WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 3, 962-996. Review Article ISSN 2277– 7105

DEVELOPMENT OF BOTANICALS TO COMBAT ANTIBIOTIC RESISTANCE

Manoj Kumar Sharma[1]* and Dhanraj Nagar[2]

[1]Professor, M.D. (Ayu), Department of Dravyaguna, Kalawati Ayurvedic Medical College & Hospital, Gorha, Kasganj (UP).

[2]Associate Professor, M.D. (Ayu), Department of Samhita Siddhant, Kalawati Ayurvedic Medical College & Hospital, Gorha, Kasganj (UP).

Article Received on
13 Jan. 2021,

Revised on 03 Feb. 2021,
Accepted on 23 Feb. 2021

DOI: https://doi.org/10.17605/OSF.IO/T9ZWH

***Corresponding Author**
Manoj Kumar Sharma[1] & Dhanraj Nagar[2]
[1]Professor, M.D. (Ayu), Department of Dravyaguna, Kalawati Ayurvedic Medical College & Hospital, Gorha, Kasganj (UP).
[2]Associate Professor, M.D. (Ayu), Department of Samhita Siddhant, Kalawati Ayurvedic Medical College & Hospital, Gorha, Kasganj (UP).

ABSTRACT

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 proteineprotein interactions. This enables the herbals 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 multidrug resistance.

KEYWORDS: Antibiotic resistance, Botanicals Synergism, Immunomodulation, Bioenhancers.

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 b-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 P. aeruginosa and Enterobacter spp. e together referred by the acronym ESKAPE which cause the majority of infections within the hospital environment.[16] The wide range of antimicrobial resistance (AMR) mechanisms used by the ESKAPE pathogens, includes enzymatic inactivation, modification of drug targets, changing cell permeability through porin loss or increase in expression of efflux pumps and mechanical protection provided by biofilm formation.

AMR in these pathogens is amajor concern to public health systems worldwide and is likely to increase as resistance profiles change.

The damaging effects of AMR are already being observed. AMR infections currently claim at least 50,000 lives annually across Europe and the US. In other areas of the world reliable estimates of the true burden are scarce but it is estimated that the deaths amount to many hundreds of thousands. It is estimated that if there is a continued rise in resistance levels, by 2050 it would lead to 10 million deaths annually.[17,18] Additionally, AMR leads to longer hospital stays, higher rates of hospitalization and rise in the treatment cost.[2,19] Preliminary research which considers only a part of the impact of AMR estimates that by 2050 the economic burden would be 100 trillion USD.[17]

2. Mechanism of development of antibiotic resistance 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- 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 S. 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 b-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 occured 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 The key mechanisms responsible for resistance to antibiotics in bacteria are listed below e_ 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. Rplasmids 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 b-lactamase that inactivates the antibiotic by hydrolyzing the b-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 Staphylococci due to the presence of mec A gene which encodes for PBP.

2A. It has low affinity for b-lactams, conferring resistance to all b-lactam antibiotics, together with b-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, ATPbinding 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 the 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]

Development of new antibiotics has not kept pace with the occurrence of resistance in antibiotics.[38] Pharmaceutical companies,in the past few decades, have shifted their development efforts to chronic diseases. In addition, many researchers are focusing on anti-viral compounds instead of antibiotics. Governments,globally, have been complacent about antibiotic research, offering only limited resources to conduct research on antibioticresistant bacteria and development of novel antibiotics. As a result, antibiotic development continues to stagnate. U.S. FDA has approved only two systemic antibacterial agents for use in humans from 2008, compared to the 16 that were approved from 1983 to 1987.[39] After a long period of stagnation, it is encouraging to see the antimicrobial 'pipeline' once again yielding results. However reports of resistance to these latest antimicrobials have been reported in select ESKAPE pathogens [40e44]. The ESKAPE pathogens,as name Suggests, are capable of 'escaping' the cidal action of antibiotics.[16] Additionally increase in the prescription of broad spectrum antibiotics is contributing to the problem of drug resistance as it is more likely that a wider spectrum of bacteria will develop resistance to them.[45]

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 [46e48]. 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), Kampo, 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, andrepresent 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 orUVstress.[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 Protoalkaloids. 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.

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 [58e60] 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 Fatoua 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 isporene 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.

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, Polygala root, Ginseng, Bupleurum root, Anemarrhena 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 proteineprotein 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 thereby 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 Rhizoma coptidis, has been reported to possess different antimicrobial activities. Anti-herpes effects of berberine was reported and the possible mechanismwas demonstrated to be inhibition of synthesis of herpes simplex viral DNA.[88]

In modest concentrations of 30-45 mg/ml, berberine showed antibacterial effect on Staphylococcus 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 herbals with anti-microbial activity [95e101] and for further details on the mechanisms of action of botanicals.[102,103] However this section oncentrates 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[110e112] 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 terpenecarnosic acid (Rosmarinus officinalis), the alkaloid reserpine (Rauvolfiavomitoria) and the diterpenetotarol (Chamaecyparis nootkatensis), have shown to inhibit NorA-induced ethidium bromide (EtBr) efflux from a NorA over expresser.[118] NorA (effuxpump) activity is inhibited by the flavonolignan 5-methoxyhydnocarpin. It synergistically increases the activity of the antimicrobial alkaloid berberine present in the same plant.

Recently a study has demonstrated that farnesol, 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 possesses an EP inhibition ability that enhanced the accumulation of EtBr and the inhibition of efflux from cells preloaded with EtBr in M. smegmatis mc2155.[116]

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[119] 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 bacterialmembrane. Brijesh et al.,[123] have demonstrated that the antidiarrhoeal 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,[125e127] 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.[134e136] 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 TNFa 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 IFN ɣ 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. officinale 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 inrecurrent 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 rotaviruswas 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 envelop 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 e 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 Tectonagrandis 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 pseudolaric acid isolated from plants used in traditional Chinese medicine and fluconazole against several Candida species has also been reported.[187]

Potentiation of antimicrobial action of berberine from 32 to 2 mg/ml, by a multidrug pump inhibitor, 50-methoxyhydnocarpin,against NorA everexpressed 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 mM), which was over 382-fold more active than the parent berberine. Berberine and b-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.[192e195] 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 antimutagenic 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 22e76%. Mutations caused by 4-nitroquinoline-N-oxide were decreased by 23e52% and those from 2-nitropropane by 8e30%. Concordant findings were reported by Jeurissen et al.[197] who showed that basil mainly worked by blocking DNA adduct formation triggered by 10-hydroxyestragole 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 Amalaki rasayana and Brahma 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,[200e202] However, recent reports suggesting that organisms can overcome bactericidal 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 0.2e100 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 anoparticles (NPs) in the work place 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. Phytoactive constituents, either unaided or in combination with antibiotics may be an effective approach to deal with the global antimicrobial resistance. The efficacy of herbals in 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 esistance.

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.

Sources of funding

The Foundation for Medical Research by the Jamsetji TataTrust (corpus grant).

Conflicts of interest

None

Acknowledgement

The authors thank Dr. Nerges Mistry for her critical reviewof the manuscript.

REFERENCES

1. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol, 2010; 1: 134. http://dx.doi.org/10.3389/fmicb.2010.00134. PMCID: PMC3109405.
2. Kapil A. The challenge of antibiotic resistance: need to contemplate. Indian J Med Res, 2005; 121: 83e91.
3. Tiwari R, Chakraborty S, Dhama K, Rajagunalan S, Singh S. Antibiotic resistance e an emerging health problem: causes, worries, challenges and solutionse a review. Int J Curr Res, 2013; 5: 1880e92.
4. Bebell LM, Muiru AN. Antibiotic use and emerging resistance: how can resource-limited countries turn the tide? Glob Heart, 2014; 9(3): 347e58. http://dx.doi.org/10.1016/j.gheart.2014.08.009.
5. Chang HH, Cohen T, Grad YH, Hanage WP, O'Brien TF, Lipsitch M. Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiol Mol Biol Rev, 2015; 79(1): 101e16. http://dx.doi.org/10.1128/MMBR.00039-14.
6. Ahmad A, Ghosh A, Schal C, Zurek L. Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community.BMC Microbiol, 2011; 11(1): 23.
7. Silbergeld EK, Price L, Graham J. Industrial farm animal production, antimicrobial resistance and human health. 2012. http://www.ncifap.org/_images/2122_AntbioRprt_FIN_web%206.7.10%202.pdf.
8. Wielinga P, Schlundt J. Combating the risk of antimicrobial resistance in animals for the benefit of human health in Denmark. A case study of emerging risks related to AMR for the International Risk Governance Council (IRGC). 2012. http://www.irgc.org/wp-content/uploads/2012/04/P.
9. Richet HM, Mohammed J, McDonald LC, Jarvis WR. Building communication networks: international network for the study and prevention of emerging antimicrobial resistance. Emerg Infect Dis, 2001; 7: 319e22.
10. http://www.medscape.com/viewarticle/846137.
11. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev, 2010; 74: 417e33.
12. Kumar S, Singh BR. An overview of mechanisms and emergence of antimicrobials drug resistance. Adv Anim Vet Sci, 2013; 1: 7e14.

13. Lowy FD. Antimicrobial resistance: the example of Staphylococcus aureus. JClin Investig, 2003; 111: 1265e73.
14. D'Souza D, Mistry NF, Vira T, Dholakia Y, Hoffner S, Pasvol G, et al. High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the revised national tuberculosis control programme in an urban metropolis (Mumbai) in Western India. BMC Public Health, 2009; 9: 211.
15. Ballal M, Devadas S, Chakraborty R, Shetty V. Emerging trends in the etiology and antimicrobial susceptibility pattern of enteric pathogens in rural coastal India. Int J Clin Med, 2014; 5: 425e32.
16. Pendleton NJ, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther, 2013; 11(3): 297e308.
17. O'Neill J. Review on antimicrobial resistance: tackling a crisis for the health and wealth of nations. 2014. http://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf.
18. Mendelson M. Role of antibiotic stewardship in extending the age of modern medicine. S Afr Med J, 2015; 105(5): 414e8. http://dx.doi.org/10.7196/samj.9635.
19. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. Lancet, 2006; 368(9538): 874e85.
20. Tiwari R, Tiwari G. Use of antibiotics: from preceding to contemporary.Scholars' Res J, 2011; 1: 59e68.
21. http://textbookofbacteriology.net.
22. Behera D. Textbook of pulmonary medicine. Published by Jaypee Brothers Medical Publishers (P) Ltd., B-3, EMCA House, 23/23B, Ansari Road, Daryaganj, New Delhi-110 002, 2010; 2.
23. Nikaido H. Multidrug resistance in bacteria. Annu Rev Biochem, 2009; 78: 119e46.
24. Liu B, Pop M. ARDBdantibiotic resistance genes database. Nucleic Acids Res, 2009; 37: D443e7.
25. Beceiro A, Tom_as M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev, 2013; 26: 185.
26. Kumar S, Varela MF. Molecular mechanisms of bacterial resistance to antimicrobial agents. 2013. http://www.formatex.info/microbiology4/vol1/522-534.pdf.

27. Kong K, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. APMIS, 2010; 118: 1e36.
28. Levy SB. The challenge of antibiotic resistance. Sci Am, 1998; 46e53.
29. Ang JY, Ezike E, Asmar BI. Antibacterial resistance. Ind J Pediatr, 2004; 71: 229e39.
30. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev, 2009; 22(4): 582e610.
31. Kourtesi C, Ball AR, Huang Y, Jachak SM, Vera DM, Khondkar P, et al. Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. Open Microbiol J, 2013; 7: 34e52.
32. Kaplan SL. Staphylococcus aureus bacteremia in children: treatment. 2003. http://www.uptodate.com/2003.
33. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents, 2010; 35: 322e32.
34. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development,dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med, 2013; 3(4): a010306.
35. Chatterjee A, Saranath D, Bhatter P, Mistry N. Global transcriptional profiling of longitudinal clinical isolates of Mycobacterium tuberculosis exhibiting rapid accumulation of drug resistance. PLoS One, 2013; 8: e54717.
36. Zar HJ, Udwadia ZF. Advances in tuberculosis 2011e2012. Thorax, 2013; 68: 283e7.
37. Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udwadia Z, et al. Global control of tuberculosis from extensively drug resist ant to untreatable tuberculosis. Lancet Respir Med, 2014; 2: 321e38.
38. Coates ARM, Gerry H, Hu Y. Novel classes of antibiotics or more of the same?Br J Pharmacol, 2011; 163: 184e94.
39. http://www.tufts.edu/med/apua/news/news-newsletter-vol-30-no-1-2.shtml.
40. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C,Venkatraman L, et al. Linezolid resistance in a clinical isolate of Staphylococcus aureus. Lancet, 2001; 358(9277): 207e8.
41. Wilson P, Andrews JA, Charlesworth R, Walesby R, Singer M, Farell DJ, et al. Linezolid resistance in clinical isolates of Staphylococcus aureus. J Antimicrob Chemother, 2003; 51(1): 186e8.

42. Hayden MK, Rezai K, Hayes RA, Lolans K, Quinn JP, Weinstein RA. Development of daptomycin resistance in vivo in methicillin-resistant Staphylococcus aureus. J Clin Microbiol, 2005; 43(10): 5285e7.
43. Mangili A, Bica I, Snydman DR, Hamer DH. Daptomycin-resistant, methicillin- resistant Staphylococcus aureus bacteremia. Clin Infect Dis, 2005; 40(7): 1058e60.
44. Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistant Acinetobacter baumannii. J Antimicrob Chemother, 2007; 59(4): 772e4.
45. http://www.thelancet.com/infection.
46. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol, 2001; 74: 113e23.
47. Narayanan S, Raja S, Ponmurugan K, Kandekar SC, Natarajaseenivasan K,Maripandi A, et al. Antibacterial activity of selected medicinal plants against multiple antibiotic resistant uropathogens: a study from Kolli Hills, Tamil Nadu, India. Benef Microbes, 2011; 2: 235e43.
48. Potroz M, Cho N. Natural products for the treatment of trachoma and Chlamydia trachomatis. Molecules, 2015; 20: 4180e203.
49. Shah A, Krishnamurthy R. Swine flu and its herbal remedies. Int J Eng Sci, 2013; 2: 68e78. 97.
50. Arora R, Chawla R, Marwah R, Arora P, Sharma RK, Kaushik V, et al. Potential of complementary and alternative medicine in preventive management of novel H1N1 flu (SwineFlu) pandemic: thwarting potential disasters in the bud. Evid Based Complement Altern Med, 2010; 1e16.
51. Gupta PD, Daswani PG, Birdi TJ. Approaches in fostering quality parameters for medicinal botanicals in the Indian context. Indian J Pharmacol, 2014; 46: 363e71.
52. Chen MC, Hao Z, Tian Y, Zhang QY, Gao PJ, Jin JL. Different effects of six antibiotics and ten traditional Chinese medicines on shiga toxin expression by Escherichia coli O157:H7. Evid Based Complement Altern Med, 2013; 2013.
53. Stefanovic O, Comic L. Synergistic antibacterial interaction between Melissa officinalis extracts and antibiotics. J App Pharm Sci, 2012; 2: 1e5.
54. Wink M, Ashour ML, El-Readi MZ. Secondary metabolites from plants inhibiting ABC transporters and reversing resistance of cancer cells and microbes to cytotoxic and antimicrobial agents. Front Microbiol, 2012; 3: 1e15.

55. Wink M. Evolutionary advantage and molecular modes of action of multicomponent mixtures used in phytomedicine. Curr Drug Metab, 2008; 9: 996e1009.
56. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics.Future Microbiol, 2012; 7(8): 979e90.
57. Carson CF, Hammer KA. Chemistry and bioactivity of essential oils. In:Thormar H, editor. Lipids and essential oils as antimicrobial agents. New York, USA: John Wiley & Sons, 2010; 203e38. ISBN-13: 9780470976678.
58. Cazarolli LH, Zanatta L, Alberton EH, Fiqueiredo MS, Folador P, Damazio RG,et al. Flavonoids: prospective drug candidates. Mini Rev Med Chem, 2008; 8(13): 1429e40.
59. Locher CP, Burch MT, Mower HF, Berestecky J, Davis H, Van-Poel B, et al. Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. J Ethnopharmacol, 1995; 49(1): 23e32.
60. Zeng F, Wang W, Wu Y, Dey M, Ye M, Avery MA, et al. Two prenylated and Cmethylated flavonoids from Tripterygium wilfordii. Planta Medica, 2010; 76(14): 1596e9.
61. Kr€amer RP, Hindorf H, Jha HC, Kallage J, Zilliken F. Antifungal activity of soybean and chickpea isoflavones and their reduced derivatives. Phytochemistry, 1984; 23(10): 2203e5.
62. Davidson PM, Naidu AS. Natural food antimicrobial systems. In: Phytophenols. CRC Press, 2000; 265e93.
63. Munyendo WLL, Orwa JA, Rukunga GM, Bii CC. Bacteriostatic and bactericidal activities of Aspilia mossambicensis, Ocimum gratissimum and Toddalia asiatica extracts on selected pathogenic bacteria. Res J Med Plant, 2011; 5: 717e27.
64. Sher A. Antimicrobial activity of natural products from medicinal plants.Gomal J Med Sci, 2004; 7(1): 65e7.
65. Ciocan D, Bara I. Plant products as antimicrobial agents. Analele S¸ tiint¸ ifice ale Univ "Alexandru Ioan Cuza" Din Ias¸ i II A Genet si Biol Mol, 2007; 8: 151e6.
66. Saura-Calixto F, P_erez-Jim_enez J. Tannins: bioavailability and mechanisms o action. In: Knasmüller S, DeMarini DM, Johnson I, Gerh€auser C, editors.Chemoprevention of cancer and DNA damage by dietary factors. Weinheim,Germany: Wiley-VCH, 2009.
67. Ya C, Gaffney SH, Lilley TH, Haslam E. Carbohydrate-polyphenol and complexation. In: Hemingway RW, Karchesy JJ, editors. Chemistry and significance of condensed tannins. New York, NY, USA: Plenum Press, 1998; 553.

68. Haslam E. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. J Nat Prod, 1996; 59(2): 205e15.
69. García A, Bocanegra-García V, Palma-Nicol_as JP, Rivera G. Recent advances in antitubercular natural products. Eur J Med Chem, 2012; 49: 1e23.
70. Termentzi A, Fokialakis N, Skaltsounis AL. Natural resins and bioactive natural products as potential antimicrobial agents. Curr Med Chem, 2012; 19: 2292e302.
71. Balzarini J, Schols D, Neyts J, van Damme E, Peumans W, de Clercq E. a-(1e3)- and a-(1e6)-D-mannose-specific plant lectins are markedly inhibitory to human immunodeficiency virus and cytomegalovirus infections in vitro. Antimicrob Agents Chemother, 1991; 35(3): 410e6.
72. Sharon N, Ofek I. Mannose specific bacterial surface lectins. In: Mirelman D, editor. Microbial lectins and agglutinins. New York, NY, USA: John Wiley & Sons, 1986; 55e82.
73. Zhang Y, Lewis K. Fabatins: new antimicrobial plant peptides. FEMS Microbiol Lett, 1997; 149(1): 59e64.
74. Lee-Huang S, Huang PL, Chen H-C, Huang PL, Bourinbaiar A, Huang HI, et al.Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon.Gene, 1995; 161(2): 151e6 [PubMed].
75. Bourinbaiar AS, Lee-Huang S. The activity of plant-derived antiretroviralproteins MAP30 and GAP31 against herpes simplex virus infection in vitro.Biochem Biophys Res Commun, 1996; 219(3): 923e9.
76. Favero J, Corbeau P, Nicolas M, Benkirane M, Trave G, Dixon JF, et al. Inhibition of human immunodeficiency virus infection by the lectin jacalin and by a derived peptide showing a sequence similarity with GP120. Eur J Immunol, 1993; 23(1): 179e85.
77. Sakagami H, Kushida T, Makino T, Hatano T, Shirataki Y, Matsuta T, et al. Functional analysis of natural polyphenols and saponins as alternative medicines, 2012; 288. http://www.intechopen.com/books/acompendium-of-essays-onalternative-therapy.
78. Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ, Saeed M. Curren approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci, 2012; 4: 10e20.
79. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov, 2005; 4: 206e20.
80. Schmidt B, Ribnicky D, Poulev A, Logendra S, Cefalu W, Raskin I. A natural history of botanical therapeutics. Metabololism, 2008; 57: S3e9.

81. Vadhana P, Singh BR, Bharadwaj M, Singh SV. Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. Pharm Anal Acta, 2015; 6: 434. http://dx.doi.org/10.4172/21532435.1000434.
82. S_anchez E, García S, Heredia N. Extracts of edible and medicinal plant damage membranes of Vibrio cholerae. Appl Environ Microbiol, 2010; 76(20): 6888e94. http://dx.doi.org/10.1128/AEM.03052-09.
83. Chitemerere TA, Mukanganyama S. Evaluation of cell membrane integrity as a potential antimicrobial target for plant products. BMC Complement AlternMed, 2014; 14: 278. http://dx.doi.org/10.1186/1472-6882-14-278.
84. Anandhi D, Srinivasan PT, Kumar G, Jagatheesh S. DNA fragmentation induced by the glycosides and flavonoids from C. coriaria. Int J Curr MicrobiolApp Sci, 2014; 3(12): 666e73.
85. Nazzaro F, Fratianni F, Martino L, Coppola R, Feo V. Effect of essential oils o pathogenic bacteria. Pharmaceuticals, 2013; 6(12): 1451e74. http://dx.doi.org/10.3390/ph6121451. PMCID: PMC3873673.
86. Radulovic NS, Blagojevic PD, Stojanovic-Radic ZZ, Stojanovic NM. Antimicrobial plant metabolites: structural diversity and mechanism of action. CurrMed Chem, 2013; 20: 932e52.
87. Mogosanu GD, Grumezescu AM, Huang KS, Bejenaru LE, Bejenaru C. Preventio of microbial communities: novel approaches based natural products.Curr Pharm Biotechnol, 2015; 16(2): 94e111.
88. Chin LW, Cheng YW, Lin SS, Lai YY, Lin LY, Chou MY, et al. Anti-herpes simplex virus effects of berberine from Coptidis rhizoma, a major component of a Chinese herbal medicine, Ching-Wei-San. Arch Virol, 2010; 155: 1933e41.
89. Wang X, Yao X, Zhu Z, Tang T, Dai K, Sadovskaya I, et al. Effect of berberine on Staphylococcus epidermidis biofilm formation. Int J Antimicrob Agent, 2009; 34: 60e6.
90. Brijesh S, Daswani PG, Tetali P, Antia NH, Birdi TJ. Studies on the antidiarrhoeal activity of Aegle marmelos unripe fruit. BMC Complement AlternMed, 2009; 9: 47.
91. Birdi T, Daswani P, Brijesh S, Tetali P, Natu A, Antia N. Newer insights into the mechanism of action of Psidium guajava L. leaves in infectious diarrhoea.BMC Complement Altern Med, 2010; 10: 33.
92. Rajasekaran D, Palombo E, Yeo T, Ley DLS, Tu CL, Malherbe F, et al. Evidence of synergistic activity of medicinal plant extracts against neuraminidase inhibitor resistant

strains of influenza viruses. Adv Microbiol, 2014; 4: 1260e77. http://dx.doi.org/10.4236/aim.2014.416136.

93. Gupta PD, Bhatter PD, D'souza D, Tolani M, Daswani P, Tetali P, et al. Evaluatin the anti Mycobacterium tuberculosis activity of Alpinia galanga (L.)Willd. Axenically under reducing oxygen conditions and inintracellular assays.BMC Complement Altern Med, 2014; 14: 84.
94. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol, 2006; 106: 290e302.
95. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plantextracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol, 2000; 31: 247e56.
96. Verma S, Singh SP. Current and future status of herbal medicines. Vet World, 2008; 1: 347e50.
97. Almagboul AZ, Bashir AK, Salih AKM, Farouk A, Khalid SA. Antimicrobial activity of certain Sudanese plants used in folkloric medicine for their antibacterial activity (in-vitro tests). J Appl Sci Res, 2011; 7: 235e56.
98. Silva NCC, Fernandes Júnior A. Biological properties of medicinal plants: areview of their antimicrobial activity. J Venom Anim Tox Trop Dis, 2010; 16: 402e13. ISSN 1678e9199.
99. Tyagi Richa, Sharma Gaurav, Jasuja Nakuleshwar Dut, Menghani Ekta. Indian medicinal plants as an effective antimicrobial agent. J Crit Rev, 2016; 3(2): 69e71.
100. Nag Mrinmoy, Mukherjee Pulok K, Biswas Rajarshi, Chanda Joydeb, Kar Amit Evaluation of antimicrobial potential of some Indian ayurvedic medicinal plants. Pharmacogn J, 2016; 8(6): 525e33.
101. Sobrinho ACN, Morais SM, Bezerra de Souza E, Santos Fontenelle. The genus Eupatorium L. (Asteraceae): a review of their antimicrobial activity. J MedPlants Res, 2017; 11(3): 43e57.
102. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan K.Combating pathogenic microorganisms using plant-derived antimicrobials:a minireview of the mechanistic basis. Biomed Res Int, 2014; 761741.
103. Srivastava J, Chandra H, Nautiyal AR, Kalra SJS. Antimicrobial resistance(AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. Biotech, 2014; 4: 451e60. http://dx.doi.org/10.1007/s13205-013-0180-y.

104. Davey ME, O'toole GA. Microbial biofilms: from ecology to molecular genetics.Microbiol Mol Biol Rev, 2000; 64(4): 847e67.
105. Amalaradjou MAR, Narayanan A, Baskaran SA, Venkitanarayanan K. Antibiofilm effect of trans-cinnamaldehyde on uropathogenic Escherichia coli. JUrol, 2010; 184(1): 358e63.
106. Amalaradjou MAR, Venkitanarayanan K. Effect of trans-cinnamaldehyde on inhibition and inactivation of Cronobacter sakazakii biofilm on abiotic surfaces.J Food Prot, 2011; 74(2): 200e8.
107. Dalleau S, Cateau E, Berg_es T, Berjeaud JM, Imbert C. In vitro activity of terpenes gainst Candida biofilms. Int J Antimicrob Agents, 2008; 31(6): 572e6.
108. Khan MS, Ahmad I. Biofilm inhibition by Cymbopogon citratus and Syzygiumaromaticum essential oils in the strains of Candida albicans. J Ethnopharmacol, 2012; 140(2): 416e23.
109. Khan MSA, Ahmad I. Antibiofilm activity of certain phytocompounds and their synergy with fluconazole against Candida albicans biofilms. J Antimicrob Chemother, 2012; 67(3): 618e21.
110. Nostro A, Roccaro AS, Bisignano G, Marino A, Cannatelli MA, Pizzimenti FC,et al. Effects of oregano, carvacrol and thymol on Staphylococcus aureusand Staphylococcus epidermidis biofilms. J Med Microbiol, 2007; 56(4): 519e23.
111. Nostro A, Marino A, Blanco AR, Cellini L, Di Giulio M, Pizzimenti FC, et al. Invitro activity of carvacrol against staphylococcal preformed biofilm by liquid and vapour contact. J Med Microbiol, 2009; 58(6): 791e7.
112. Knowles JR, Roller S, Murray DB, Naidu AS. Antimicrobial action of carvacrol at different stages of dual-species biofilm development by Staphylococcus aureus and Salmonella enterica serovar typhimurium. Appl Environ Microbiol, 2005; 71(2): 797e803.
113. Moore-Neibel K, Gerber C, Patel J, Friedman M, Ravishankar S. Antimicrobial activity of lemongrass oil against Salmonella enterica on organic leafy greens.J Appl Microbiol, 2012; 112: 485e92.
114. Sibanda T, Okoh A. The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr J Biotechnol, 2007; 6: 2886e96.
115. Stavri M, Piddock L, Gibbons S. Bacterial efflux pump inhibitors from natural sources. J Antimicrob Chemother, 2007; 59: 1247e60.

116. Jin J, Zhang J, Guo N, Sheng H, Li L, Liang J, et al. Farnesol, a potential efflux pump inhibitor in Mycobacterium smegmatis. Molecules, 2010; 15: 7750e62.
117. Li XZ, Pl_esiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin Microbiol Rev, 2015; 28(2): 337e418. http://dx.doi.org/10.1128/CMR.00117-14.
118. Holler JG, Christensen SB, Slotved H, Rasmussen HB, GuzMan A, Oslen CE,et al. Novel inhibitory activity of the Staphylococcus aureus NorA efflux pump by a kaempferol rhamnoside isolated from Persea lingue Nees. J AntimicrobChemother, 2012; 1e7.
119. Sharma S, Kumar M, Sharma S, Nargotra A, Koul S, Khan IA. Piperine as an inhibitor of Rv1258c, a putative multidrug efflux pump of Mycobacterium tuberculosis. J Antimicrob Chemother, 2010; 65: 1694e701.
120. Birdi T, D'souza D, Tolani M, Daswani P, Nair V, Tetali P, et al. Assessment of the activity of selected Indian medicinal plants against Mycobacterium tuberculosis: a preliminary screening using the Microplate Alamar Blue Assay. Eur J Med Plants, 2012; 2: 308e23.
121. Adonizio A, Leal SM, Ausubel FM, Mathee K. Attenuation of Pseudomonas aeruginosa virulence by medicinal plants in a Caenorhabditis elegans model system. J Med Microbiol, 2008; 57: 809e13.
122. Thakur P, Chawla R, Narula A, Goel R, Arora R, Sharma RK. Anti-hemolytic, hemagglutination inhibition and bacterial membrane disruptive properties of selected herbal extracts attenuate virulence of Carbapenem Resistant Escherichia coli. Microb Pathog, 2016; 95: 133e41. http://dx.doi.org/10.1016/j.micpath.2016.04.005 [Epub 2016 Apr 4].
123. Brijesh, Daswani P, Tetali P, Antia N, Birdi T. Studies on the antidiarrhoeal activity of Aegle marmelos unripe fruit: validating its traditional usage. BMC Complement Altern Med, 2009. http://dx.doi.org/10.1186/1472-6882-9-47.
124. Othman AS. Bactericidal efficacy of omega-3 fatty acids and esters present in moringa oleifera and portulaca oleracea fixed oils against oral and gastro enteric bacteria. Intern J Pharmacol, 2017; 13(1): 44e53.
125. Upadhyay A, Johny AK, Amalaradjou MAR, Baskaran SA, Kim KS,Venkitanarayanan K. Plant-derived antimicrobials reduce Listeria monocytogenes virulence factors in vitro, and down-regulate expression of virulence genes. Int J Food Microbiol, 2012; 157: 88e94.

126. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan K. Antibiofilm effect of plant derived antimicrobials on Listeria monocytogenes. Food Microbiol, 013; 36: 79e89.
127. Mooyottu S, Kollanoor-Johny A, Flock G, Bouillaut L, Upadhyay A,Sonenshein AL, et al. Carvacrol and trans-cinnamaldehyde reduce Clostridium difficile toxin production and cytotoxicity in vitro. Int J Mol Sci, 2014; 15: 4415e30.
128. Upadhyaya I, Upadhyay A, Kollanoor-Johny A, Darre MJ, Venkitanarayanan K.Effect of plant derived antimicrobials on Salmonella enteritidis adhesion to and invasion of primary chicken oviduct epithelial cells in vitro and virulence gene expression. Int J Mol Sci, 013; 14: 10608e25.
129. Yin H, Chen C, Kollanoor-Johny A, Darre MJ, Venkitanarayanan K. Controllin Aspergillus flavus and Aspergillus parasiticus growth and aflatoxin production in poultry feed using carvacrol and trans-cinnamaldehyde. Poult Sci, 2015. http://dx.doi.org/10.3382/ps/pev207.
130. Goh EB, Yim G, Tsui W, McClure J, Surette MG, Davies J. Transcriptional modulation of bacterial gene expression by sub-inhibitory concentrations of antibiotics. Proc Natl Acad Sci USA, 2002; 99: 17025e30.
131. Qiu J, Feng H, Lu J, Xiang H, Wang D, Dong J, et al. Eugenol reduces the expression of virulence-related exoproteins in Staphylococcus aureus. ApplEnviron Microb, 2010; 76: 5846e51.
132. Koh CL, Sam CK, Yin WF, Tan LY, Krishnan T, Chong YM, et al. Plant-derived natural products as sources of anti-quorum sensing compounds. Sensors, 2013; 13: 6217e28.
133. Ahmad A, Viljoen AM, Chenia HY. The impact of plant volatiles on bacterial quorum sensing. Lett Appl Microb, 2015; 60: 8e19.
134. Mukherjee PK. GMP for Indian system of medicine. In: Verpoorte R,Mukherjee PK,editors. GMP for botanicals. New Delhi: Business Horizons Ltd, 2003; 99e112.
135. Gauniyal AK, Rawat AKS, Pushpangadan P. Interactive meeting for evidenced-based complementary and alternative medicines: a report. EvidBased Complement Altern Med, 2005; 2: 249e52.
136. Kumar UA, Manjunath C, Thaminzhmani T, Ravi kiran Y, Brahmaiah Y. A review on immunomodulatory activity of plants. Indian J Nov Drug Deliv, 2012; 4: 93e103.
137. Spellberg B, Bartlett J, Wunderink R, Gilbert DN. Novel approaches are needed to develop tomorrow's antibacterial therapies. Am J Respir Crit CareMed, 2015; 191(2): 135e40. http://dx.doi.org/10.1164/rccm.201410-1894OE.

138. Serafino A, Vallebona PS, Andreola F, Zonfrillo M, Mercuri L, Federici, et al.Stimulatory effect of Eucalyptus essential oil on innate cell-mediated immune response. BMC Immunol, 2008; 9: 17.
139. Nascimento FRF, Barroqueiro ESB, Azevedo APS, Lopes AS, Ferreira SC,Silva LA, et al. Macrophage activation induced by Orbignya phalerata Mart. J Ethnopharmacol, 2006; 103(1): 53e8.
140. Cruz GVB, Pereira PVS, Patrício FJ, Costa GC, Sousa SM, Frazao JB, et al. Increase of cellular recruitment, phagocytosis ability and nitric oxide production induced by hydroalcoholic extract from Chenopodium ambrosioides leaves. J Ethnopharmacol, 2007; 111(1): 148e54.
141. Yousofi A, Daneshmandi S, Soleimani N, Bagheri K, Karimi MH. Immunomodulatory effect of Parsley (Petroselinum crispum) essential oil on immune cells: mitogen-activated splenocytes and peritoneal macrophages. ImmunopharmacolImmunotoxicol, 2012; 34(2): 303e8.
142. Ryul J-H, Ahn H, Kim JY, Kim Y-K. Inhibitory activity of plant extracts on nitric oxide synthesis in LPS-activated macrophages. Phytother Res, 2003; 17(5): 485e9.
143. Mistry NF, Birdi TJ, Antia NH. M. leprae phagocytosis and its association with membrane changes in macrophages from leprosy patients. Parasite Immunol, 1986; 8: 129e38.
144. Pugh N, Ross SA, ElSohly MA, Pasco DS. Characterisation of aloeride, a new high molecular-weight polysaccharide from Aloe vera with potent immune stimulatory activity. J Agric Food Chem, 2001; 49: 1030e4.
145. Song QH, Kobayashi T, Xiu LM, Tie H, Cyong JC. Effects of Astragalus root and Hedysari root on the murine B and T cell differentiation. J Ethnopharm, 2000; 73: 111e9.
146. Wang YY, Khoo KH, Chen ST, Lin CC, Wong CH, Lin CH. Studies on the immuno-modulating and antitumor activities of Ganoderma lucidum (Reishi) polysaccharides: functional and proteomic analyses of a fucosecontaining glycoprotein fraction responsible for the activities. Bioorg Med Chem, 2002; 10: 1057e62.
147. L_opez-García S, Casta~neda-Sanchez JI, Jim_enez-Arellanes A, Dominquez-Lopez L, Castro-Mussot ME, Hernandez-Sanchez J, et al. Macrophage activation by ursolic and oleanolic acids during mycobacterial infection. Molecule, 2015; 2(0): 14348e64.
148. Chen J, Du CY, Lam KY, Zhang WL, Lam CT, Yan AL, et al. The standardized extract of Ziziphus jujuba fruit (jujube) regulates pro-inflammatory cytokine expression in

cultured murine macrophages: suppression of lipopolysaccharide-stimulated NF-kB activity. Phytother Res, 2014; 28: 1527e32.

149. Jen HC, Ing SL, Ming YS, Chiu HL, Chiu AC, Lui WY, et al. Tumor necrosis factor-producing activity of wogonin in RAW 264.7 murine macrophage cell lines. Planta Med, 2002; 68: 1036e9.
150. Suja RS, Nair AM, Sujith S, Preethy J, Deepa AK. Evaluation of immunomodulatory potential of Emblica officinalis fruit pulp extract in mice. Indian J Anim Res, 2009; 113: 103e6.
151. Birdi TJ, Brijesh S, Daswani PG. Bactericidal effect of selected antidiarrhoeal medicinal plants on intracellular heat-stable enterotoxineproducing Escherichia coli. Indian J Pharm Sci, 2014; 79: 229e35.
152. Kayser O, Masihi KN, Kideden AF. Natural products and synthetic compounds as immunomodulators. Exp Rev Anti Infective Ther, 2003; 1: 319e35.
153. Naik S, Mohanty S, Padhi A, Pati R, Sonawane A. Evaluation of antibacterial and cytotoxic activity of Artemisia nilagirica and Murraya koenigii leaf extracts against mycobacteria and macrophages. BMC Complement Altern Med, 2014; 14: 87.
154. Kim JY, Germolec DR, Luster MI. Panax ginsengas a potential immunomodulator studies in mice. Immunopharmacol Immunotoxicol, 1990; 12: 257e76.
155. Jae YC, Ae RK, Eun SY, Kyong UB, Myung HP. Ginsenosides from Panax ginseng differentially regulate lymphocyte proliferation. Planta Med, 2002; 6: 497e500.
156. Li ZC, Zhi BL. Regulation on maturation and function of dendritic cells by Ganoderma lucidum polysaccharides. Immuno Lett, 2002; 83(3): 163e9.
157. Sang BH, Young HK, Chang WL, Park SM, Lee HY, Ahn KS, et al. Characteristi immunostimulation by angelan isolated from Angelica gigas. Immunopharm, 1998; 40(1): 39e48.
158. Zhang JS, Tang QJ, Martin ZK, Reutter W, Fan H. Activation of B lymphocytes by GLIS, a bioactive proteoglycan from Ganoderma lucidum. Life Sci, 2002; 71: 623e38.
159. Shivaprasad HN, Kharya MD, Rana AC, Mohan S. Preliminary immunomodulatory activities of the aqueous extract of Terminalia chebula. Pharm Biol, 2006; 44: 32e4.
160. Hori Y, Miura T, Hirai Y, Fukumura M, Nemoto Y, Toriizuka K, et al. Pharmacognostic studies on ginger and related drugs-part 1: five sulfonated compounds from Zingiberis rhizome (Shokyo). Phytochem, 2003; 62: 613e7.
161. Kwang SS, Kiyohara H, Matsumoto T, Yamada H. Rhamnogalacturonan II dimers cross-linked by borate diesters from the leaves of Panax ginseng C.A.Meyer are responsible for

expression of their IL-6 production enhancing activities. Carbohydr Res, 1998; 307: 97e106.

162. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicin, 2001; 8: 401e9.
163. Raskin I, Ripoll C. Can an apple a day keep the doctor away? Curr Pharm Des, 2004; 10: 3419e29.
164. Aqil F, Ahmad I, Owais M. Evaluation of anti-methicillin-resistant Staphylococcus aureus (MRSA) activity and synergy of some bioactive plant extracts. J Biotechnol, 2006; 1: 1093e102.
165. Kamatou GPP, Viljoen AM, van Vuuren SF, van Zyl RL. In vitro evidence of antimicrobial synergy between Salvia chamelaeagnea and Leonotis leonurus. S Afr J Bot, 2006; 72: 634e6.
166. Kicklighter CE, Kubanek J, Barsby T, Hay ME. Palatability and defense of some tropical in faunal worms: alkyl pyrrole sulfamates as deterrents to fish feeding. Mar Ecol Prog Ser, 2003; 263: 299e306.
167. Gomez Castellanos JR, Prieto JM, Heinrich M. Red lapacho (Tabebuia impetiginosa e a global ethnopharmacological commodity? J Ethnopharmacol, 2009; 121: 1e13.
168. Lila M, Raskin I. Health-related interactions of phytochemicals. J Food Sci, 2005; 70: 20e7.
169. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine, 2009; 16: 97e110.
170. Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgiris. Indian J Med Res, 2004; 120: 24e9.
171. Vlietinck AJ, Van den Berghe DA. Can ethnopharmacology contribute to the development of antiviral drugs? J Ethnopharmacol, 1991; 32: 141e53.
172. Taylor RS, Manandhar NP, Hudson JB, Towers GH. Antiviral activity of Nepalese medicinal plants. J Ethnopharmacol, 1996; 52(3): 157e63.
173. Kitazato K, Wang Y, Nobayashi K. Viral infectious disease and natural products with antiviral activity. Drug Discov Ther, 2007; 10: 14e22.
174. Guo S, Kenne L. Characterization of some O-acetylated saponins from Quillaja saponaria Molina. Phytochemistry, 2000; 54: 615e23.
175. Roner M, Tam KI, Kiesling-Barrager M. Prevention of rotavirus infections in vitro with aqueous extracts of Quillaja Saponaria Molina. Future Med Chem, 2010; 2: 1083e97.

176. Birdi T, Daswani P, Brijesh S,Tetali P. In vitro antigiardial and antirotaviral activity of Psidium guajava L. leaves. Indian J Pharmacol, 2011; 43: 616e7.
177. Balasubramanian G, Sarathi M, Rajesh Kumar S, Sahul Hameed AS. Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. Aqua Cult, 2007; 263: 15e9.
178. Abdallah EM. Plants: an alternative source for antimicrobials. J App Pharm Sci, 2011; 1: 16e20.
179. Chanda S, Rakholiya K. Indian combination therapy: synergism between natural plant extracts and antibiotics against infectious diseases. In: Mendez-Vilas A, editor. Science against microbial pathogens: communicating current research and technological advances, vol. 1. Spain: Formatex Research Center; 2011; 520e9.
180. Darwish R, Aburjai T. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on Escherichia coli. BMCComplement Altern Med, 2010; 10: 9.
181. Dudhatra G, Mody S, Awale M, Patel HB, Modi CM, Kumar A, et al.Comprehensive review on pharmacotherapeutics of herbal bioenhancers. SciWorld J, 2012; 1e33.
182. Tatiraju D, Bagade V, Karambelkar P, Jadhav V, Kadam V. Natural bioenhancers: an overview. J Pharmacog Phytochem, 2013; 2: 55e60.
183. Shiota S, Shimizu M, Mizusima T, Ito H, Hatano T, Yoshida T, et al. Restoration of effectiveness of beta- lactams on methicillin-resistant Staphylococcus aureus by tellimagrandin I from rose red. FEMS Microbiol Lett, 2000; 185: 135e8.
184. Shimizu M, Shiota S, Mizushima T, Ito H, Hatano T, Yoshida T, et al. Marked potentiation of activity of beta-lactams against methicillin-resistant Staphylococcus aureus by corilagin. Antimicrob Agents Chemother, 2001; 45: 3198e201.
185. Souto OSM, Falc~ao SVS, Siqueira Jr JP, Costa MJ, Melo DMF. Modulation of drug resistance in Staphylococcus aureus by extract of mango (Mangifera indica) peel. Rev Bras Farm, 2011; 21: 190e3.
186. Purushotham KG, Arun P, Jayarani JJ, Vasnthakumari R, Sankar L, Reddy BR. Synergistic in vitro antibacterial activity of Tectona grandis leaves with tetracycline. Int J Pharm Tech Res, 2010; 2: 519e23.
187. Yan Z, Hua H, Xu Y, Samaranayake LP. Potent antifungal activity of purecompounds from traditional Chinese medicine extracts against six oral Candida species and the synergy with fluconazole against azole-resistant Candida albicans. Evid Based Complement Altern Med, 2012; 106583.

188. Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 50-methoxyhydnocarpin,a multidrug pump inhibitor. Proc Natl Acad Sci USA, 2000; 97: 1433e7.
189. Samosorn S, Tanwirat B, Muhamad N, Casadei G, Tomkiewicz D, Lewis K,et al. Antibacterial activity of berberine-NorA pump inhibitor hybrids with a methylene ether linking group. Bioorg Med Chem, 2009; 17: 3866e72.
190. Yu HH, Kim KJ, Cha JD, Kim HK, Lee VE, Choi NY, et al. Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant Staphylococcus aureus. J Med Food, 2005; 8: 454e61.
191. Odunbaku OA, Ilusanya OA, Akasoro KS. Antibacterial activity of ethanolic leaf extract of Ficus exasperata on Escherichia coli and Staphylococcus albus.Sci Res Essay, 2008; 3: 562e4.
192. Chulet R, Pradhan P. A review on rasayana. Pharmacog Rev, 2009; 3: 229e34.
193. Singh AK. Rationality of rasayana therapy as adaptogenic, antioxidant and anti-inflammatory agent. Int Res J Pharm, 2011; 2(12): 259e60. ISSN2230e8407.
194. Kulkarni R, Girish KJ, Kumar A. Nootropic herbs (Medhya Rasayana) in Ayurveda an update. Pharmacog Rev, 2012; 6(12): 147e53. http://dx.doi.org/10.4103/0973-7847.99949. PMCID: PMC3459457.
195. Talekar M, Mandal SK, Sharma R. Rasayana as a public health tool in communicable diseases: a review. Int J Ayur Med, 2015; 6(1): 1e7.
196. Stajkovic O, Beric-Bjedov T, Mitic-Culafic D, Stankovic S, Vukovic-Gacic B,Simic D, et al. Antimutagenic properties of basil (Ocimum basilicum L.) in Salmonella typhimurium TA100. Food Technol Biotechnol, 2007; 45: 213e7.
197. Jeurissen SM, Punt A, Delatour T, Rietjens IM. Basil extract inhibits the sulfotransferase mediated formation of DNA adducts of the procarcinogen 1'-hydroxyestragole by rat and human liver S9 homogenates and in HepG2 human hepatoma cells. Food Chem Toxicol, 2008; 46: 2296e302.
198. Swain U, Sindhu KK, Boda U, Pothani S, Giridharan NV, Raghunath M, et al. Studies on the molecular correlates of genomic stability in rat brain cells following Amalaki rasayana therapy. Mech Ageing Dev, 2012; 133(4): 112e7. http://dx.doi.org/10.1016/j.mad.2011.10.006.
199. Guruprasad K, Subramanian A, Singh VJ, Sharma RSK, Gopinath PM,Sewram V, et al. Brahma rasayana protects against Ethyl methane sulfonate or Methyl methane sulfonate

induced chromosomal aberrations in mouse bone marrow cells. BMC Complement Altern Med, 2012; 12: 113. http://dx.doi.org/10.1186/1472-6882-12-113.

200. Nostro A. Activity of plant extracts and plant-derived compounds against drug-resistant microorganisms. In: Ahmad I, Aqil F, Owais M, editors.Modern phytomedicine: turning medicinal plants into drugs. Weinheim,Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2006. http://dx.doi.org/10.1002/9783527609987.ch10.
201. Reiner VA. Essential oils versus MRSA: viruses, bacteria can not develop resistance to natural ingredients. 2008. http://suite101.com/article/essentialoils-versus-mrsa-a50478#ixzz275BnVJ1q.
202. Warnke PH, Becker ST, Podschun R, Sivananthan S, Springer IN, Russo PA,et al. The battle against multi-resistant strains: renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. J Cranio Maxillofac Surg, 2009; 37: 392e7.
203. Ait-Ouazzou A, Espina L, Gelaw TK, de Lamo-Castellví S, Pag_an R, Garcia Gonzalo D, et al. New insights in mechanisms of bacterial inactivation by carvacrol. J Appl Microbiol, 2013; 114: 173e85.
204. Paul S, Bhattacharyya SS, Boujedaini N, Khuda-Bukhsh AR. Anticancer potentials of root extract of Polygala senega and its PLGA nanoparticlesencapsulated form. Evid Based Comp Altern Med, 2011; 517204: 13 pages,http://dx.doi.org/10.1155/2011/517204.
205. Wong IY, Bhatia SN, Toner M. Nanotechnology: emerging tools for biology and medicine. Genes Dev, 2013; 27: 2397e408.
206. Jena M, Mishra S, Jena S, Mishra SS. Nanotechnology-future prospect in recent medicine: a review. Int J Basic Clin Pharmacol, 2013; 2: 353e9.
207. Ravishankar Rai V, Jamuna Bai A. Nanoparticles and their potential application as antimicrobials. In: M_endez-Vilas A, editor. Science against microbial pathogen: communicating current research and technological advances vol. 2. Badajoz, Spain: Formatex Research Center, 2011; 197e209.
208. Rudramurthy GR, Swamy MK, Sinniah UR, Ghasemzadeh A. Nanoparticles: alternatives against drug-resistant pathogenic microbes. Molecules, 2016; 21: 836. http://dx.doi.org/10.3390/molecules21070836.