

Potential of Herbal Drug and Antibiotic Combination Therapy: A New Approach to Treat Multidrug Resistant Bacteria

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Received date: October 24, 2016; Accepted date: November 16, 2016; Published date: November 20, 2016

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Abstract

The word antibiotic emerges from antibiosis mean against life; it is also because of the fact that antibiotics affect our life. Antimicrobial drug use is rampant not only in therapeutics but also in livestock production leading to continuous contamination of our environment. Indiscriminate use of antibiotics has led to emergence and spread of antimicrobial drug resistance in microbes including pathogens leading to an imbalance in the microbiota of our system and the environment. Ominous distribution of antimicrobial drug resistance in microbes, and still then the use of antimicrobials cause several social, economic and psychological complications. At present the challenges are several with us, viz.: 1) The antibiotics, once considered miracle drugs, are now not miraculous and there is hardly any antibiotic available to treat Multiple Drug Resistant (MDR), Extended Drug Resistant (XDR), and Total Drug Resistant (TDR) microbes with a promise to cure; 2) There are no alternatives to antibiotics at hand or insight at present; 3) There are no policies to regulate antimicrobial drug use in most of the developing world if any they have not been implemented; 4) Antimicrobial drug-resistant strains are everywhere even in environment naive to human invasion; 5) Those are susceptible may get MDR strains from their clinicians; and 6) Clinicians usually ignore the physiological and psychological effects of antimicrobials while recommending the therapy and are least bothered for the side effects of antimicrobial chemotherapy. In treatment of number of infections, two or more drugs are however, the therapeutic efficacy of the combination depend interactions of drugs combined. Synergistic combinations help to reduce emergence of resistant mutants, toxicity, exhibit more antimicrobial activity and more effective against mixed infections. However, only little is understood about the combination of herbal antimicrobials and antibiotics. Herein the existing information on the synergy between antibiotics and herbal antimicrobials is reviewed.

Keywords: Bacteria; Herbs; Antibiotics; Resistance; Synergy; Antagonism; Interaction

Introduction

Due to emergence of bacterial strains resistant due to inappropriate prescribing, inappropriate use, use of antibiotics as substitute for sanitation, as growth enhancers, aerial spray of antibiotics to control plant diseases [1] and as vaccine preservative [2]. The antibiotics are also responsible for large number of side effects, so plant products are currently of growing interest as future antimicrobial agents [3]. The shortcomings of antibiotic therapy lead to the generation of new antibacterial drugs from natural resources like herbs.

The plant products proved as more promising antimicrobials in spite of the fact that their antimicrobial activity is milder than commercially available antibiotics [4,5]. The essential oils have been used for many years for their curative potential. Herbal drugs alternatively can be used in combination with antibiotics with enhanced activity against bacterial infections. In support of it, Abascal and Yarnell [1] indicated that there are various mechanisms due to which microbes usually do not become resistant to herbs viz., herbal drugs may act synergistically with drugs to kill microbes, herbs may inactivate/destroy enzymes produced by bacteria to degrade antibiotics, herbal drugs may inhibit the action of efflux pumps making bacteria unable to remove antibiotics from their body, etc. The research indicates that the combination therapy should be applied in veterinary and human medicine as an alternative therapy against bacterial infections. To

exploit the synergistic combinations, it is important to determine the optimal ratio and dose of antimicrobials. The evaluation of the synergistic or antagonistic effect of antimicrobials can improve the use of antibiotics. Previously, the synergistic activity of amoxicillin with clavulanic acid against β -lactamase-producing strains of *Haemophilus ducreyi* was determined by agar dilution susceptibility testing [6]. Along with it, sulbactam and ampicillin were combined against methicillin-resistant *staphylococci* by the agar fixed ratio method, checkerboard dilution and by time killing curves [7]. Another β -lactamase inhibitor tazobactam combined with piperacillin and cefepime to access their activity by using time-kill assay against *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* [8]. This approach was helpful in thinking about other drugs which can be combined with antibiotics.

It is important to know various types of effects that occur due to use of different drug combinations. The combined therapy has numerous benefits that include treatment of mixed infections, infection caused by specific causative organism, to increase antimicrobial activity, preventing need for long term antibiotic use, prevent emergence of multidrug resistant bacteria [9]. In other words, use of combination therapy would provide a better chance that at least one drug will be effective, thereby preventing the resistant mutant population from emerging as the dominant strain and causing therapeutic failure. In case of intra-abdominal infections that are usually caused by multiple organisms with a variety of aerobic and anaerobic bacteria. Antimicrobial combinations, such as a third-generation cephalosporin or a fluoroquinolones with metronidazole, can be used as a potential

treatment option in these cases and can sometimes be more cost-effective than a comparable single agent.

The present review summarizes the interactions between plant extracts/herbal drugs and standard antibiotics. It is observed that the drugs in combination affect differently as compared to use of the same drug(s) alone. Therefore, it is important to understand the potential interaction between herbal drugs and antibiotics for the selection of an appropriate combination.

Antibiotic resistance

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day [10]. With the continuous use of antibiotics, resistant microorganisms have emerged as a great threat. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host which include hypersensitivity, immunosuppressant and allergic reactions. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics. Drug resistance is driven by the misuse and overuse of antibiotics, which encourages bacteria to develop new ways of overcoming their presence. It has been a feature of medicine since Alexander Fleming's discovery of the first antibiotic, penicillin, in 1928. The spread of multiple antimicrobial-resistant pathogenic bacteria has been recognized by the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health threat. The development of Antimicrobial Resistance (AMR) in bacteria is neither an unexpected nor a new phenomenon. It is, however, an increasingly troublesome situation because of the frequency with which new emerging resistance phenotypes are occurring among many bacterial pathogens and even commensal organisms. Resistance has been observed to essentially all of the antimicrobial agents currently approved for use in human and veterinary clinical medicine [11].

Antibiotics along with some feed additives have been used in the livestock and poultry industry in a large scale since a long time [12]. Implementation of a ban on antibiotics as the feed additive, forced us to go for some alternative treatment [12]. Emergence and spread of AMR and Multiple Drug Resistant (MDR) infections and antibiotic-associated health ailments as a sequel of antibiotic therapy made researcher focus alternative medicine. Among alternates, the traditional herbal medicines making possible to identify novel bioactive compounds has attracted the attention of researchers' world over.

Herbs and their therapeutic effects

Herbal drugs have been used for several medication purposes throughout the world due to presence of important properties like antimicrobial, immunomodulatory, good health, therapeutic and healing properties that ensure prevention and cure of animal and human diseases [13]. In last few years, the requirement of herbal drugs has been increased due to their efficacy and potency in the treatment of various bacterial infections [13]. Due to the adaptation of civilized life over rural life, people have started to live along with companion animals which led to increasing in infectious and non-infectious agents, creating the problem of zoonotic pathogens. There are a number of herbal remedies which are constantly used as folk medicine

in various tribal communities. Some of the most common herbal drugs are there like Tulsi (*Ocimum sanctum*), Cinnamon (*Cinnamomum cassia*), Black cohosh (*Cimicifuga racemosa*), Turmeric (*Curcuma longa*), Black pepper (*Piper nigrum*), Thyme (*Thymus vulgaris*), Ginger (*Zingiber officinale*), Ashwagandha (*Withania somnifera*), Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Clove (*Syzygium aromaticum*) etc. [12]. There are number of herbal drugs which have shown adverse effects when used on experimental animals. Akande [14] investigated hepatological, histological and renal function tests of Sprague-Dawley albino rats, to determine the possible effects on rat kidney and liver on oral exposure to Agyanom mixture, Bolex bitters and Remedia mixture. Histological features of mild to severe tubular necrosis were evident in the kidney tissues of all the experimental groups compared to the control, unlike in the liver tissues. The different type of herbs exhibit different properties shown in the Table 1.

Herbs as antimicrobial

Herbs have been used for many years as food additives and traditional medicine against a number of infectious agents. The most commonly used herbs that possess antimicrobial properties are garlic, black cumin, cloves, cinnamon, thyme, mustard etc. Herbs are potent antimicrobials and can replace synthetic preservatives like Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) used in the food industry [15]. According to WHO, herbs can be the best source to obtain a variety of drugs [16]. The herbs should be studied in a better way to understand their properties, safety, and efficiency [17-19].

A number of pathogenic bacteria have developed resistance to antibiotics that results into multi-drug resistance among bacteria [20]. Several studies have reported on potent antimicrobial properties of essential oils [21]. It is observed that there are complex interactions between different functional groups of herbal drugs like phenols, aldehydes, ketones, alcohols etc. [22]. It is a fact that the herbs containing cinnamaldehyde, citral, carvacrol, eugenol or thymol (aldehyde or phenol as functional groups) possess the higher antibacterial activity in comparison to other herbs/essential plant oils [23-25].

Lambert et al. [26] assessed minimum inhibitory concentration (MIC) of oregano essential oil (OEO) and two of its principal components, i.e., thymol and carvacrol, against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. They observed an additive antimicrobial effect of carvacrol and thymol and thus the overall inhibition by OEO was much more than any of the two components used alone. Inhibition due to OEO is due to damage of membrane integrity, further affecting pH, homeostasis and equilibrium of inorganic ions in the cytoplasm. Abascal and Yarnell [1] and recently Bhardwaj and coworkers [27] reported a synergistic effect of herbs with antibiotics to kill microbes.

Herbal drugs: More effective in combination

The herbal drugs including carvacrol, eugenol, cinnamaldehyde, thymol, terpenes are found effective against a number of bacteria like *Staphylococcus aureus* and *Escherichia coli*. The combinations of carvacrol, menthol, eugenol and thymol have been found to have synergistic, additive or antagonistic effects against different microbes. The antibacterial activities of eugenol, cinnamaldehyde, thymol, carvacrol estimated as MICs were 1600, 400, 400, and 400 mg/L,

respectively. After combination, cinnamaldehyde/eugenol, thymol/eugenol, carvacrol/eugenol, and thymol/carvacrol the MIC got reduced to 400, 100, 100, and 100 mg/L, respectively, showing synergistic effects [28]. Again in the year 2010, the study was conducted to assess the susceptibility of foodborne bacteria to single and paired combinations of the EOs of *Lippia multiflora* Moldenke, *Mentha x piperita* L. and *Ocimum basilicum* and their major monoterpene alcohols, to detect synergistic, additive or antagonistic effects. The FIC indices ranged from 0.11 to 2.47 for paired combinations of *L. multiflora*, *M. x piperita* and *O. basilicum* EOs. It was found that all paired

combinations had synergistic effects on the inhibition of *Enterococcus faecalis*, *Listeria monocytogenes* and *Escherichia coli*. But eugenol with carvacrol and thymol did not show any synergistic action on any of the three bacteria and *P. aeruginosa* and *Enterobacter aerogenes* [29].

There are several kinds of drug interactions might be positive (synergy), negative (antagonism) or additive (Figure 1). The interactions are measured in terms of fractional inhibitory concentration (FIC).

Herbal drug	Active constituent	Therapeutic action	Therapeutic use
<i>Ocimum sanctum</i>	Eugenol, Urosolic acid	Analgesis, Anti-inflammatory, Antipyretic, Immunomodulatory	Against Enteric organisms pathogens and fish
<i>Cinnamomum cassia</i>	Cinnamaldehyde, 2-Hydroxy Cinnamaldehyde	Antimicrobial	Food borne pathogens, Skin infection
<i>Cimicifuga racemosa</i>	Cimicifugin, Racemosin, Actaealactone, Fukinolic acid	Anti-inflammatory	Dysmenorrhea, Discomfort of menopause, Discomfort in late pregnancy
<i>Curcuma longa</i>	Cucurmin	Antimicrobial, Anti-inflammatory, Anti-neoplastic	Detoxification, Insecticidal, Wound Healing
<i>Piper nigrum</i>	Piperine, Pentadienylpiperidine	Antimicrobial (Anti- Mycobacterial)	Asthama, Toxins
<i>Thymus vulgaris</i>	Thymol and carvacrol	Antibacterial, Anticandidal, Antioxidant	Digestive disorders
<i>Zingiber officinale</i>	6,8,10-gingerol, 6- shogoal	Analgesis, Anti-inflammatory, Antipyretic, Antimicrobial	Helicobacter pylori infection
<i>Withania somnifera</i>	Winthanolides, Withaferins, Dimeric thiowinthanolide	Analgesis, Anti-inflammatory, Antimicrobial, Anti-tumor	Alzheimer's disease, Arthritis
<i>Azadirachta indica</i>	Nimbin, Azadirachtin, gedunin, gallic acid	Antibacterial, Anti-malarial, Anti-leprotic, Anti-tuberculosis	To treat skin conditions, dental tartar and caries, endometritis
<i>Allium sativum</i> and <i>Allium cepa</i>	Allin, allicin, allyl sulfides	Antibacterial	MDR pathogens causing nosocomial infections
<i>Syzygium romaticum</i>	Eugenol, Eugenol acetate, alpha and beta caryophyllene	Antibacterial (Food borne pathogens), Anti-inflammatory, Analgesic	Periodontal problems
<i>Morinda citrifolia</i>	Anthraquinones glycosides, Flavanoids, Iridoids	Anti-inflammatory, Antimicrobial, Anti-oxidant, Anti-helminthic, Immunomodulating	Sickle cell anaemia, Anti-cancer
<i>Resveratrol</i>	CIS, Trans forms	Anti-oxidant, Cardioprotective, Anti-ageing, Anti-tumour, Immunomodulating	Growth promoter

Table 1: Different type of herbs, their active constituents, properties and therapeutic uses.

There are number of methods to access the drug interactions:

Checkerboard method.

Time-kill assay.

Diffusion methods (disks, paper strips, E- test, keyhole effect).

Checkerboard assay

The checkerboard microtiter plate assay is used to test the activities of several drugs in combination against bacteria by determining the FICs of all combinations tested.

Determination of Fractional Inhibitory Concentration (FIC Index)

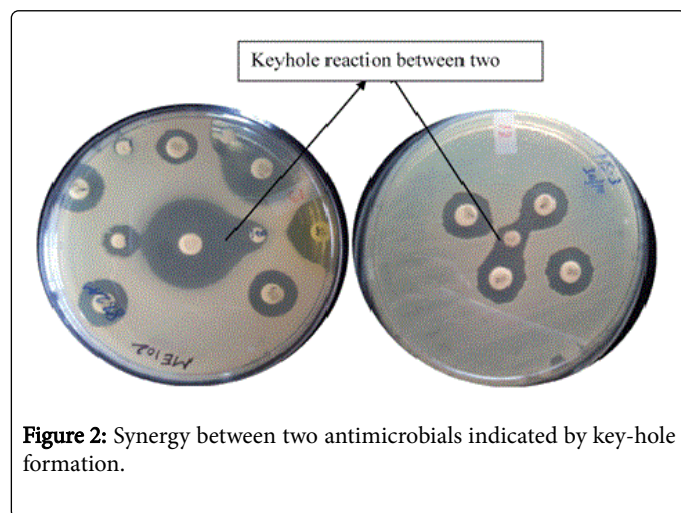
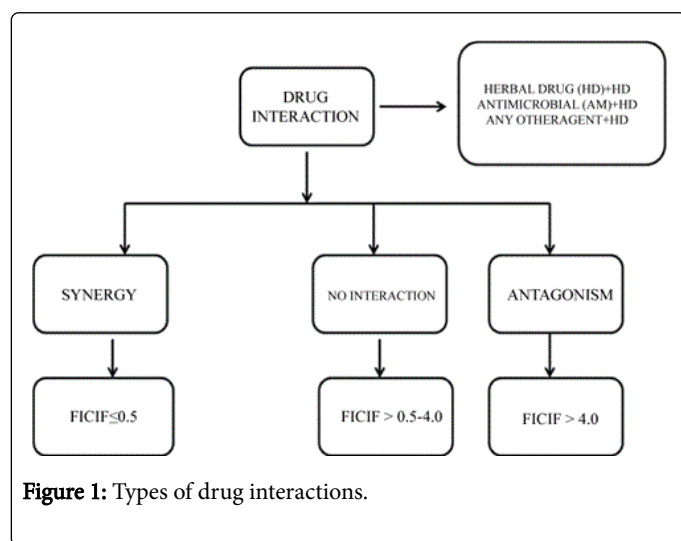
The combined effect is analyzed by using measurements of the MIC to calculate the fractional inhibition concentration index (FIC Index) according to the formulas defined by Davidson and Parish [30]:

$$\text{FIC Index} = \text{FICA} + \text{FICB}$$

Where $\text{FICA} = \text{MICA} + \text{B} / \text{MICA}$, $\text{FICB} = \text{MICB} + \text{A} / \text{MICB}$. The $\text{MICA} + \text{B}$ value is the MIC of compound A in the presence of compound B, and vice versa for $\text{MICB} + \text{A}$. Calculating the FIC value (Figures 2 and 3) for either substance A or B then requires determination of the MIC for the individual components. FIC Index results are interpreted as synergistic if $\text{FIC Index} < 0.5$, additive if $0.5 > \text{FIC Index} < 4$, or antagonistic if $\text{FIC Index} > 4$ [31].

Time kill assay

Synergy is defined as showing a 2-log₁₀ decrease in colony count after 24 h with the combination compared to that with the most active single agent alone, “indifference” as showing less than 2-log₁₀ decrease or some increase in colony count at 24 h with the combination compared to that with the most active single agent alone and “antagonism” as showing a 2-log₁₀ increase in colony count after 24 h with the combination compared to that with the most active single agent alone [32]. Time-kill and checkerboard methods are the most widely used techniques to assess synergy but are time-consuming and labor-intensive.

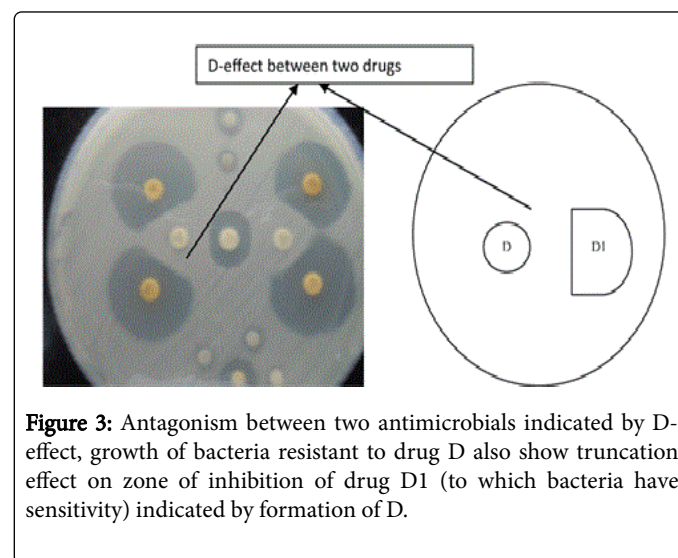


Disc Diffusion Methods

E-test (Epsilometer test)

This test is new agar diffusion method for performing antimicrobial susceptibility testing. The E-test strip is placed onto the surface of an agar plate inoculated with bacteria and incubated overnight and an elliptical zone of inhibited growth is formed around the strip. The MIC is read at the intersection of the zone with the strip. This new method of performing synergy tests would represent an attractive alternative to its labor intensive predecessors. White et al. [33] performed synergy

testing of *Escherichia coli* ATCC 35218, *Enterobacter cloacae* ATCC 23355, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 with various combinations of cefepime or ceftazidime with tobramycin or ciprofloxacin using time-kill, checkerboard, and E test techniques. Sopirala et al. [34] compared *in vitro* susceptibility testing by epsilometric (E test) methods and the checkerboard (CB) method with testing by time-kill analysis for clinically isolated *Acinetobacter baumannii* strains. They were tested *in vitro* with tigecycline, colestimethate, imipenem, and amikacin. This study indicates the utility of synergy testing to predict activity of specific antibiotic combinations against pan-drug resistant *A. baumannii*.



Keyhole reaction

The test is also known as double disk synergy test. The test was described by Jarlier and coworkers [35] for detection of ESBL enzymes in *E. coli* and *Klebsiella pneumoniae* isolates. In this method, a standardized inoculum of test isolate is swabbed on MHA. An amoxiclav (amoxicillin/clavulanic acid 30/10 µg) disc is placed at the centre of plate, surrounded by ceftazidime, cefotaxime, ceftriaxone, aztreonam are placed 20-30 mm away from this central disk. An extension in zone of inhibition around peripheral disks towards central disk indicates ESBL production. This extension is known as 'keyhole reaction'. The keyhole reaction can be seen in Figure 2.

D-effect

The D-effect can be seen in disk diffusion method between different antibiotics placed in close proximity. The effect is also used to determine some mutations or drug actions so named as D-Test or Double Disk diffusion test. If two antibiotics are tested in close proximity in disc diffusion method, then inducing agent diffuses in media and induces some particular gene expression. This gene expression inhibits the sensitivity zone of another antibiotic in its proximity. Due to this effect, that side of the zone of inhibition is compressed and flattened which forms D-shape zone of inhibition. The ideal distance between antibiotic discs is not properly known but a distance of 15-20 mm is used in routine testing. Plates are analyzed after 18 h of incubation at 35°C. There are chances of false positive as well as false negative results. The effect can be seen in Figure 3.

The effect is commonly seen in staphylococcal species. *Staphylococcus aureus* which is susceptible to Clindamycin but resistant to erythromycin may possess resistance to clindamycin [36]. In the case of clindamycin, resistance to *Staphylococcus* species can be inducible or constitutive [37]. Staphylococcal strains with inducible resistance to clindamycin appear erythromycin resistant and clindamycin sensitive in disk diffusion method when used far from each other, difficult to detect routinely in a laboratory. The isolates which possess clindamycin resistance with inducible phenotype can undergo a quick *in vitro* and *in vivo* conversion to a constitutive resistance phenotype [38].

Synergy among herbal antimicrobials

The essential oils are widely known for their antimicrobial properties. The essential oil has been indicated for the treatment of conditions like rhinitis, wet coughs, and minor burns and in the emergency treatment of wounds [39]. The aqueous and ethanolic extracts of fruits and leaves (1000 ppm) of *Foeniculum vulgare*, *Priminella anisum*, *Carum carvi*, *Majorana hortensis*, *Mentha longifolia* and *Salvia officinalis* have been tested individually and in combination for their antibacterial activity against drug resistant *E. coli* O157:H7 isolates from cattle, human and food. Zone of inhibition of 1.4 and 1.2 cm for aqueous extracts of *Foeniculum vulgare* and ethanolic extracts of *Salvia officinalis*, respectively was observed against *E. coli* O157:H7 on testing individually. On another hand the combination of aqueous extracts of *Foeniculum vulgare*, *Priminella anisum*, and *Carum carvi* in the ratio of 1:1:1 showed 2.5 cm Zone of inhibition against *E. coli*. The results showed the synergistic activity of plant extracts against *E. coli* O157:H7 isolates [40]. The drug interaction can be of different types as we have already discussed. The interaction between different drugs can be shown in graphical form in Figures 4 and 5.

Lavender oil (*Lavandula angustifolia*) is one of the commercially available and popular essential oil. The oil is used as antibiotic or antiseptic in combination with other oils like so that it can act synergistically to further enhance these effects (bitter orange, caraway, cedar wood, chamomile, geranium, grapefruit, lemon, marjoram, patchouli, rosemary, sage, sweet orange, and ylang-ylang). They are responsible for healing in a holistic manner by stimulating the mind, body, and senses. *Cinnamomum zeylanicum* combination with *L. angustifolia* has been associated with the treatment of topical infections, as a general antimicrobial agent [41]. *Lavandula angustifolia* was combined with other different 45 oils (in ratio 1:1) to find any possible antimicrobial interactive properties. Most of the interactions were additive (48.9%) than synergistic (26.7%) followed by non-interactive (23.7%) and antagonistic (0.7%). The best interaction was found between *L. angustifolia* with *Cinnamomum zeylanicum* against *Candida albicans* and with *Citrus sinensis* against *S. aureus*, respectively. When placed in various ratios the combination of *L. angustifolia* and *C. sinensis* essential oil demonstrated the best antimicrobial effect with synergy identified for all ratios against the microorganisms tested. The combination has shown a greater antifungal effect when placed in various ratios as five of the nine ratios of different oils against *C. albicans* [42].

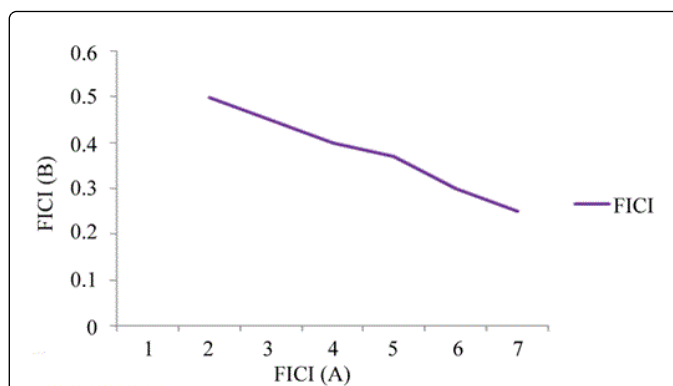


Figure 4: Fractional inhibitory concentration (FICI) showing value less than 0.5 showing synergy.

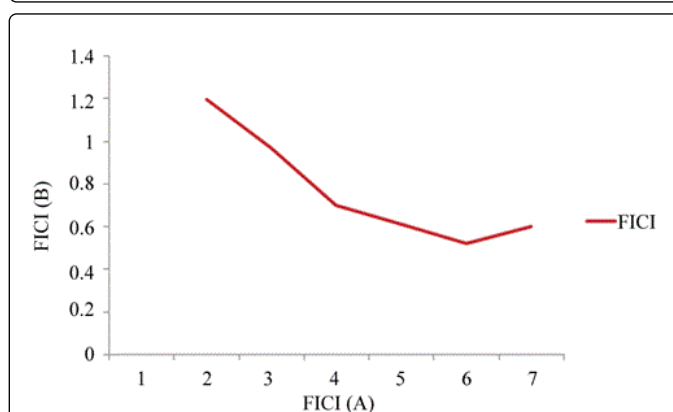


Figure 5: Fractional inhibitory concentration (FICI) showing value more than 0.5 showing antagonism.

Combined therapy is traditionally used to increase antimicrobial activity and reduce toxic effects of agents [43]. Thyme plant is having a property of healing, antiseptic fumigator, food preservation etc. [44]. *Thymus vulgaris* EO exhibits wide range of antimicrobial activity [45]. Its two main components thymol and carvacrol was demonstrated against *Staphylococcus aureus* [46], including methicillin-resistant isolates [47] etc. Studies on antibacterial activity of *Thymus vulgaris* essential oil in combination with other essential oils against *S. aureus* and *E. coli* indicated synergistic effect. The combination of *T. vulgaris* and *Cinnamomum zeylanicum* against *S. aureus* had FIC index of 0.26. The essential oils of *Juniperus communis* and *Picea abies* EOs showed additive effect (FIC indexes were 0.74 and 0.78, respectively). *Thymus vulgaris* in combination with *Anibarosaeodora* and *Melissa officinalis* had synergistic antimicrobial effect against *E. coli* with FIC of 0.23 and 0.34, respectively, whereas combination of *T. vulgaris* and *Mentha piperita* was found additive (FIC index 0.55). The combinations of essential oils are suggested as an effective and economic alternative to treat various infections instead of antibiotics [48].

The carvacrol and p-cymene in combination have been tried on *Bacillus cereus*. It was suggested that p-cymene induces swelling of bacterial cell membranes that enable easier entry of carvacrol into the cell membrane [49]. García-García et al. [50] found the most

synergistic binary combination against *L. innocua* to be carvacrol and thymol, and the most active ternary combination to be carvacrol, thymol and eugenol.

Allium sativum (Garlic) belongs to family Liliaceae is a perennial bulb-forming plant. Garlic has been used for dietary and therapeutic purposes [51]. *Gongronema latifolium* belongs to family Asclepiadaceae. Extracts of *Allium sativum* (E1) and *Gongronema latifolium* (E2) when combined against *E. coli* and *S. aureus* had reduced zone of inhibition than induced by anyone them in isolation. Besides, both plant extracts were antagonistic with ciprofloxacin against *E. coli* whereas synergistic against *S. aureus*. The similar additive effect was seen on a combination of *Gongronema latifolium* and ampicillin against *S. aureus*. They have observed synergism between *Allium sativum* and ampicillin against *S. aureus* [52]. Similarly, the antibacterial activity of orientin and vicenin flavonoids of *Ocimum sanctum* had synergistic activity against *E. coli*, *Proteus*, *S. aureus*, *S. cohnii* and *Klebsiella pneumonia* [53].

Ginkgo biloba belongs to division Ginkgo phyta and is used as traditional Chinese Medicine. *Ginkgo biloba* Leaves contain many kinds of bioactive components like flavonoids, biflavones, proanthocyanidins, alkylphenols, carboxylic acids, sterols, polyphenols etc. [54]. The non-saponifiable lipids of GBL contained terpenoids, polyphenols, sterols, chainlike alcohols. *Ginkgo biloba* polyphenols is generally composed of 15 to 21 unsaturated isoprene units [55]. The synergistic interactions were evaluated between *Ginkgo biloba* polyphenols (GBP) and lipids (extracted from *Ginkgo biloba* leaves) against *Salmonella enterica*, *S. aureus* and *Aspergillus niger*. Total eight compounds were extracted from GBL i.e., isophytol, nerolidol, linalool, β -sitosterol acetate, β -sitosterol, stigmasterol, ergosterol, β -sitosterol-3-O- β -D-glucopyranoside and *Ginkgo biloba* polyphenols (GBP). The combinations between various components have been tested for synergy. The FIC of 0.25 showed that GBP with isophytol mixture had the strongest synergistic effect against *Salmonella enterica* among all the three tested strains [56].

The synergistic interactions of honey with curcuma starch were evaluated on *E. coli* and *P. aeruginosa*. The MIC for the six varieties of honeys ranged between 12 and 18% (vol/vol) and 11 and 15% (vol/vol) against *E. coli* and *P. aeruginosa*, respectively in combination. However, MIC of kinds of honey without curcuma starch was 5-70% (vol/vol) and 5-40 % (vol/vol) against *E. coli* and *S. aureus*, respectively [57].

Interaction between herbal drugs and antibiotics

With an increase in antibiotic resistance, the idea of synergy between herbal drugs and antibiotics is a new approach to treating multidrug-resistant bacteria. The combination of two drugs can be synergistic, additive or antagonistic. The effect is said to be synergistic if the effect of the combination is more than it would be if the concentration of the second drug is replaced by the first drug, whereas antagonistic if combined effect will be less than alone effect. Synergy results in increased killing rate, potentiating of drug, prevention of drug elimination, a better effect *in vivo* etc. Understanding of synergy mechanism may provide a new strategy for the treatment of infectious diseases by reducing the side effects produced by high doses of antibiotics. The herbal extracts and antibiotics are tested for synergistic association against multi-drug resistant bacteria [18].

Various mechanisms of synergy are described such receptor blocking or active site modification [58], enzymatic degradation and

modification of the drug [59], accumulation of the antibiotic within the bacterial cell due to decreased outer membrane permeability [60], active efflux [61] etc. The success of combination therapy against various infections has been observed for many years. Due to this, the combination approach has been applied in the treatment of cancer stress-induced insomnia, hypertension, arthritis, AIDS etc. The treatment approach can be single targeted called as "silver bullet" or multitargeted called as "herbal shotgun" [62]. For the treatment of multifactorial diseases, 3-5 drugs can be used together to treat them. Synergistic combinations are composed of antibiotics with EOs of plants [63], antibiotics and bacteriophages [64], antibiotics and nano-scaled metals [65] antibiotics with quorum-sensing inhibitors [66], antipsychotic and anti-inflammatory drugs Lehtinen and Lilius [67] and many more.

Nisin and thymol alone and in combination were tested for antibacterial activity against *L. monocytogenes* and *Bacillus subtilis*. It was found that the antibacterial effect of nisin Z, produced by *Lactococcus lactis* was enhanced by sub-inhibitory concentrations of thymol for both strains of bacteria. Moreover, the use of low concentrations of nisin reduces the chances of growth of nisin-resistant bacteria sub-populations [68]. The synergistic antimicrobial activity of tea and antibiotics has also been evaluated against enteropathogens like *Salmonella Typhimurium*1402/84, *S. Typhi*, *S. Typhi*Ty2a, *Shigella dysenteriae*, *Yersinia enterocolitica* C770, and *E. coli* (EPEC P2 1265). A combination of chloramphenicol and tea extract inhibited the growth of *S. dysenteriae* at the chloramphenicol concentration of 2.5 μ g/ml (MIC 5 μ g/ml) and 5.094 mg/ml black-tea extracts (MIC 9.089 mg/ml). Tea extract also showed synergistic activity with other antibiotics like gentamicin, methicillin and nalidixic acid [69].

The biofilm inhibitors, acetic acid and corosolic acid evaluated for effect on *P. aeruginosa* biofilms growth in rotating disk reactors in combination with antibiotics tobramycin and ciprofloxacin revealed selective synergy. A non-mucoid strain of *P. aeruginosa* formed biofilm tolerating ciprofloxacin but could not in presence of both the acids without affecting the viable cell density of *P. aeruginosa*. Both compounds potentiated the activity of antibiotic tobramycin against biofilm bacteria [70]. The crude extract from *Salvia officinalis* containing carnosol reduced the MIC of aminoglycosides in vancomycin-resistant enterococci (VRE). Similar compound carnosic acid had synergistic effect with gentamicin too [71].

The *in vitro* antibacterial activity of kaempferol against antibiotic-resistant *Propionibacterium acnes* alone and in combination with erythromycin and clindamycin was tested. The combination effect analyzed by checkerboard test revealed MICs for both compounds were ≤ 32 μ g/ml for clindamycin-sensitive strains and ≥ 64 μ g/ml for clindamycin resistant *P. acnes* strains. A combination of kaempferol with quercetin showed an indifferent effect whereas a combination of clindamycin with kaempferol or quercetin showed a greater synergistic effect than that of erythromycin with kaempferol or quercetin [72]. The essential oil of *Helichrysum italicum* reduced the multidrug resistance of different bacteria like *Enterobacter aerogenes*, *E. coli*, *P. aeruginosa* and *Acinetobacter baumannii*. Geraniol significantly increases the efficacy of beta-lactams, quinolones, and chloramphenicol [63]. It is observed that at 40 mM, ellagic and tannic acids enhanced the activity of novobiocin, coumermycin, clorobiocin, rifampicin and fusidic acid against *A. baumannii* [73].

Salvia officinalis (sage) is an aromatic plant belongs to family Lamiaceae and used as flavoring agent in a number of foods. Its main constituents are diterpenoids, triterpenoids, flavonoids and phenolic

glycosides. It was found that *Salvia* is strongly antioxidant and strong use of *Salvia* tea for 14 days has improved liver antioxidant status [74]. Studies on antibacterial activity of aqueous extracts of *Salvia officinalis* alone and with the preservatives like sodium nitrite, sodium benzoate and potassium sorbate against some of the food spoiling bacteria have shown synergistic antimicrobial action against *Agrobacterium tumefaciens*, *Bacillus subtilis* and *Proteus* sp. The synergism was accessed by using checkerboard method. Synergism was observed up to 1/8 MIC values. The FIC index was varied from 0.25-0.50. Around 33.3% bacterial species have shown synergy for the combination of aqueous extract and sodium benzoate. Also, 33.3% of bacteria have shown indifference and additive effect. The combination of aqueous extract and potassium sorbate exhibits additive effect against 66.6% of bacterial species, indifferent effect against 22.2% bacteria whereas single isolate was showing synergy. Sodium nitrite combination with *Salvia* sp. extract exerted synergistic effect on 55.5% of bacteria and 33.3% showed indifference in effect. The study concluded utility of combination as a natural preservative in food [75]. Ge et al. [76] found the synergy of Oleanolic Acid (OA), a triterpenoid, with isoniazid, rifampicin or ethambutol anti-mycobacterial effects against drug-resistant strains of *M. tuberculosis* with FICs of 0.121-0.347, 0.113-0.168 and 0.093-0.266, respectively.

The *in vitro* antimicrobial effects of different spices and herbs namely, *Piper nigrum*, *Coriandrum sativum*, *Cuminum cyminum*, *Mentha piperita* and *Rosmarinus officinalis* (Rosemary) against different bacteria and fungi species was studied. The possible effects between herbs and antibiotics were also studied. Standard antibiotic such as gentamicin (10 Cg), amoxicillin/clavulanic acid (20/10) (30 µg), erythromycin (15 µg), norfloxacin (10 µg) discs were used for comparison with the antimicrobial activities of essential oils of these plants. The study was carried on *Micrococcus luteus*, *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogens*, *Escherichia coli*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans*. A synergistic effect was seen in all microorganisms when combination of *Piper nigrum* essential oil (2 µl) and gentamicin antibiotic discs was used. In the case of *Listeria monocytogens* and *Lactobacillus acidophilus* additive effect was seen. A synergistic effect was seen highest against bacteria *Listeria monocytogens*, *Salmonella Typhimurium* and *Lactobacillus acidophilus*. The additive effect was observed in *S. aureus* on combining *C. sativum* essential oil (2 µl) and gentamicin antibiotic discs whereas an antagonistic effect was seen in a combination of *C. sativum* essential oil (2 µl) and erythromycin antibiotic discs in all tested bacteria [77].

A member from family Piperaceae, *Piper* species have a number of biologically active natural products, such as pyrenes, lignans, neolignans, terpenes, propenylphenols, chalcones, flavones, benzopyrenes, chromenes, lactones, and amides [78]. The antimicrobial activity and synergism of *Piper gaudichaudianum* Kuntze with different antibiotics were studied. The crude extract, fraction F2 (dichloromethane-ethyl acetate 95:5), and fraction F3 (dichloromethane-ethyl acetate 50:50) showed good activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida tropicalis* and showed synergism with ceftriaxone, tetracycline, and vancomycin. The

synergism was accessed by isobologram and checkerboard method. Morphological changes in form and structure were found by scanning electron microscopy in *S. aureus* treated with the combination of fraction F2 and vancomycin [79]. Bharti and Vasudeva [15] evaluated the antimicrobial activity of *Oreganum vulgare* essential oil and phenolic and non-phenolic fractions of chloroform extract alone and in combination with ciprofloxacin against *S. aureus* and with fluconazole against *A. terreus*. The volatile oil and the phenolic fraction of chloroform extract have shown synergistic antibiotic and antifungal effects.

The synergistic activity between *Salvia officinalis* and *Cichorium intybus* extracts and commonly used antibiotics like amoxicillin and chloramphenicol against bacteria *S. aureus*, *E. coli*, *P. aeruginosa*, *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Proteus mirabilis* has been reported. *Salvia officinalis* extract was found more synergistic than *Cichorium intybus* to enhance the antimicrobial activity of both the antibiotics. Synergistic interactions were observed between ethyl acetate extract of *Salvia officinalis* with amoxicillin and chloramphenicol. At the sub-inhibitory concentration (1/4-1/32 MIC) of *Salvia officinalis* extracts, the MIC values of antibiotics were decreased by 2-10 fold. The combinations of *Cichorium intybus* acetone and ethyl acetate extract with antibiotics showed either additive or indifferent effects. The study concluded that synergistic activity of extracts of *S. officinalis* and some of the tested antibiotics can be an alternative treatment method [80].

The interaction between ampicillin and polyphenol theaflavin against *Stenotrophomonas multophila* isolates was determined by using checkerboard assay. It was found that MIC of ampicillin was reduced from 12.5-22.9 µg/mL to 3.125-6.25 µg/mL when used in combination [81]. FIC index was calculated as 0.22-0.35 confirms synergy. In continuation, enhancement of antibiotic efficacy of antibiotic combinations with antimicrobials derived from plants, metal ions and nanoparticles and bacteriophage lytic enzymes has been described [82]. Studies in Iran indicated that the combination of antibiotic with oil possessing antimicrobial potential could possibly result in a reduction in the amount of antibiotics to be used [83].

Researchers examined aquo-ethanolic extract of *Camellia sinensis* standardized using FTIR analysis. Seven common functional groups were found in comparison with marker compound, quercetin. The extract was tested in combination with third line antibiotics (tigecycline, ertapenem, meropenem, colistin, and augmentin) against carbapenem resistant *E. coli*. The FIC was calculated to be less than one. The combination of aquo-ethanolic extract of *Camellia sinensis* with ertapenem or meropenem showed maximum augmentative potential at 50 µg/mL and 100 µg/mL, respectively, with 5 fold decrease in MICs [84]. Bioenhancers are combined with antibiotics like tetracycline combined with drug loperamide to enhance the bioavailability and efficacy of drug by increasing its permeability, without its own pharmacological activity [85,86]. Also, Cow Urine Distillate (CUD) enhances rifampicin activity against *E. coli* by 57 times as well as against gram positive bacteria by 311 times [85]. Some of the drug interaction examples will be shown in Table 2.

Herbal Drug+Herbal Drug/Antibiotic/any other	Tested against	Type of Interaction	Reference
<i>Foeniculum vulgare</i> , <i>Priminella anisum</i> and <i>Carum carvi</i>	<i>Escherichia coli</i>	Synergistic	[40]
<i>L. angustifolia</i> + <i>Cinnamomum zeylanicum</i>	<i>Candida albicans</i>	Synergistic	-
<i>L. angustifolia</i> + <i>Citrus sinensis</i>	<i>Staphylococcus aureus</i>	Synergistic	[42]
<i>L. officinalis</i> and <i>M. alternifolia</i>	Methicillin resistant <i>S. aureus</i> (MRSA)	Antagonism	[42]
<i>Cymbopogon citratus</i> and <i>L. angustifolia</i>		Antagonism	[42]
<i>T. vulgaris</i> and <i>Cinnamomum zeylonicum</i>	<i>S. aureus</i>	Synergistic	[48]
<i>T. vulgaris</i> and <i>Mentha piperita</i>	<i>E. coli</i>	additive	[48]
Carvacrol and thymol	<i>Listeria innocua</i>	Synergistic	[50]
Orientin and vicenin flavanoids of <i>Ocimum sanctum</i>	<i>E. coli</i> , <i>Proteus</i> , <i>S. aureus</i> , <i>S. cohnii</i> and <i>Klebsiella pneumoniae</i>	Synergistic	[103]
<i>Ginkgo biloba</i> polyphenols (GBP) and lipids (extracted from <i>Ginkgo biloba</i> leaves)	<i>Salmonella enterica</i> , <i>S. aureus</i> and <i>Aspergillus niger</i>	Synergistic	[56]
Honey with curcuma starch	<i>E. coli</i> and <i>Pseudomonas aeruginosa</i>	Synergistic	[57]
Chloramphenical and tea extract	<i>Shigella dysenteriae</i>	Synergistic	[69]
Kaempferol with quercetin	<i>Propionibacterium acnes</i>	Indifference	[72]
Ellagic and tannic acids+novobiocin, coumermycin, clorobiocin, rifampicin and fusidic acid	<i>Acinetobacter baumannii</i>	Synergistic	[73]
<i>C. sativum</i> essential oil (2 µl) and Erythromycin	<i>Micrococcus luteus</i> , <i>Bacillus cereus</i> , <i>Salmonella Typhimurium</i> , <i>Listeria monocytogenes</i> , <i>E. coli</i> , <i>Lactobacillus acidophilus</i> , <i>S. aureus</i> , <i>Streptococcus pneumoniae</i> and <i>C. albicans</i> .	Antagonism	[77]
<i>Salvia officinalis</i> and <i>Cichorium tybus</i> extracts and commonly used antibiotics like amoxicillin and chloramphenicol	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>Bacillus subtilis</i> , <i>Enterobacter cloacae</i> , <i>K. pneumoniae</i> and <i>Proteus mirabilis</i>	Synergistic	[80]
<i>Camellia sinensis</i> +ertapenem, meropenem	Carbapenem resistant <i>E. coli</i>	Synergistic	[84]
Cinnamon and other herbal drugs	<i>L. monocytogenes</i> , <i>B. cereus</i> and <i>Yersinia enterocolitica</i>	Synergistic	[108]
Cinnamon and other herbal drugs	<i>E. coli</i>	Antagonism	[108]
<i>Oreganum vulgare</i> essential oil and phenolic and non-phenolic fractions of chloroform extract in combination with ciprofloxacin	<i>S. aureus</i>	Synergistic	[15]
<i>Oreganum vulgare</i> essential oil and phenolic and non-phenolic fractions of chloroform extract in combination with fluconazole	<i>Aspergillus terreus</i>	Synergistic	[15]
Imipenem and trans-cinnamaldehyde	<i>E. coli</i>	Synergistic	[27]

Table 2: Herbal drugs showing interaction with other agents.

Interaction between Cinnamon and Other Antimicrobials

Cinnamon and its properties

Cinnamomum zeylanicum is one of the oldest herbal medicines known, which is mentioned in Chinese texts since 4000 years ago [87]. *Cinnamomum zeylanicum* is natural spice and remedy to combat bacterial infection, belongs to family Lauraceae [28,66]. The plant is native to Sri Lanka, South East India, Indonesia, South America and West Indies. The active components of the cinnamon oil are cinnamaldehyde [88], ethyl cinnamate, eugenol, β-caryophyllene, and

linalool and methyl chavicol [89]. The cinnamon oil is rich in trans-cinnamaldehyde with antimicrobial effects against animal and plant pathogens, food poisoning and spoilage bacteria and fungi [90,91]. Cinnamon barks and leaves are used as flavoring agent in foods and also for the medicinal purpose [92]. It plays an important role in pharmacological effects such as anti-inflammatory, antimicrobial, antioxidant, anti-diabetes, antispasmodic, antiulcer and cytotoxic properties [93]. It possesses antimicrobial activity also against protozoans [94] and viruses, with minimal mammalian toxicity [95]. The EO of cinnamon has been found to be the most effective antimicrobial, followed by the essential oil of oregano and thyme [96].

Mechanism of action

The antimicrobial activity of cinnamon is due to the presence of aromatic aldehyde i.e., cinnamaldehyde that inhibits amino acid decarboxylase activity [97]. It is active against many pathogenic bacteria [98]. Cinnamaldehyde is highly electro-negative that interferes in biological processes like electron transfer and reacts with nitrogen-containing components, e.g., proteins and nucleic acids. The aldehyde groups of cinnamaldehyde are reactive and have the ability to cross-link covalently with DNA and proteins through amine groups, thereby interfering with their normal function [99]. At low concentrations, cinnamaldehyde inhibits different enzymes involved in cytokinesis. At higher, sub-lethal concentrations, it acts as an ATPase inhibitor and at lethal concentrations it disturbs cell membrane. According to Kwon et al. [100] cinnamaldehyde inhibited cytokinesis in *Bacillus cereus* by inhibiting the septum formation. It has been established that cinnamaldehyde binds to the FtsZ protein, inhibiting its GTP-dependent polymerization and thereby preventing cell division [101]. Besides, enzymes like histidine decarboxylase are also inhibited by cinnamaldehyde [97].

Cinnamon: Antimicrobial action

Helander et al. [102] reported the inhibition of *E. coli* O157:H7 and *S. Typhimurium* by the essential oil of cinnamon. Friedman et al. [89] found essential oil of cinnamon active against *Campylobacter jejuni* and *E. coli*. Ali et al. [103] observed 3.5 log reduction of *E. coli* O157:H7 in broth containing 1 ppm cinnamaldehyde after 75 min. Yossa et al. [104] observed that cinnamaldehyde exhibits strong antimicrobial properties on *E. coli* O157:H7 and *Salmonella*. Antibacterial activity of essential oil of cinnamon was maximum against *Staphylococcus aureus* [105] followed by *E. coli* and *Campylobacter jejuni* [106].

Cinnamon and other antimicrobials

The combined effect of essential oils of cinnamon bark, lavender, marjoram, tea tree, peppermint and ampicillin, piperacillin, cefazolin, cefuroxime, carbenicillin, ceftazidime, meropenem, analyzed by checkerboard method against beta-lactamase-producing *E. coli* indicated useful synergy. Two to four-fold reductions in MIC of the antibiotics was noted. Piperacillin+cinnamon and piperacillin +lavender FIC indices were 0.5 and 0.38, respectively [107]. The effect of combination of cinnamon and other herbal drugs was also observed on *E. coli*, *Yersinia enterocolitica*, *P. aeruginosa* and *Salmonella choleraesuis*, *S. aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Enterococcus faecalis*. The combination of two showed a synergistic effect for inhibiting the growth of *L. monocytogenes*, *B. cereus*, and *Y. enterocolitica* whereas antagonism was observed when tested against *E. coli* [108]. Recently, the synergy between imipenem and trans-cinnamaldehyde has been reported in the study on 168 *E. coli* isolates, 42 (25%) revealed synergy [27].

Studies on combination of cinnamon oil, thyme oil, nigella extract and clove extract with imipenem against *S. aureus* 5 S and *P. aeruginosa* 30 S revealed a significant increase in growth inhibition zones indicating synergy. In the case of *S. aureus* 5 S, combination between imipenem and cinnamon oil (MIC) decreased the MIC value of imipenem from 62.5-12.5 µg/ml. In the case of *E. coli*, a combination between imipenem and cinnamon oil (MIC) decreased the MIC value of imipenem from 15.625-6.25 µg/mL [109].

Carvacrol in Combination with Other Antimicrobials

Carvacrol and its properties

Carvacrol [2-methyl-5-(methylethyl) phenol] is one of the major components of oregano oil which is known for a wide spectrum of antimicrobial activity *in vitro* or *in vivo* [110,111]. Carvacrol is a well-known constituent of some medicinal plants, like black cumin (*Nigella sativa*), oregano (*Origanum compactum*), *Monarda didyma*, *Origanum dictamnus*, *Origanum microphyllum*, *Origanum onites*, *Origanum scabrum*, *Origanum vulgare*, thyme (*Thymus glandulosus*), savory (*Satureja hortensis*) [112]. The percent composition of carvacrol in oregano ranges from trace amounts to 82% and 2-45% in thyme. Carvacrol [2-methyl-5-(1-methylethyl) phenol] is biosynthesized from p-cymene and g-terpinene [113]. The hydrophobic phenolic compound in its structure is thought to be the main contributor to its antimicrobial properties; however, other components of its structure may also be important in this regard [114]. Carvacrol possesses multiple biological properties such as anti-inflammatory, anti-leishmanial, antioxidant, hepatoprotective, antimicrobial, antitussive, antispasmodic and anti-tumoral activities [115].

Carvacrol is considered safe for consumption and has been approved by the federal drug administration for addition in food. Similarly, the council of Europe included carvacrol in the list of chemical flavorings that can be found in alcoholic beverages, baked goods, chewing gum. In food industry, carvacrol is added to different products, e.g., baked goods (15.75 ppm), nonalcoholic beverages (28.54 ppm/0.18 mM), chewing gum (8.42 ppm) [116].

Mechanism of action

The effectiveness of carvacrol as a natural antimicrobial is well established; however, the mechanism of action is less understood and is believed to be associated with damage to the cell membrane. The phenolic component of carvacrol has prompted research focused on its effect on structural and functional damage to cellular membranes [117]. Permeability of the cell membrane is dependent on the hydrophobicity and the composition of the solutes that have to cross the membrane [118].

According to Lambert et al. [26] the possible mode of action of carvacrol is by disintegrating the outer membrane of bacterial cells. The underlying mechanism of carvacrol induced inhibition of growth of both Gram-positive and Gram-negative bacteria by similar to that of other phenolic compounds and occurs via membrane damage resulting in an increase in membrane permeability and disruption of the cell wall. Helander et al., [102] also reported a similar mechanism for carvacrol by releasing lipopolysaccharides (LPS) thereby increasing the permeability of the cytoplasmic membrane to ATP. However, +, 1997, opined that carvacrol act through disruption of proton motive force (PMF), electron flow, active transport and coagulation of cell contents.

Carvacrol: Antimicrobial action

Carvacrol exerts a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria isolated from food and clinical isolates and showed that it exerts bacteriostatic and bactericidal activities against *Vibrio cholerae*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enteric serovar Typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Lactobacillus sakei*, *Pseudomonas aeruginosa*, *Pseudomonas putida*,

Streptococcus mutans, and *Bacillus subtilis* [118]. Botelho et al., [119] reported its antifungal activity against *Candida albicans*. Nostro et al. [110], proved it effective even against meticillin-resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Several studies have proved that carvacrol has both bacteriostatic and bactericidal activity against microorganisms such as *Escherichia coli* O157:H7 [119,120], *Pseudomonas fluorescens* [121] etc.

Carvacrol and other antimicrobials

Carvacrol has been combined with other preservatives or preservative methods to control foodborne microorganisms. It has been shown to have stronger antimicrobial effect with increased temperature and storage time, and at a lower pH [122]. Some antimicrobials that have been combined with carvacrol to control foodborne microorganisms include p-cymene [123], nisin [124], thymol [28], eugenol [28], citral [125], cinnamaldehyde [126] and EDTA [127]. Encapsulated carvacrol in surfactant micelles has been proven effective in inhibiting the growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* [120]. Karatzas et al., [128] reported that carvacrol and high hydrostatic pressure (HPP) acted synergistically in inactivating *Listeria monocytogenes*. The combinations of *Origanum vulgare* EO and different antibiotics against a MDR strain of extended-spectrum β -lactamase-producing *E. coli* was examined [129] and a combination of whole oregano oil with penicillin had no synergistic antimicrobial effect against *E. coli* [129,130]. However, the synergistic activity of thymol and carvacrol with penicillin against *E. coli* and *S. Typhimurium* has been reported [131].

Herbal drugs and antibiotics in combination: as therapeutics *in vivo*

Mostly the studies of drug interaction are carried out *in vitro*. But in human medicine, some of the studies are carried out *in vivo* to determine effect of combined therapy. The anticancer drugs like Polyphyllin I (rhizome of Parispolyphyllin) and evodiamine (*Evodiarutae carpa*) are less effective individually as compared to other anticancer drugs but on combination, they are significantly more effective in cancer patients. The CI values were <1 in all combinations at fraction affected (Fa)=80%, indicating a synergistic anti-cancer effect [132]. *Salvia Miltiorrhiza*, *Panax Notoginseng* and *Borneol* in combination proved to be more effective in case of cardiovascular diseases [133]. In case of hepatic injury syndrome, *Artemisia annua* L. *Gardenia jasminoids Ellis*, and *Rheum palmatum* L. exhibit more strong therapeutic effect as compared to individual effect by targeting multiple sites in rat hepatic injury [134]. During the year 2012-2013, in cattle farm of IVRI, Izatnagar, screening of diarrhoeic calves was done to identify pathogenic bacteria. The calves were treated with three different combinations that included group I (antibiotics), group II (antibiotic+herbal drug) and group III (herbal drugs). In test group-I diarrhoeic calves received gentamicin (4 mg/kg body weight) intramuscular twice daily for 3-5 days. Test group-II diarrhoeic calves received gentamicin (4 mg/kg body weight) intramuscular twice daily and herbal extracts i.e., ethanolic extract of *Holarrhena antidysenterica* (bark) (10 mg/kg body weight)+leaves of *Dalbergia sissoo* (10 mg/kg body weight) orally twice daily for 3-5 days. Test group-III diarrhoeic calves received herbal extracts-*H. antidysenterica* (10 mg/kg body weight) and leaves of *D. sissoo* (10 mg/kg body weight) orally twice daily for 3-5 days. The combined therapy (in group II) had a profound effect on biochemical profile of diarrhoeic calves, the altered values as recorded on day 0 in respect of

number of parameters viz. PCV, TSP, serum creatinine, serum albumin, serum urea nitrogen and serum potassium come towards normal values by day 7 post therapy. As regards to haemoglobin SGOT, SGPT, A:G ratio and serum gamma globulin in group II diarrhoeic calves values recorded before treatment were in the normal range for the species and these values did not change significantly consequent to muscular gentamicin and oral herbal therapy on Day 7 of post treatment. So, the combined therapy being more useful over individual therapy was advocated in clinical management of diarrhea in calves [135].

Conclusion

Herbal drugs have great potential as an antimicrobial agent. In combination with each other or with other antimicrobial agents they may of immense value in decreasing use of antibiotics. The synergistic effects between herbal drugs and antibiotics against resistant bacteria provide a new and alternative way of treatment of resistant microbes. The synergistic action is of more importance in case where antibiotic(s) is no longer effective as a therapeutic agent. Combinations of herbal drugs in form of essential oils provide an effective and economical way in combating antibiotic-resistant bacteria. The analysis of a combination of herbal drugs with other aroma-therapeutic essential oils has largely demonstrated against the pathogens tested. Their FIC indexes have indicated that the essential oils have favourable antimicrobial interactions. The essential oils in combination demonstrate the best antimicrobial effect with synergy identified with all ratios against the microorganisms can be the alternative therapy.

The combinations of essential oils have not been well investigated, so further studies are required to evaluate the effect of herbal drugs in combination. The combination therapies increase the treatment options and reuse of antibiotics in a case where resistance has developed. The use of antibiotics producing side effects can be made by reduced dose exploiting their synergy with herbal antimicrobials. Moreover, to determine whether the drugs were showing synergism or antagonism, it is important to know the molecular mechanism of the antibiotic in presence as well as an absence of herbal drugs for further selection of herbal drugs. On the other hand, with benefits always there are some disadvantages also. The herbal drugs like ginseng saponins are very costly and require 4-6 years to purify it. The methods of extraction and preparation of crude extracts, their purification process influence the inhibition activity of herbal drugs. There is still scope for exploring the problems like stability, selectivity, and bioavailability of the herbal drugs and any of the side effects that may be possible during combined use. The review opens a window to look at the significant potential for the development of novel antimicrobial combinations of antibiotics and herbal antimicrobials. It may be an alternative method for the treatment of several diseases caused by multidrug-resistant bacteria.

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