Review Article

Development of botanicals to combat antibiotic resistance

Pooja D. Gupta, Tannaz J. Birdi*

The Foundation for Medical Research, 84-A, R.G. Thadani Marg, Worli, Mumbai, 400 018, Maharashtra, India

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A B S T R A C T

The discovery of antibiotics in the previous century lead to reduction in mortality and morbidity due to infectious diseases but their inappropriate and irrational use has resulted in emergence of resistant microbial populations. Alteration of target sites, active efflux of drugs and enzymatic degradations are the strategies employed by the pathogenic bacteria to develop intrinsic resistance to antibiotics. This has led to an increased interest in medicinal plants since 25–50% of current pharmaceuticals are plant derived. Crude extracts of medicinal plants could serve as an alternate source of resistance modifying agents owing to the wide variety of secondary metabolites. These metabolites (alkaloids, tannins, polyphenols etc.) could act as potentials for antimicrobials and resistance modifiers. Plant extracts have the ability to bind to protein domains leading to modification or inhibition protein–protein interactions. This enables the herbs to also present themselves as effective modulators of host related cellular processes viz immune response, mitosis, apoptosis and signal transduction. Thus they may exert their activity not only by killing the microorganism but by affecting key events in the pathogenic process, thereby, the bacteria, fungi and viruses may have a reduced ability to develop resistance to botanicals. The article is meant to stimulate research wherein the cidal activity of the extract is not the only parameter considered but other mechanism of action by which plants can combat drug resistant microbes are investigated. The present article emphasizes on mechanisms involved in countering multi drug resistance.

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1. Background

Antibiotics, the wonder drugs of the 20th century, play a critical role in treating bacterial infections.

The synthesis of Salvarsan an arsenic based drug for syphilis in 1910 and development of Prontosil, a sulpha drug in 1935 and penicillin purified and produced in early 1940s, set up the paradigms for future drug discovery research. The period from 1950s to 1970s was considered the golden era of discovery of novel antibiotics classes [1]. This resulted in a major reduction in mortality and morbidity, due to infectious diseases leading to a euphoria [2,3]. However, with each passing decade, bacteria that could defy multiple antibiotics started becoming increasingly common, leading to an increase in morbidity, mortality and cost of health care. The increase of drug resistant organisms stemmed from a multitude of factors [4,5]. The improper use of antibiotics in patients contributed to the emergence of drug resistance. Additionally extensive use of antibiotics in the animal industry has also resulted in strong selective pressure for the emergence of antibiotic-resistant bacteria [6–8]. With increasing patient movement and travel throughout the world, transmission of the drug-resistant organisms from one country to another also increased [9,10].

The wide spread antibiotic resistance observed is now posing a serious public health concern, with medical scholars warning of a return to the pre-antibiotic era [11]; be it community or hospital acquired infections due to Vancomycin Intermediate Staphylococcus aureus (VISA), Vancomycin Resistant Enterococci (VRE), Methicillin Resistant S. aureus (MRSA) or ESBL (extended spectrum β-lactamase) enzyme producing Gram negative bacteria [12]. Thus effective antimicrobials were no longer available which could cure virtually all bacterial infections. This optimism was shaken further by the emergence of resistance to multiple antibiotics amidst enteric pathogens, Pseudomonas aeruginosa, Streptococcus pneumonia, S. aureus and Mycobacterium tuberculosis [13–15]. Additionally a high level of drug resistance is reported in Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii,
The origin of genes for antibiotic resistance is due to a natural process. The source could be genes encoding resistance in the antibiotic producing bacteria themselves as a mechanism for their own protection or generally due to spontaneous mutations in the bacterial chromosome. The spontaneous mutation frequency for antibiotic resistance is on the order of about $10^{-8}$ to $10^{-9}$. Whilst mutation is a rare event, it does not take long for resistance to develop in a bacterial population owing to the fast growth rate of bacteria and the absolute number of cells attained [20].

Once the development of resistance has occurred, the mutated gene is directly transferred to the bacteria's progeny during replication. In the selective environment of the antibiotic, the wild type are killed and the resistant mutant allowed to flourish, influenced by the rate and pattern of antibiotic use (selective pressure) and influence of the particular resistance on bacterial fitness [21]. Resistance to penicillin in *Staphylococcus aureus* was observed as early as 1942 after penicillin came into use [11].

As the next generations of antibiotics were developed to overcome the problems of resistance against the available ones, bacteria developed resistance mechanisms to the newer antimicrobial agents [22]. For example, the production of an enzyme penicillinase by *S. aureus* led to penicillin resistance initially. To resist penicillinase, cloxacillin was developed. To contest this antibiotic, the bacteria altered the target site for binding of β-lactam antibiotics, i.e., the penicillin binding proteins (PBPs) and this led to the development of MRSA. Presently the bacteria have been reported to be resistant to not only methicillin but also to chloramphenicol, macrolides, aminoglycosides, tetracycline and lincosamides [23].

Multidrug resistance in bacteria occurs by accumulation, of resistance (R) plasmids, transposons, or genes with each coding for resistance to a particular agent, and/or due to the action of multidrug efflux pumps (EP) which can pump out more than one drug type [23]. Development of plasmids for multi drug resistance in pathogenic organisms is a comparatively recent phenomenon which occurred after the introduction of antibiotics in the 1940s [22].

A recent database lists more than 20,000 potential resistance genes of nearly 400 different types, predicted mainly from available bacterial genome sequences [24]. Fortunately, the number existing as functional resistance determinants in pathogens is much smaller [11].

The key mechanisms responsible for resistance to antibiotics in bacteria are listed below—

- **Plasmids:** Whilst both chromosomal mutations and/or genetic transfer are responsible for acquisition of resistance, it is the transferable resistance which poses a greater threat as it can achieve much wider dimensions due to rapid dissemination. R plasmids play a vital role in carrying this transferable resistance. A single plasmid can harbor several genes coding for multiple drug resistance. Horizontal gene transfer (HGT) is responsible for the development of antibiotic resistance through the transfer of the so-called mobile genetic elements (MGEs) [25].

- **Inactivation of antibiotic:** Bacteria may produce enzymes that chemically modify or degrade antibiotics and inactivate the drugs [26]. For example, Penicillin resistance in *S. aureus* is because of the production of the enzyme β-lactamase that activates the antibiotic by hydrolyzing the β-lactam ring [27].

- **Target site modification:** The molecules that are normally bound by an antibiotic are normally altered or replaced and thus essentially eliminate the drug's targets in bacterial cells. An example of this mechanism is Methicillin resistance in *Staphylococcus aureus* due to the presence of mec A gene which encodes for PBP 2A. It has low affinity for β-lactams, conferring resistance to all β-lactam antibiotics, together with β-lactamase inhibitor combinations (ampicillin/sulbactam), cephalosporins and carbapenems [28].

- **Prevent drug uptake:** The entry ports for the drugs can be eliminated by bacteria by altering permeability [29]. It has been reported that *P. aeruginosa* can develop resistance to imipenem by mutational loss of porin proteins thereby modifying the outer membrane permeability [30].

- **Efflux pumps (EP):** There are 5 super - families of microbial efflux systems viz. NorM, multi-antimicrobial extrusion protein family (MATE), QacA major facilitators (MFS), LmrA, ATP-binding cassettes (ABC), MexAB, QacC small multidrug resistance family (SMR), resistance-nodulation cell division (RND) [31]. These EPs are responsible for the export of antibiotics before they find their intracellular targets. Kaplan [32] has demonstrated that an active drug EP is an effective mechanism of macrolide resistance in *Streptococcus pyogenes*. The resistance is encoded by a mefA gene and is specific for 14- and 15-membered macrolides.

- **Biofilm formation:** Biofilm is formed by a complex aggregation of microbes, wherein the cells are embedded matrix of extracellular polymeric substance (EPS)(self-produced). Production of biofilms through adherence of bacteria to human tissues and medical devices is a major virulence factor associated with increased antibiotic resistance, reduced phagocytosis, and overall persistence of the microorganisms [33]. Additionally, these biofilms being difficult to eradicate, are a source of many intractable infections. The inherent resistance of biofilms to the antibiotics can be attributed to failure of antibiotic to penetrate or slow growth rate of organisms owing to slower metabolism [34].

The combined effects of genetic processes of mutation and selection, fast growth rates and the ability to exchange genes, sum up for the extraordinary rates of adaptation and evolution that can be observed in bacteria. For these reasons bacterial resistance or adaptation to the antibiotic environment seems to occur very quickly in an evolutionary timeframe [21]. Bacteria established mechanisms to resist the next generation antimicrobials that were developed to overcome the difficulties associated with resistance [2]. Tuberculosis which is now considered a global emergency, exemplifies this scenario with *M. tuberculosis* becoming increasingly resistant to first and second line drugs [35–37].
FLAVONOIDS- Photosynthesizing cells are store house of flavonoids. These phenolic structures are usually found in common edible plant parts such as: Vegetables, seeds, fruits and nuts. So far 14 classes of flavonoids have been identified; they vary based on the chemical nature and position of substituents on the different rings [56,57]. The antimicrobial activity of flavonoids against an array of bacterial [58–60] and fungal pathogens [61] is resultant of their action on the microbial cell membranes [62]. Increase in permeability of membrane and disruption is observed due to their interaction with membrane proteins present on bacterial cell wall. Flavonoids are known to possess antioxidant, anti-inflammatory and antitumor activity. Various plants that have been studied for their antimicrobial activity against MRSA and P. aeruginosa contain flavonoids in plenty [63].

QUINONES- Quinones are compounds that possess aromatic rings with two ketone substitutions. Researchers have been able to identify at least 400 naturally occurring quinones that are found in all plant parts. Naphthoquinones being one of the largest groups of plant secondary metabolites exhibit a wide range of biological activities. With nucleophilic amino acids in protein, quinones are known to complex irreversibly, which often leads to their inactivation and loss of function [64]. Surface-exposed adhesin proteins, cell wall polypeptides, and membrane-bound enzymes are the major targets in the microbial cell [65].

TANNINS- The polymeric phenolic substances, tannins, are found in almost all plant parts. Earlier studies have shown that tannins exhibit different biological activities including antibacterial and antifungal [56,57]. The mechanism of antimicrobial efficacy of tannins is possibly due to inactivation of cell envelope transport proteins and microbial adhesins [66–68].

COUMARINS- Coumarins are a group of aromatic benzopyrones consisting of fused benzene and alpha pyrone rings. Coumarins such as scopoletin and chalcones have been recently isolated as antitubercular constituents from Fatooa pilosa. [69] Additionally, phytoalexins, which are produced in plants as a response to microbial infections, exert remarkable antifungal activity.

TERPENES- With over 30,000 known structures, terpenes form the largest group of natural compounds. They are identified on the basis of the number of isoprene units they possess; therefore, 1 isoprene unit is called hemiterpene, 2 isoprene units are called monoterpenes and so on. Essential oils are majorly made up of monoterpenes or sesquiterpenes (3 isoprene units). Terpenoids, one of the terpene derivative, are antibacterial in nature. The mode of antimicrobial action of terpenoids is not clearly defined, but it is ascribed to disruption of the membrane in microbes [70].

LECTINS AND POLYPEPTIDES- Lectins are larger than polypeptides and include mannose-specific molecules obtained from various plants [71]. The mode of action of the peptides and lectins is assumed to be due to competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors or due to the formation of ion channels in the microbial membrane [72,73]. Lectins viz MAP30 from bitter melon [74], GAP31 from Gelonium multiflorum [75], and jacalin [76] inhibit viral proliferation, including HIV and cytomegalovirus by preventing viral interaction with critical host cell components. Although, they possess versatile antifungal, antibacterial, and antiviral functions, the exact mechanism of their action remains unclear.

3. Exploring botanicals as an alternative

In view of the above, Traditional Medicine (TM) may offer a plethora of interesting possibilities to combat drug resistance [46–48]. Herbs demonstrate a varied range of biological activities and may be efficiently harnessed for managing diseases. Nutritional and botanical approaches, combined, may provide powerful tools for controlling an array of infections [49]. The traditional systems of medicine which include Ayurveda, Traditional Chinese medicine (TCM), Kampoo, Unani, Siddha have so far been unable to enter mainstream medicine due to various reasons [50,51]. In view of their effectiveness, efforts are being made to develop strong evidence-based standardization of these traditional systems of medicine so that they can appropriately satisfy the criteria to fit into the modern medicinal framework. In China, TCMs are already playing an important role in treating infectious diseases [52].

Plants produce a wide range of compounds which may not be important for their primary metabolism, but signify an adaptive capability of a plant to adverse abiotic and biotic environmental conditions [53]. These organic compounds are biologically active substances, and represent ‘secondary metabolites’, given the fact that they are resultant of secondary plant metabolism and occur as an intermediate or end products [53]. The structures of secondary metabolites have been optimized during evolution so that they can act as defense mechanisms by interfering with molecular targets in herbivores and microbes [54]. In addition some secondary metabolites affect cell signaling or protect against oxidative or UV stress [55]. Enlisted below are the important secondary plant metabolites:

ALKALOIDS- Alkaloids are organic heterocyclic nitrogen compounds that are basic-forming water-soluble salts. They contain nitrogen, which is usually derived from an amino acid. Alkaloids are grouped into three classes- True alkaloids, Pseudoalkaloids and Prototalkoids. They tend to exhibit analgesic effects; morphine alkaloids are pain relievers and are used as narcotics. The alkaloids possess the ability to intercalate with DNA thereby resulting in impaired cell division and cell death. The mechanism of action of alkaloids such as harmane and berberine is attributed to this property [56].

PHENOLICS AND POLYPHENOLS- These are a diverse group of aromatic secondary metabolites involved in plant defense. They consist of flavonoids, quinones, tannins, and coumarins.
SAPONINS—Saponins are naturally occurring glycosides with the ability of forming a soapy lather when shaken with water, and classified as triterpene saponins or steroidal saponins on the basis of the structural features of the aglycone moieties. Several important Chinese herbal medicines such as Astragalus root, Licorice root, Polygonal root, Ginseng, Bupleurum root, Anemar-rhena rhizome and Ophiopogon tuber contain saponins as the main secondary metabolites, which may be responsible for their specific pharmacological activities [77].

The use of plant extracts and phytochemicals, possessing both known and unknown antimicrobial activities, could be of great importance in therapeutic treatments. Organic chemists are amazed with the structural diversity of phytochemicals and have investigated their chemical properties extensively since the 1850s [78]. In this way, organic chemistry contributed significantly to the collection of knowledge on plant secondary metabolites that was necessary for the better understanding of their biological importance. In recent years, based on leads from Ayurvedic and other traditional medicines and chemical and pharmacological studies, isolation of several antimicrobial agents from plants has been reported [49].

4. Evidence based research on mode(s) of action of botanicals

Synthetic drugs and natural products significantly differ in terms of frequency of different radicals and spatial configuration [79]. The latter have less nitrogen, sulfur, phosphorus, halogens and exhibit overall enhanced scaffold variety, molecular complexity, stereo chemical abundance, diversity in the ring system and carbohydrate contents [80]. Additionally, plant products have the ability to modify or inhibit protein–protein interactions, thus presenting themselves as effective modulators of immune response, mitosis, apoptosis and signal transduction [79]. Bacteria are unable to easily develop resistance to the multiple and/or chemically complex phytochemicals present in plant extracts [51]. However a recent review by Vandhana et al. [81], draws attention to the increasing reports of bacterial resistance to herbal antimicrobials.

Whilst bacterial resistance to antibiotics as discussed earlier, typically involves inactivation or modification of the drug, alteration of target and decrease in drug accumulation there by decreasing the permeability and/or an increase in efflux, the microbial cell can be affected by plant secondary metabolites in several different ways. These include the disruption of membrane function and structure (including the efflux system) [82,83], interruption of DNA/RNA synthesis and function [84] interference with intermediary metabolism, induction of coagulation of cytoplasmic constituents and interruption of normal cell communication (quorum sensing) [85–87].

A single plant alkaloid berberine, an active ingredient of Rhi-zoma coptidis, has been reported to possess different antimicrobial activities. Anti-herpes effects of berberine was reported and the possible mechanism was demonstrated to be inhibition of synthesis of herpes simplex viral DNA [88]. In modest concentrations of 30–45 μg/ml, berberine showed antibacterial effect on Staphylo-coccus epidermidis and significant inhibition of its biofilm formation [89].

However it is more commonly noted that multiple components in a crude extract act at different sites thereby contributing to the overall activity of the extract. The plant extracts may exert their anti-microbial activity not by killing the microorganism alone but also by affecting key events in the pathogenic process [90]. The anti-diarrhoeal activity of the guava leaf extract is one such example. The guava leaf extract is not bactericidal but affects crucial pathogenic events of colonization and toxin production by diarrhoeal pathogens [91]. The study by Rajasekaran et al. [92] also demonstrates the presence of multiple antiviral components in plant extracts that act against different viral proteins or interfere with different stages of viral replication. A study by Gupta et al. [93], has demonstrated that Alpinia galanga extracts are effective against multi drug resistant isolates of M. tuberculosis. The efficacy of extracts under aerobic and anaerobic conditions is suggestive of varied mode(s) of action by phytoactive components present in the plant extract. Thus a crude extract which contains multiple active compounds is less likely to generate antimicrobial resistance than isolated active fractions [94].

A number of recent reviews can be referred for obtaining an exhaustive list of herals with anti-microbial activity [95–101] and for further details on the mechanisms of action of botanicals [102,103]. However this section concentrates on mechanisms which are responsible for resistance to multiple drugs.

4.1. Inhibition of biofilm formation

Bacterial biofilms are surface–associated microbial communities enclosed in a self-generated exopolysaccharide matrix [104] which protects the microbes from anti-microbial agents. Thus extensive research has been undertaken to explore the potential of alternative mechanisms to control microbial biofilm. This has resulted in identifying several plant extracts in controlling biofilm formation in major pathogens. Trans-cinnamaldehyde, an aromatic aldehyde from bark of cinnamon trees [105,106], terpenes such as carvacrol, thymol, and geraniol have been identified. The essential oils of Cymbopogon citratus and Syzygium aromaticum were found to exhibit marked antibiofilm activity against both fungal [107–109] and bacterial biofilms [110–112]. The components of lemongrass oil inhibited biofilm formation, destroyed the pre-formed biofilms and had multiple targets on the bacterial cell [113].

4.2. Efflux pump (EP) inhibitors

It is now generally accepted that EPs are becoming a vital resistance mechanism, both alone and in combination with changes in the permeability of the outer membrane. Medicinal plants have been reported to not only have the ability of inhibiting EPs but also disrupt the cytoplasm by affecting the permeability of membranes [114–117].

Numerous phytoactive components, including the terpene-carnosic acid (Rosmarinus officinalis), the alkaloid reserpine (Rau-volvia vomitoria) and the diterpenetotarol (Chamaecyparis nootkatensis), have shown to inhibit NorA-induced ethidium bromide (EtBr) efflux from a NorA over expresser [118]. NorA (efflux pump) activity is inhibited by the flavonolignan 5-methoxyhydrocacin. It synergistically increases the activity of the antimicrobial alkaloid berberine present in the same plant. Recently a study has demonstrated that farneisol, a natural plant metabolite, not only augmented the intrinsic susceptibility of Mycobacterium smegmatis to EtBr but also demonstrated relatively good anti-mycobacterial activity compared to the reference EP inhibitors; farnesol was found to be more effective than N-methylpyruvate against Mycobacterium smegmatis [119].

M. tuberculosis has one of the largest numbers of putative drug efflux pumps which are mainly members of the MFS or ABC super families [31]. Piperine has been reported to modulate Rv1258c, an efflux protein belonging to the MFS super family of efflux systems, thereby increasing the bioavailability and consequently the susceptibility of M. tuberculosis to rifampicin [119]. Thus addition of piperine increases susceptibility to rifampicin in M. tuberculosis strains in which Rv1258c is responsible for drug
resistance. No effect of piperine was seen in other resistant strains [120], which could be due to efflux pumps other than Rv1258c being responsible for resistance.

4.3. Attenuating bacterial virulence

A growing body of evidence suggests that plant extracts without being bactericidal are capable of attenuating virulence factors in bacteria thereby affecting pathogen survival. Extracts of various plants exhibit an effect on virulence factors of *P. aeruginosa* including QS gene expression and autoinducer production [121]. Thakur et al. [122], reported that extracts of *Berberis aristata* and *Camellia sinensis* show noteworthy antibacterial potential by targeting hemolysin and bacterial hemagglutination on the bacterial membrane. Brijesh et al. [123], have demonstrated that the anti diarrhoeal activity of *Aegle marmelos* is not due to its bactericidal activity but the ability to prevent binding of the bacterial toxin and colonization of the intestinal epithelial cells. Omega 3 and oleic acids are known for their inhibitory effect on Gram negative bacteria. They are incorporated to the outer cell membrane to increase permeability. Concentration gradient necessary between the organism and its environment may thus be dissipated causing death of the organism [124]. Additionally, various studies reveal that at sub-lethal or sub-inhibitory concentrations, phytoactive compounds affect virulence in Gram-positive [125–127] and Gram-negative bacteria [128] and fungal pathogens [129] by modulation of gene transcription [130,131], expression of proteins [131] and quorum sensing [132,133].

4.4. Immunomodulation

TM has been used for treating various diseases by modulating the immune response [134–136]. In a scenario of increasing bacterial drug resistance where the choice of antibiotics available becomes a limiting factor, botanicals with immune-stimulatory properties which can stimulate the immune system to kill the pathogen can be considered [137].

There is a long history of the use of plant products for both stimulation and suppression of host immunity. However for the purpose of this review the focus will be on stimulants of both innate and acquired immunity.

4.4.1. Innate immunity

Macrophage activation is a key component of innate immunity. *Eucalyptus* oil [138], babassu mesocarp extract [139], and oil from seeds of *Chenopodium ambrosioides* L [140], have been reported to enhance the phagocytic activity of macrophages, whereas essential oils from *Petroselinum crispum* [141] and *Artemisia iwayomogi* [142] were found to suppress phagocytosis by macrophages. Increased phagocytosis by macrophages is not always a beneficial response as reflected in the observations by Mistry et al. [143] in macrophages from lepromatous leprosy patients. The enhanced phagocytosis of *M. leprae* by lepromatous macrophages was considered to be one of the reasons for intracellular survival of *M. leprae*. Thus regulation of phagocytosis is desirable. Additionally, other markers of macrophage activation should be considered viz. ability to kill intracellular pathogens, increase in production of NO and ROS and cytokine secretion.

Enhanced secretion of IL1 by macrophages with extracts of *Aloe vera*, [144] *Astragalus radix*, [145] *Ganoderma lucidum*, [146] and increase in TNFα secretion in the presence of Ursolic and Oleanolic acids [147] and *Ziziphus jujube* extracts [148] have been reported. Increase of NO production by wagonin, a flavonoid of *Scutellaria baicalensis* has been reported by Jen et al. [149] The aqueous extract of *Emblica officinalis* enhanced ROS generation [150]. The ability of plant extracts to enhance killing of intracellular organisms has been demonstrated for *Psidium guajava*, [151] *Pelargonium sidoides* [152] and *Artemisia nilagirica*. [153].

4.4.2. Acquired immunity

Enhancement of both B and T cell mediated immune responses have been reported with saponins of *Panax ginseng* [154,155] and the aqueous extract of *E. officinalis*. [150] T cell stimulation by plant extracts resulting in IFNγ secretion [146] can enhance the functioning of antigen presenting cells (macrophage and dendritic cell) leading to further stimulation of the immune response. Plant extracts (eg. *G. lucidum*) can also directly stimulate dendritic cells which can then present processed antigen to T cells and activate them [156].

A number of plant extracts have been reported to preferentially increase humoral immunity. It was reported that angelan isolated from *Angelica gigas* increases T cell dependent antibody production [157]. A proteoglycan, GLIS isolated from the fruiting bodies of *G. lucidum* induces B cell activation and subsequent increase in circulating antibody production [158]. The aqueous extract of *Terminalia chebula* has also been reported to increase the antibody titres in mice against *Salmonella typhimurium* [159]. Extracts of *Z. officinalis* and *P. ginseng* are stimulators of IL 6, a B cell stimulant [160,161].

4.5. Contesting resistance through synergism between phytoconstituents

As a practice, several studies have investigated medicinal plants just to discover a single chemical compound responsible for the therapeutic effect [162]. However it has been demonstrated that the process of isolation often leads to loss or reduction in activity [163–167]. The enhanced activity of extracts could be due to the fact that the plant secondary metabolites play a role in defense and cell signaling both at the cellular and organic level [168], resulting in increased total activity of the plant [78]. The combined or synergistic actions exhibited by the compounds present in a single herbal extract could be attributed to the multiplicity of targets that these constituents can act on, including receptors, enzymes, ion channels, antibodies, transport proteins and others [169]. Since plant extracts are a mixture of various phytoactive components, development of bacterial resistance to such synergistic combinations may be much slower than those for single chemical compounds [94].

4.6. Botanicals as anti-virals

The exploration of new antiviral compounds for treatment of viral infections is gaining pace owing to the problem of viral resistance coupled with viral latency and conflicting efficacy in recurrent infection in immunocompromised patients [170]. Natural plant products have proved to be an imperative source of principal molecules and many extracts and compounds with antiviral activity [171–173].

Aqueous extracts of the Chilean soap bark tree (*Quillaja saponaria* Molina) contain many physiologically active tri terpenoid saponins [174]. These saponins have been explored for use in animal and human vaccines as they exhibit strong adjuvant activity [175]. The strong immune-enhancing activity Quillaja extracts may lead to a reduction in virus infection in vivo. Roner et al., [175], have suggested that Quillaja saponins prevent attachment of rotavirus by forming a ‘coat’ on the epithelium of the host’s small intestine.

Birdi et al. [176], reported that the decoction of *P. guajava* leaves possess anti-rotaviral activity. Since in presence of the extract there was decrease in cell death in infected cultures it was concluded that
entry and subsequent survival of simian rotavirus was prevented by the decoction of guava leaves.

Balasubramaniam et al. [177], studied the antiviral activity of Indian medicinal plants against white spot syndrome virus (WSSV) in shrimp. They reported that the antiviral activity of these plant extracts could be suggestive of three possible mechanisms. Firstly, the reaction between the extract and the viral envelope proteins may result in its inactivation, followed by prevention of virus entry into the host. Secondly, effect of plant extract on the virus replication in the host cell and lastly the immune stimulant property of the plant extract which enhances innate immunity like superoxide anion, prophenol oxidase, nitric oxide of shrimp against WSSV and an antioxidant property of the plant extract protects the cells from the free radicals which arise due to WSSV infection.

5. Combining traditional and modern medicine — bioenhancers

Despite the progress in pharmacology and conventional chemistry in developing new synthetic antibiotics, structurally altering existing antibiotics or finding suitable enzyme targets against which inhibitors can be developed; current global drug development programs may not be able to provide new effective antibiotics for the next decade [178]. As increased acquired resistance to conventional antibiotics is observed, it is rational to attempt combination therapy of standard antibiotics with plant extracts that possess bioenhancing activity to attain bactericidal synergism [95,179–182]. Use of such combination therapy against resistant bacteria may lead to newer options for the treatment of infectious diseases. Combination therapy can be used for expansion of the antimicrobial spectrum, prevention of the emergence of resistant mutants, minimizing the toxicity [179,181]. Bioenhancers may act by (1) increasing drug absorption (2) modulating biotransformation of drugs in the liver or intestines (3) modulating active transport (4) decreasing elimination (5) immune modulatory activity [181,182].

Various studies have reported that the bioavailability of phenolic compounds like tellimagrandin I, present in Rosa canina [183] and corilagin, found in Arctostaphylos uva-ursi, [184] enhance the inhibitory effect of antibiotics. In addition, a 4-fold reduction of the MIC of tetracycline and erythromycin on combining with ethanolic extract of Mangifera indica has been demonstrated by Souto et al. [185] Combination of methanolic extract of Tectona grandis and tetracycline, stimulated a synergistic effect against S. typhimurium and K. pneumoniae strains causing a 2-fold reduction and a 4-fold reduction on the MIC respectively [186]. Synergistic effect of pseudaloric acid isolated from plants used in traditional Chinese medicine and flunoxalone against several Candida species has also been reported [187].

Potentiation of antimicrobial action of berberine from 32 to 2 μg/ml, by a multidrug pump inhibitor, 5’-methoxyhydrocarnocip, against NorA overexpressed S. aureus has also been demonstrated [188]. Samosorn et al. [189], coupled berberine with the NorA inhibitor 5-nitro-2-phenyl-1H-indole, to further augment the efficacy of berberine, via a methylene ether linking group. They found that this hybrid exhibited remarkable antibacterial activity against S. aureus 1199B (MIC: 1.7 μM), which was over 382-fold more active than the parent berberine. Berberine and β-lactam antibiotics have shown synergistic effect against MRSA [190].

Piperine is the major plant alkaloid present in P. nigrum Linn (Black pepper) and P. longum Linn (Long pepper). Besides being an efflux pump inhibitor, it is also well known for its bioavailability enhancing activity of multiple drugs and nutraceuticals [181,182].

Odunbaku et al. [191], have reported the synergistic activity between antibiotics and ethanolic extract of Ficus exasperata leaf on Escherichia coli and Staphylococcus albus. The antibiotics selected had different targets on bacteria (protein synthesis, cell wall synthesis, nucleic acid). The study revealed that the combination of the protein synthesis inhibitors and crude plant extract had the highest inhibitory activity.

6. Way forward

The effect of rasayana is not restricted to a specific pharmacological action instead is a complex phenomenon operating via a comprehensive holistic mechanism. Some of the possible modes of action of rasayanas include: hemopoetic effect, antioxidant action, adaptogenic action, immunomodulatory action, anabolic action, nutritive function, DNA repair action & neuroprotective action [192–195]. The anti-oxidant activity of rasayana may play a vital role in prevention of DNA damage thereby minimizing mutations which lead to drug resistance. Recently, modulating factors from plants have been studied with respect to their ability to prevent or minimize toxic effects produced by an increasing number of mutagenic and carcinogenic environmental agents. The anti-mutagenic effects of basil, which has rasayana activity, on mutagenicity in S. typhimurium TA98, TA100, and TA102 cells in the presence or absence of liver microsomal activation were studied by Stajkovic et al. [196]. The basil essential oil inhibited mutations from ultraviolet irradiation by 22–76%. Mutations caused by 4-nitroquinoline-N-oxide were decreased by 23–52% and those from 2-nitropropane by 8–30%. Concordant findings were reported by Jeurissen et al. [197], who showed that basil mainly worked by blocking DNA adduct formation triggered by 1’-hydroxyestracol in the human hepatoma (HePG2) cell line, possibly by promoting phase II enzymes which resulted in conjugation and elimination of the carcinogen. This relatively new approach of using medicinal plants for their anti-mutagenic properties could be applied to bacteria by testing the plants ability to prevent mutations in bacteria thereby reducing bacterial antibiotic resistance. Thirdly, the DNA repair mechanism of rasayanas such as Amalakai rasayana and Brahama rasayana [198,199], can be further evaluated to explore the potential of rasayanas to counteract the spontaneous or induced mutations in bacteria that confer drug resistance in them.

It is often believed that bacteria can not develop resistance to botanicals [200–202]. However, recent reports suggesting that organisms can overcome bacterial or bacteriostatic action of herbal drugs cannot be ignored. Deletion of rpoS gene in E. coli and deletion of the sigB gene in Listeria monocytogenes were associated with decreased resistance to carvacrol [203]. It is possible that bacteria may develop resistance to a herbal if only one active principal with a specific target is involved; a situation similar to an antibiotic. It may be less likely if multiple active principals are involved. Nevertheless since the literature available on bacteria developing resistance to botanicals is limited further research on mechanisms to study the development of this resistance is required.

Major hindrances in use of plant extracts for clinical applications include their complex composition, extract instability and toxicity risks. It has been observed that encapsulation could be effectively utilized to decrease toxicity, if any, of plant extracts, to achieve stability and enhance targeted drug delivery. Researchers [204] have demonstrated that PLGA encapsulation helps to enhance the cellular uptake and anticancer potentials of Polygala senega, presumably by increasing drug bioavailability. Nanoscale materials/nanocomposites have emerged as significant and novel antimicrobial agents. Enhanced durability, performance, strength, flexibility and the inimitable physicochemical properties of nanomaterials are being explored in the health industry. They can be used in treatment modalities including targeted drug delivery,
prognostic visual monitoring of therapy, and even the detection of tumors [205,206]. Nanomaterials, typically 0.2–100 nm in size, have a high surface-to-volume ratio [207]; this increases their interaction with microbes enhancing their antimicrobial activity. This ability of nanoparticles can be explored further to target the concern of antibiotic resistance. However, continuous exposure of humans to nanoparticles (NPs) in the workplace can cause unpredictable human health risks [208].

7. Conclusion

The current problem of emerging MDR bacteria is posing a global medical threat and is continuously challenging the scientific community. The reducing efficacy and increasing toxicity of synthetic drugs is further aggravating the problem. This has led researchers to seek plant based antimicrobials for solution as they are now known to play a vital role in the development of effective therapeutics. Phytoconstituent actives, either unaided or in combination with antibiotics may be an effective approach to deal with the global antimicrobial resistance. The efficacy of herbs in the treatment of diseases for decades suggests that bacteria, fungi and viruses may have a reduced ability to adapt to a plant based antimicrobial regime.

The article is meant to stimulate research wherein the cidal activity of the extract is not the only parameter considered but other mechanism of action by which plants can combat drug resistant microbes are investigated. The present article emphasizes on mechanisms involved in countering multi drug resistance.

In conclusion there is an urgent need for new business models to be developed to support development of botanicals to counter drug resistant microbes as well as regulatory reforms so that clinical development programs are equitable, feasible, rigorous, and clinically relevant.

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Conflicts of interest

None.

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