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Research Article

PHYTOCHEMICAL SYNERGY: ENHANCEMENT/ SUPPRESSION OF ANTIMICROBIAL ACTIVITY & CHROMATOGRAPHIC ANALYSIS OF SELECTED HERBS AND SPICES

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ABSTRACT

Among the 12 methanolic extracts of selected spices and herbs tested for their synergistic enhancement/ suppressive antimicrobial (Kirby & Bauer method) potential against clinical strains of human bacterial pathogens, pure extracts of *Allium sativum* (Garlic), *Citrus limon* (Lemon), *Syzygium aromaticum* (Cloves) and *Ocimum basilicum* (Sweet Basil) at 25% concentration exhibited remarkable inhibition. Lemon & Sweet Basil showed a broad-spectrum of activity against all the pathogens, with pronounced inhibition against *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Proteus vulgaris*. But, in the presence of equal concentrations of other 3 extracts 100% suppression of bioactivity was observed. Garlic and cloves showed no activity against the Gram +ve cocci, but synergistically showed inhibition. Combination of garlic-clove-sweet basil was and effective cocktail against *Escherichia coli* and *Klebsiella pneumonia*. The activity of cloves and sweet basil was totally suppressed by lemon. Reversal of bioactivity was seen with the addition of garlic. The introduction of lemon in the combination of garlic and sweet basil augmented the inhibitory activity against *Salmonella paratyphi*-B. Ironically, lemon and cloves, in equal combinations, suppressed the activity of the garlic. Thin-layer chromatography of the bioactive extracts and cocktails revealed the presence of several chromogens. These observations indicated that bioactivity of phytochemical combinations precisely gets enhanced/ suppressed. Further purification & characterization of the 'lead cocktails' would generate novel antibacterial formulations.

Keywords: *Allium sativum*, *Citrus limon*, *Syzygium aromaticum*, *Ocimum basilicum, S. faecalis*, *S. aureus* and Thin-layer chromatography

INTRODUCTION

Antibiotics have undoubtedly made a major contribution to improvements in both human and animal health and welfare. The recent years have brought an alarming rise in the prevalence of resistance to some agents among certain groups of bacteria[1]. Changes in the antimicrobial target, inactivation by enzymes, changes in cellular permeability, antimicrobial active efflux, overproduction of target enzymes and bypass of the antimicrobial have been common mechanisms of antimicrobial resistance[2]. Currently, 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation, because of the absence of contraindications[3].

Plant products are characterized for a wide range of volatile compounds, some of which are important flavor quality factors[4]. Among them spices & herbs have been documented for its antimicrobial properties, since 1980's, and the interest continues to be pursued[5,6,7,8]. Crude aqueous extracts of asafoetida, ginger, cinnamon and cardamom showed a broad spectrum of antimicrobial activity against *Bacillus subtilis*, *Staphylococus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicians* & *Pseudomonas chrysogenum*[9].The MIC value ranged between 12.5 mg/ml to 3.13 mg/ml, with an exception of cinnamon alcohol extract against *E. coli* (25mg/ml).

The effectiveness of Garlic cloves and Onion stem extracts against Potato Virus Y (PVY) *in vitro* and *in vivo* showed reduced infectivity of PVY, expressed as the number of local lesions induced by PVY on *Chenopodium amaranticolor* plant as a local lesion host. The effect of these two extracts (10^{-1} to 10^{-3}) was the highest (63.6% & 51.5%) in the crude extract[10]. Components of the neem tree (*Azadirachta indica*) has been proved as promising candidates for the control of *Crinipellis perniciosa* and *Phytophthora* species[11]. Essential oils from the genus *Ocimum* has been reported to exhibit antimicrobial activity[12]. *Ocimum* extracts are used in traditional medicine and have been shown to contain biologically active constituents that are insecticidal, nematicidal, fungicidal and generally antimicrobial[13].

Eventhough the synergism between phytochemicals and antibiotics has been well documented against dreadful diseases[14]. Combined effect of spices and herbs against bacterial human pathogens is yet to be explored. The synergistic activity of spice and herbal extracts, without any commercial antibiotic interference, has never been attempted. Thus, our study was focused on evaluation of various combinations of aqueous methanolic extracts of selected spices and herbs against a set of bacterial pathogens. Standardization of thin-layer chromatograms (TLC) for the promising leads to identify the number of compounds and its phytochemical class has been done.

Chemicals and Glassware

Analytical grade (AR) grade solvents were used for extraction (Merck®, Mumbai, India), Bacteriological media (HiMedia®, Chennai, India). All the glassware were autoclaved at 121°C/ 15 mins before use.

Media and Antibiotics

Nutrient broth was used for preparation of the inoculum for the assays, Mueller-Hinton agar was used for the assay (Kirby and Bauer). Antibiotics against Gram +ve organisms: Ampicillin (A), Cephotaxime (Ce), Clotrimoxazole (Co), Tobramycin (Tb), Amoxyclav (Ac) and Gentamicin (G). Antibiotics against Gram-ve organisms were as follows: Amoxyclav (Ac), Erythromycin (E), Penicillin-G (P), Oxacillin (Ox), Cephalothin (Ce) and Clindamycin (Cd) were purchased from HiMedia®.

TLC Spray Reagents: Anisaldehyde-H_2SO_4 (for detection of terpenoids) and Iodine reagent (universal reagent for detecting compounds with functionalities).

Plant Source and Extraction

Allium cepa (Onion), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), *Syzygium aromaticum* (Clove), *Citrus limon* (lemon) were freshly bought from the local organic vegetable market. Leaves of *Azadirachta indica* (Neem) was collected from a fully grown tree in the prefectures of Chennai city. The 3 species of Basil (Tamil Name: *Tulasi*) i.e. *Ocimum sanctum* (Sacred Basil), *O. canum* (Camphor Basil) *and O. basilicum* (Sweet Basil) were freshly purchased from Anna Botanical Farm, Arumbakkam, Chennai. All the herbs were washed thoroughly with tap water and freshly used for extraction.

Human Bacterial Pathogens

Clinical strains of *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi-A, Salmonella paratyphi-B, Shigella boydii, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus faecalis* were obtained from the Department of Microbiology, A.L.Mudaliar Post Graduate Institute of Basic Medical Sciences (A.L.M.PGIBMS), Velachery, Chennai. The organisms were sub-cultured in sterile nutrient agar slants that were used as a source for further assays. Routine sub-culturing was done every fortnight to prevent contamination.

Extraction and Preliminary Bioassay with the Crude Extracts

Fresh spices and herbs were chosen for the study to specifically extract the phytochemicals in its native form, devoid of the influence of any temperature gradient. The 500 g of plant material was extracted with methanol : water (Ratio: 1:1, 250 ml x 2 times), by percolation process at room temperature (34°C). The material were soaked for 3 days, after which they were decanted and filtered using filter papers (Scholl and Schultz®). The filtrate was condensed in a rotary evaporator (Buchi® R-200 rotavapor) and dried under air draft for three days to completely remove methanol. The crude aqueous extract is readily used for the assay. One part was refrigerated (5°C) for further assays.

Antibacterial disc diffusion assay (Kirby and Bauer) against selected human pathogens

Preparation of extracts discs

2 ml of the crude extracts (25% conc.) were taken in separate glass vials and sterile whatman filter paper discs (7 mm diameter, HiMedia®) were soaked in it for overnight (16 hrs). Then they were aseptically removed using sterile forceps and thoroughly dried for 2 hrs under air draft to remove minute traces of the solvent. Separate positive controls- Antibiotic discs (HiMedia®) and negative controls-plain solvent were compared with the activity of the extracts.

Preparation of starter culture and bioassay

A loopful of culture from different pathogens was derived from the slant culture and inoculated in 2 ml of nutrient broth and incubated at 37°C/18 hrs. They were vortexed before the assay. Sterile Mueller-Hinton agar (MHA) plates were seeded with 100 µl of the inoculum and spread as a 'lawn' using a sterile cotton swab. The fortified discs were placed equidistant using a template, such that, 6 discs were accommodated in a single plate. Triplicates were made and the plates were incubated at 37°C/24 hrs. The zone of inhibition around the discs was measured using ruler and the average of the triplicates was tabulated. Bacteriostasis was also observed by checking the growth of resistant colonies within the zone of inhibition after 24 hrs of incubation.

Assay with Botanical Combinations

Out of nine extracts employed in the preliminary assay, 4 turned out to be promising. Hence, they were subjected to further assay in which 11 possible combinations (Table: 1) were tested for its efficacy against the same set of pathogens. 2 ml of equal concentrations of the different combinations of the most promising crude extracts were taken in separate glass vials. They were vortexed thoroughly for complete homogenization to assure even consistency, before the addition of discs. Separate positive controls i.e. commercial antibiotic discs (HiMedia®) and negative controls (plain solvent) were prepared along with the extract combinations and tested for its detrimental activity. The assay was carried out at room temperature in a biosafety level -II laminar airflow unit (Klenzaids®), as per the earlier procedure.

Tlc standardization of promising extracts and combinations

The promising pure extracts and botanical combinations were analyzed by Thin Layer Chromatography (TLC). Ready made silica gel plates (SiO_2 F-254, 230-400 mesh) of dimension (20 x 10 cm) were used as the sorbent. 20 *µl* from each working standard solution was spotted and eluted with various combinations of hexane: EtOAc and Pet. ether: EtOAc were employed to get the best separation of compounds. Finally, the mobile phase was standardized as petroleum ether: EtOAc (7:3) that gave convincing separation of solutes. The dried plates were detected for fluorescence under 254 nm. UV active chromogens were marked for its R_f values.

Table 1: Combinations of promising extracts tested

Extract Code	Ingredients
1.	$E_1 + E_2 + E_3 + E_4$
2.	$E_1 + E_2$
3.	$E_2 + E_3$
4.	$E_3 + E_4$
5.	$E_2 + E_4$
6.	$E_2 + E_3 + E_4$
7.	$E_1 + E_2 + E_3$
8.	$E_1 + E_3 + E_4$
9.	$E_1 + E_2 + E_4$
10.	$E_1 + E_3$
11.	$E_1 + E_4$

Key: E_1- *Citrus limon*, E_2- *Allium sativum*, E_3- *Syzygium aromaticum*, E_4- *Ocimum basilicum*

RESULTS

Out of 12 extracts of the spices, herbs and fruits tested against 11 isolates of bacterial human pathogens, garlic, lemon, clove and sweet basil exhibited conspicuous inhibitory activity against 10 pathogens. Lemon demonstrated remarkable broad-spectrum activity against 10 isolates with pronounced inhibition against *S. aureus* (Fig. 1a), *S. epidermidis* (17.3 mm), *P. vulgaris* (14.6 mm), *E. coli* (13.3 mm) and *S. faecalis* (18.6 mm), compared to all the antibiotic controls, except Clindamycin (28.6 mm). Whereas the activity was totally suppressed by garlic, clove and sweet basil against *E. coli* (Fig. 1b & 1c), *P. vulgaris* and *S. aureus*. Lemon extract was also effective against *S. paratyphi*-A. Growth of *P. vulgaris* was effectively suppressed by the triple combination of garlic, clove & sweet basil (Fig. 1e) Solvent controls showed no effect on the organisms tested (Table: 2).

Table 2: Anti-bacterial activity of extracts against Gram +ve bacteria

Treatments	*S. aureus*	*S. epidermidis*	*S. faecalis*
A. sativum	-	-	-
C. limon	12.6±0.2	17.3±0.2	18.6±0.2
S. aromaticum	-	-	-
O. basilicum	14.6±0.3	14.6±0.5	11.3±0.2
Amoxyclav®	R	10.3±0.2	21±0.5
Erythromycin®	R	16.6±0.2	11±0.5
Penicillin-G®	R	10.3±0.3	12.6±0.3
Oxacillin®	R	10.3±0.5	8.6±0.2
Cephalothin®	R	8.3±0.2	-
Clindamycin®	R	28.6±0.3	18.3±0.3
Solvent Cont.	-	-	-

* treatments were given at conc. of 5 mg/ml. R- Resistant. 100 µl of the culture is plated in each assay plate. The average of the triplicates is tabulated.

Clove effectively exhibited bactericidal activity against Salmonella and Shigella (Table: 3), the best among all the extracts tested. Nevertheless, it did not show any inhibition against the Gram +ve isolates. Similarly, Garlic was found to be ineffective at the given concentration against the Gram +ve bacterial isolates, but exhibited notable activity against the Gram –ve organisms, with maximum efficacy against *E. coli* and *P. vulgaris* (11 mm). Sweet basil was the only herbal extract that showed effective inhibition against *S. aureus* (14.6 mm) compared to other extracts, followed by lemon. It is also important to note that none of the broad spectrum antibiotic controls were inhibitory to *S. aureus*, indicating the resistance of the isolate.

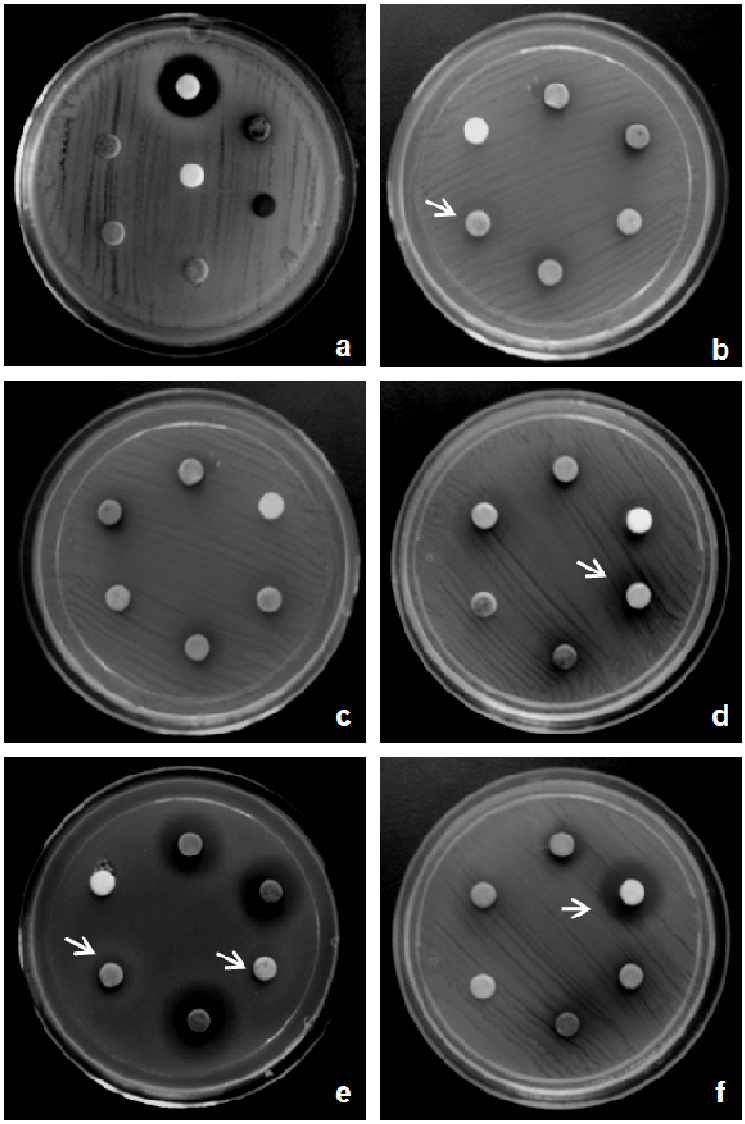

Fig. 1: Synergistic suppression & enhancement of anti-bacterial activity of aqueous methanolic extracts of herbs & spices

a. *S. aureus* inhibited by pure *C. limon* extract; b. *C. limon* activity suppressed by *A. sativum, S. aromatocum* & *O. basilicum*; c. *S. faecalis* treated with extracts of *A. sativum* & *S. aromaticum*; d. Synergistic enhancement; e. *P. vulgaris* inhibited by combinations of *A. sativum, O, basilicum* & *S. aromaticum* (zones of inhibition), while *C. limon* nullifies the activity of *O. basilicum & S. aromaticum* combination (arrows); f. *S. paratyphi-B* inhibited by *C. limon & A. sativum* (arrow) while suppression is seen along with other 2 extracts

Table 3: Anti-bacterial activities of extracts against Gram -ve bacteria

Treatments*	*E. coli*	*K. pneumonia*	*P. vulgaris*	*S. boydii*	*S. paratyphi-A*	*S. paratyphi-B*
A. sativum	11±0.2	-	11±0.2	10±0.2	10.3±0.2	8.6±0.2
C. limon	13.3±0.2	9.6±0.3	14.6±0.3	11.3±0.5	14.6±0.5	11.3±0.3
S. aromaticum	-	-	-	15.6±0.5	15±0.3	13.6±0.5
O. basilicum	-	-	-	-	-	13±0.4
Ampicillin®	-	-	-	11.6±0.2	21.6±0.4	21.6±0.4
Amoxyclav®	-	-	-	17.3±0.2	23±0.4	23±0.2
Cephotaxime®	22.6±0.3	-	36.3±0.2	23.6±0.3	26±0.3	26.6±0.2
Cotrimoxazole®	-	-	-	-	28.3±0.2	31.3±0.3
Gentamicin®	20.3±0.5	10.6±0.2	-	18.3±0.3	18.6±0.2	23.3±0.2
Tobramycin®	16.6±0.5	8.6±0.3	-	14.3±0.2	17±0.5	15.3±0.3
Solvent Cont.	-	-	-	-	-	-

* Treatments were given at conc. of 5 mg/ml. 100 µl of the culture is plated in each assay plate.

The average of the triplicates is tabulated.

TLC Fingerprinting and the influence of Phytochemical Synergy

15 different formulations of the promising extracts and combinations that were eluted through TLC suggested 6 major spots, in which the first 3 spots were predominantly found in all the combinations. 2 ice blue chromogens at the R_f 0.85 and 0.5 were typically found in the lemon extract and all of its combinations, except E1+E3 mixture. These chromogens failed to undergo any change in the fluorescence property along with any of the mixtures, except for the clove extract. Corollary to this, the antibacterial activity of lemon (E1) that was very much evident against *E. coli* and *K. pneumoniae*, got completely diminished in the presence of clove. Moreover, whether these 2 molecules are singly acting on the bacterial system or they act together, was still a question to be addressed.

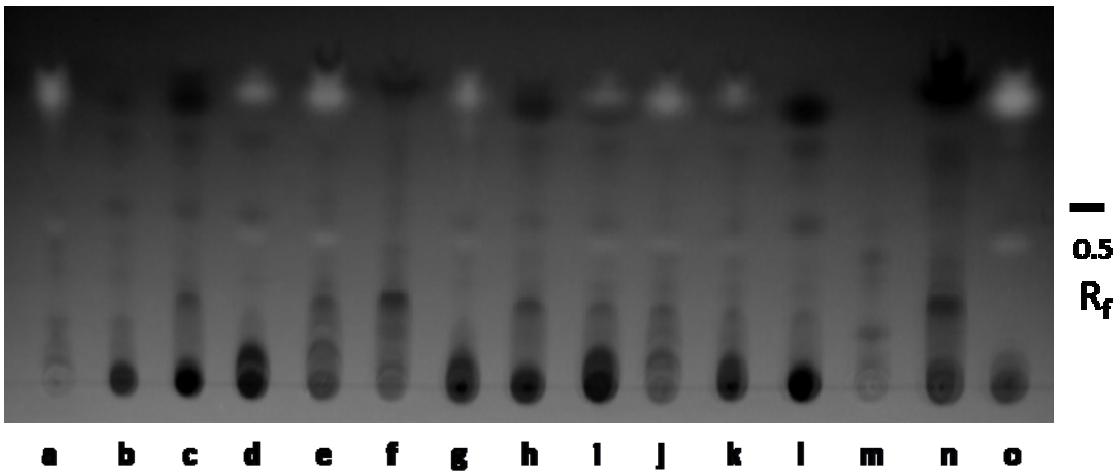

Fig. 2: Thin-Layer chromatography of bioactive extracts & combinations

a. E1+E2; b. E2+E3; c. E1+E3; d. E1+E2+E3; e. E1+E2+E3+E4; f. E3+E4; g. E2+E4; h. E2+E3+E4; i. E1+E3+E4; j. E1+E2+E4; k. E1+E4; l. E3; m. E2; n. E4; o. E1

Key: (E1- *C. limon*, E2- *A. sativum*, E3- *S. aromaticum*, E4- *O. basilicum*)

Extract of clove (E3) featured 3 predominant fluorescence spots (Dark Violet) at R_f 0.75, 0.62 and 0.53 respectively (Fig: 2, lanes D and I). All the combinations showed the presence of these compounds. However, E1+E2+E3 and E1+E3+E4 mixtures lacked the first major compound. It is also important to note that the first ice blue chromogen in lemon extract shares the same R_f (0.77) as that of the first spot of clove. But the spot found in lemon has a very imminent fluorescence that either masked or quenched the latter, to form a new compound in the same R_f. Another observation in both the mixtures (E1+E2+E3 & E1+E3+E4) was the visibility of another compound near the origin. Specifically, this chromogen was not found in the pure extracts, which might be attributed to the observation that pure clove extracts which was inactive against *K. pneumoniae* and *P. aeruginosa*, gained bactericidal activity.

Pure clove and sweet basil were not detrimental to *E. coli, K. pneumoniae* and *P. vulgaris* at the tested concentration. But, as a mixture (E2+E3+E4) with garlic, inhibits the first 2 organisms. Garlic and sweet basil independently inhibited *S. paratyphi*-B (Table: 3). But, the activity was suppressed in combination, except against *P. aeruginosa* & *P. vulgaris* (Table: 4).

The chromatogram of garlic showed 2 compounds of higher percent abundance. Spraying of the developed plates with Anisaldehyde- H_2SO_4 and Iodine reagent suggested the presence of bioactive terpenoids (pink and purple spots with the former and brown spots with the latter). The combinatorial activity of the active extracts were detrimental to *S. epidermidis* & *S. faecalis* (Table: 5)

Table 4: Anti-bacterial activities of botanical combinations against Gram -ve bacteria

Treatments	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*P. vulgaris*	*S. boydii*	*S. paratyphi* A	*S. partyphi* B
E1+E2+E3+E4	-	15.4±0.3	15.3±0.3	-	-	10.2±0.2	12.2±0.2
E1+E2+E3	-	11±0.3	12.3±0.5	16.2±0.2	10.4±0.3	10.5±0.3	-
E1+E2+E4	-	8.6±0.2	11.5±0.5	16.2±0.3	11.7±0.5	12.3±0.2	10.3±0.4
E1+E3+E4	-	9.5±0.2	10.3±0.4	-	7.5±0.3	11.3±0.4	9.6±0.3
E2+E3+E4	9.4±0.2	19.2±0.2	14.3±0.2	-	-	-	-
E1+E2	-	-	7.4±0.2	-	10.2±0.2	16.2±0.2	12.4±0.2
E1+E3	-	-	11.5±0.2	17.3±0.5	10.7±0.3	17.4±0.3	8.3±0.4
E1+E4	-	-	15.1±0.5	-	11.3±0.2	12.3±0.3	11.5±0.2
E2+E3	-	14±0.2	11.2±0.5	-	-	-	-
E2+E4	-	-	17.3±0.3	16.2±0.3	-	-	-
E3+E4	-	18.3±0.2	14.1±0.3	7.3±0.3	-	-	-
Solvent Cont.	-	-	-	-	-	-	-

* treatments are given at conc. of 5 mg/ml. 100 µl of the culture is plated in each assay plate. The average of the triplicates istabulated.

(Key: E1- *C. limon*, E2- *A. sativum*, E3- *S. aromaticum*, E4- *O. basilicum*)

Table 5: Anti-bacterial activity of botanical combinations against Gram +ve bacteria

Treatments	*S. epidermidis*	*S. faecalis*
E1+E2+E3+E4	11.6±0.2	-
E1+E2+E3	12±0.3	7.5±0.3
E1+E2+E4	8.5±0.2	14.7±0.4
E1+E3+E4	10.3±0.2	7.6±0.4
E1+E2	11.2±0.3	9±0.2
E1+E3	11.3±0.4	11±0.2
E1+E4	8.2±0.2	15.1±0.3
E2+E3	8.3±0.2	-
E2+E4	-	-
E3+E4	7.6±0.3	-
E2+E3+E4	7.4±0.3	-
Solvent Cont.	-	-

* treatments are given at conc. of 5 mg/ml. 100 µl of the culture is plated in each assay plate.

The average of the triplicates is tabulated.

(Key: E1- *C. limon*, E2- *A. sativum*, E3- *S. aromaticum*, E4- *O. basilicum*)

DISCUSSION

Among the studied extracts and combinations, the broad-spectrum of activity of lemon should be due to its acidic pH and the presence of essential oils α-pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, linalool, β-bisabolene, limonene, trans-α-bergamotene, nerol and neral that contains proven antimicrobial properties. Allicin, the main ingredient in garlic was a proven antimicrobial compound[15]. Sweet basil was the only herb extract that showed effective inhibition against *S. aureus* compared to other extracts. Garlic and Clove showed no activity against the Gram +ve cocci, but synergistically showed inhibition. In a previous synergistic study, 3% (v/v) aqueous extract of *M. myristica* or *Z. officinale* reduced the growth of *Fusarium nivale, Rhizopus stolonifer* and *Aspergillus fumigatus* in the deteriorating sweet potato juice. However, a 2% combination of both extracts retarded the growth more effectively[16].

Synergistic activity of crude aqueous and methanolic extracts of unripe fruits & brown leaves of *Carica papaya*, *Citrus aurantifoliia*, *Anana sativus*, *Citrus paradisi*, *Cymbopogon citratus*, *Cocos nucifera* chaffs, leaves *of Euphorbia heterophylla* and *Gossypium* spp. in various combinations against multi-drug resistant *S. typhi* ranged from 10-33 mm zone of growth inhibition. The antibacterial efficacy of the mixture of extracts from plant parts increased considerably compared to the low activities recorded with the extract of individual plant parts. Methanolic extracts of each plant material and mixture produced greater antimicrobial activity than the aqueous extracts at all concentrations[17]. In our study, the introduction of lemon in the combination of garlic and sweet basil augmented the inhibitory activity against *S. paratyphi*-B.

In a previous study, garlic and turmeric (70%) extracts were detrimental to *S. aureus* compared to Clotrimoxazole, Ampicillin-A, Cloxacillin, Chloramphenicol[18]. Whereas, in our study, the clinical isolates were resistant to the same combination. Addition of black thyme to fennel, sage, wild tea and wild mint mixture improved the antibacterial activity against *E. coli*, *S. aureus, E. faecalis, K. pneumonia, M. smegmatis, S. enteritidis*[19].

Lemon independently inhibited the growth of *E. coli* in the screening assay, whereas, the activity was totally nullified by the presence of other three extracts. Extracts of *Rosmarinus officinalis* (rosemary) and *Glycyrrhiza glabra* (liquorice) mutually enhanced their antimicrobial activities against *Listeria monocytogenes*, *E. coli*, *Pseudomonas fluorescens* and *Lactobacillus sake*, when compared to combinations with Clove and Cassia bark[20]. Whereas, in the present study clove along with garlic and sweet basil was the only effective cocktail against *E. coli*. All of the 11 combinations inhibited *P. aeruginosa*, where the combination of all the 4 leads proved to be the one of the most efficacious (15.3 mm). Nevertheless, the mixture of garlic and sweet basil outweighed the activity of this tetra-combination (17.3 mm). The secondary growth of resistant organisms was predominantly observed within the zone of inhibition in some of the plates.

It is also evident that lemon and clove extracts, at equal combinations, suppresses the activity of the garlic. In another study, mixtures of *ortho* and *meta*-coumaric acids and combinations of *meta*-coumaric and *trans*-cinnamic acids inhibits the growth *Erwinia carotovora* subsp. *carotovora.* Whereas, *ortho*-coumaric and *trans*-cinnamic acid combinations exhibit synergistic suppression reducing the activity to one fifth[20].

Garlic, clove and sweet basil combination was the best among all the herbal cocktails tested against *K. pneumonia* (19.2 mm), followed by clove - sweet basil and the mixture of all the 4 lead extracts. Unusually, lemon in combination with garlic, clove and sweet basil showed no evident inhibition. This observation very clearly shows that phytochemicals precisely gets suppressed, revealing the activity of the ever-changing functionalities. Similar results were seen in *S. paratyphi*-A, B and *S. faecalis* when assayed with garlic-clove, garlic-clove-sweet basil and clove-sweet basil combinations, where there was no evident inhibition. Ironically, extract of lemon in combination with other 3 extracts showed demonstrable inhibition of the cocci. Among the Gram +ve organisms tested, the behavior of *S. aureus* to the extracts suggested a new dimension of thought on antimicrobial botanicals. Lemon and sweet basil inhibited the growth of *S. aureus* independently. Whereas, none of the 11 cocktails, derived from the 4 leads, demonstrated inhibition. This total suppression was quite interesting to note.

Our attempt to study the bactericidal activity of the promising crude extracts of the spices and herb species belonging to the genus *Ocimum*, revealed many interesting and intriguing observations. From time immemorial, it has been observed and thoroughly demonstrated that phytochemicals from various plants, when treated as a mixtures, exhibits augmented/ suppressed biological activities, under *in vitro* conditions. This observation was strengthened by our experiments wih selected herbs and spices. Further, it is quite convincing to observe that extracts of pure compounds that were totally resisted by the pathogens, gains the capacity to inhibit the same set of organisms when administered as cocktails, where the 'synergy' plays a vital role in creation of new small molecular entities that acquires functional groups to influence the growth of organisms, under the *in vitro* conditions. In another view, addition of inactive extracts to the active ones doesn't suppress the bioactivity of the latter. MIC's of the bioactive mixtures has to be calculated for further applications. During formulation, the 'Phytosome' process could be applied for an effective product with maximum absorbance *in vivo*. Phytosome technique has been followed in many popular herbal extracts including *Ginkgo biloba, grape seed, hawthorn, milk thistle, green tea,* and *ginseng* [21].

This study strengthens the practice of traditional Indian & Chinese medicine, where the physicians rely on botanical combinations for

treatments. Nevertheless, characterization of these molecular entities, optimization of their dosages and removal of innate toxicities will pave way for an arsenal of drugs & bases for an 'ever-green therapeutic system'.

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