*, 2002

1.19 Cinnamon

Cinnamon is a member of family Lauraceae and genus cinnamomun having around 250 species. Cinnamon is commonly used herb having beneficial characteristics for health improvement. It also possesses effective antimicrobial and anticancer properties. Different strains treated with cinnamon essential oil have faced prominent reduction in their growth. Cinnamon is mostly used against food borne pathogens (Mau *et al.*, 2001). Cinnamaldehyde is the major constituent in cinnamon responsible for its bactericidal properties. Cinnamaldehyde targets bacterial cell membrane, changes its morphology and permeability. Electrical conductivity of cell membrane increases. Consequently, traffic against cell membrane also faces a major shift disturbing metabolic activities of bacterial cell and bacterial cell membrane gets wrinkled or swollen (Blondeau, 2004).

1.20 Nutmeg

Nutmeg is the hard kernel of the seed of an evergreen tree native to Molucca. Around 54 components found to be present in nutmeg. Most prominent ones are sabinene (39.12%), beta-pinene (10.10%) and alpha-pinene (11.94%). Nutmeg has bactericidal properties against many food spoilage and pathogenic bacteria. Nutmeg has specific use in meat industry. It is very effective against meat degrading bacteria, therefore majorly used for the preservation of meat and meat products (Forgea and Schachtb, 2000) Bioactive constituents of nutmeg majorly target bacterial cell membrane and DNA synthesis. Permeability of cell membrane changes movement across cell membrane. Its integrity lost, it gets wrinkled or in some cases outer membrane gets dissolved out by the application of nutmeg oil. Disintegration of cellular membrane also disrupts the production of cellular adenosine tri-phosphate (Adewole *et al.*, 2013). Sabinene is the major constituent of nutmeg essential oils. Sabinen is a type of terpenoids which are recognized for their antibacterial properties because of the presence of hydroxyl group and delocalized electrons (Gupta *et al.*, 2013).

1.21 Oregano, thyme and basil

These herbs belong to the lamiaceae family and are excessively used in cooking and seasoning of food. Their essential oils have remarkable antimicrobial properties due to the presence of phenolic components, carvacrol and thymol. Combination of oregano and thyme essential oils produces very effective results against *Campylobacter jejuni, E. coli, Salmonella enteritidis and L. monocytogenes*. Thymol, carvacrol and phenolic components make it really effective against fungal strains therefore; it is used in baking processes. Thymol interferes with the permeability of fungal cell membranes, disrupts the metabolic process of ergosterol synthesis and also depletes concentration of cellular components essential for survival (Blondeau, 2004).

1.22 Neem

Neem is an evergreen tree native to sub-continent. It has been used in Ayurvedic medicines for approximately 4000 years. Its various parts are being used by herbalists due to many beneficial factors of neem including their antiviral, antibacterial, antifungal, antiseptic and anti-inflammatory properties (Brahmachari, 2004). More than 135 different medicinally important compounds have been isolated from different parts of neem. Neem is also being used as an eco-friendly insecticide that does not impart catastrophic damage to the environment (Biswas *et al.*, 2002). A number of fungal infections are being treated by using neem plant. 14 common fungal species are susceptible towards neem extracts. Athlete's foot and ring worm of nails and skin are the most common. Being antibacterial agent, it can treat infections of *Staphylococcus aureus* and because of its antiviral properties, neem products proved beneficial to treat viral disease as small pox, chicken pox and foul pox. Neem leaf extracts also has antimutagenic properties (Ogbuewe *et al.*, 2011).

Conclusions and Recommendations

With the passage of time, more and more antibiotic resistant strains of pathogenic microbes are becoming prevalent in spreading different human, animal and plant related diseases. A number of social and economic practices are still increasing the number of these resistant pathogens. There is an urgent need of new and improved antibiotics which is a costly procedure with an absolute risk that microorganisms will also develop resistance against them after some time. On the other hand, natural resources provide cheap and effective alternatives. Different experiments have highlighted the effectiveness of medicinal plants against many pathogens. These plants, including neem, clove, cinnamon, nutmeg, basil, thyme and oregano, has the ability to reverse the bacterial defense mechanisms and kill them due to the

presence of many complex phytochemicals that have good antimicrobial properties with minimum side effects to the human race and environment and their chemical complexity does not allow microorganisms to develop resistance easily.

Novelty Statement

Application of phytochemicals for microbial-resistance reversal is a recently evolving research. This review aims to provide updates to the scope and can prove insightful towards advance studies in combating the present and any possible future MDR outbreak.

Author's Contribution

Adeen Kiran and Ayesha Farooq: Literature survey.
Malik Muhammad Asif: Data collection.
Umar Farooq Gohar: Article write up.
Hamid Mukhtar: Script reading.

Conflict of interest

The authors have declared no conflict of interest.

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