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Combination Effects of *Cymbopogon* sp. Essential Oil on Selected Bacteria

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***Abstract*— Combination effects between *Cymbopogon flexuosus* and *Cymbopogon nardus* essential oils were studied to determine whether the combination could emerge as better and more powerful antimicrobial agents against six selected bacteria includes *Bacillus subtilis, Escherichia coli, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus,* and *Staphylococcus epidermidis.* This combination study exhibited 40.67% additive, 28.67% antagonistic, 16.00% indifferent and 14.66% synergistic effects. *C. flexuosus* and *C. nardus* essential oils in combination showed a high inhibitory activity against *S. aureus* with 16% synergistic, 64% additive and 20% indifferent effects.**

***Keywords*— *Cymbopogon* sp.; essential oils; combination effects; synergism; additive.**

I. Introduction

Plant extracts and essential oils constitute a natural source of antimicrobial mixtures or pure compounds for centuries [1]. More than 60 different plant essential oils were reported to have great potential to become good antimicrobial agents against both spoilage and pathogenic organisms [2]. There has been a considerable interest in extracts and essential oils from common culinary herbs, spices and aromatic plants characterized by a notable antimicrobial activity [3]. Among the essential oils tested and proven to demonstrate a high potential as antimicrobial agent are *C. flexuosus* and *C. nardus* essential oils [2].

Cymbopogon sp. is widely used as the alternative remedies for the treatment of many infectious diseases especially related with bacterial infections [4]. *C. nardus* was reported to be used in the treatment of rheumatism fever, intestinal parasites, digestive and menstrual problems. Besides, this species exhibited some pharmacological properties such as antifungal and repellent against mosquito [5]. *C. flexuosus* commonly known as East Indian lemongrass is the other most significant species to treat various diseases. It was reported that this species is useful in the treatment of arthritis problems, cellulites, skin related problem problems and serve as digestive tonic [2]. Therefore, searching for the traditional healing systems around the world that utilize herbal remedies such as *Cymbopogon* sp. is very important for the discovery of new antibiotics for certain pathogenic bacteria [6].

Many pharmacological investigations are carried out to identify new lead structures for the development of novel therapeutic agents in the antibiotic treatments [7]. Interestingly, antimicrobial spectrum can be further characterized by studying the combination therapy. The combination therapy is very useful in preventing the emergence of resistant mutants, minimizing toxicity and obtaining the synergistic activity. Besides, it could be used as an alternative to monotherapy for patients with invasive infections that are difficult to treat, such as those due to multi-resistant species and for those who fail to respond to the standard treatment [8]. Additionally, drug synergism between two different compounds is a novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome) [9].

Few studies revealed that the combination therapy possessed high inhibitory activities against most pathogenic bacteria compared to the standard antibiotic. The combination effects between *Thymus vulgaris* and *Pimpinella anisum* essential oils exhibited the antibacterial enhancement (additive effects) against most pathogenic bacteria [10]. However, the study on combination effects of the other essential oils like *C. nardus* and *C. flexuosus* against certain bacteria is still limited. Hence, it becomes necessary to evaluate the combination effects of *Cymbopogon* sp. essential oils against pathogenic bacteria such as *B. subtilis, E. coli, S. typhimurium, S. marcescens, S. aureus,* and *S. epidermidis.*

II. Material and Method

A. Test Bacteria

Test bacteria used include *B. subtilis, E. coli, S. typhimurium, S. marcescens, S. aureus,* and *S. epidermidis*

were obtained from Microbiology Laboratory, Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM), Nilai, Negeri Sembilan, Malaysia. Each bacterial strain was streaked onto Mueller-Hinton agar (MHA, Oxoid) and incubated at 37°C overnight. The isolated single colony of each bacterial was then cultured in Mueller-Hinton broth (MHB; Oxoid) and incubated overnight at 37°C. The density of bacterial growth was then determined at 600 nm using BioPhotometer (Eppendorf AG, Hamburg, Germany). By referring to McFarland Standard Formula, the concentration of each bacterial inoculum was determined. The bacterial inoculum was diluted to the concentration of 10^6 cells / ml for further study on combination effect.

B. Test Samples

Cymbopogon nardus and *C. flexuosus* essential oils used were obtained from Universiti Putra Malaysia (UPM), Selangor, Malaysia. The series of essential oil concentration tested ranging from 1/10 to 1/50 (v/v) were prepared by combining certain volumes of each pure essential oil with Mueller-Hinton broth (MHB).

C. Combination Effect

Combination effect of *C. flexuosus* and *C. nardus* essential oils was done according to [11] and [12]. About 50 µl of each essential oil with different concentrations ranging from 1/10 to 1/50 (v/v) were mixed together. The mixtures were then added with 100 µl of diluted bacterial culture containing 10^6 cells / ml. As a control, 100 µl of the diluted bacterial culture was also mixed with 100 µl of *C. flexuosus* and *C. nardus* essential oils with different concentrations ranging from 1/10 to 1/50 (v/v). After an overnight incubation at 37°C, 20 µl of each mixture was removed and mixed with 180 µl of MHB before being plated on MHA. The agar plates were incubated overnight at 37°C and the colonies produced were counted.

D. Combination Analysis

Analysis on combination effect was done based on method by [13]. If a combination of *C. flexuosus* and *C. nardus* essential oils caused decrease in viable cell count of ≥ 2 $\log_{10}$ cfu/ml compared with the most active single treatment, the effects of the combination were considered as synergistic. If the decrease in viable cell count is 1-2 $\log_{10}$ cfu/ml, the effects of combination were considered additive. If there were no differences in viable cell count between combination treatment and individual treatment, the relationship is defined as indifferent. Antagonism was defined as an increasing of viable cell count > 2 $\log_{10}$ cfu/ml in the combination treatment compared with the most active single treatment [13]. The data of synergism, additive, indifferent and antagonistic for each combination treatment on selected bacteria were concluded in percentage (%) form.

III. Results and Discussion

The healing power of the plants was believed to be responsible from the active compound constituents. Studies of the compositional components of the essential oil from *C. flexuosus* discovered many bioactive compounds such as citral, geranial, neral, isointermedeol, sesquiterpenoids, and methyl eugenol, which are highly enriched with terpenes that could serve as potential [2]. The abundant of phytochemical in *C. nardus* essential oil such as citronella, neral, citronellol, elemicine, geranyl acetate, borneol, bournonene, camphere, camphor, citronellabutyrate, ethanol, menthol, myrcene, linalool, phellendral, trans-ocimene and tricyclene might be responsible for imparting them with the future of being therapeutically effective with the advantage of synergistic and additive effects [14]. It seems that combination study between *C. flexuosus* and *C. nardus* essential oils may show various effects since they contain abundant and different phytochemical with biological activity.

Combination effects between *C. flexuosus* and *C. nardus* essential oils were studied to determine whether the combination could emerge as better and more powerful antimicrobial agents against six selected bacteria includes *B. subtilis*, *E. coli*, *S. typhimurium*, *S. marcescens*, *S. aureus*, and *S. epidermidis*. The combination therapy is very useful in preventing the emergence of resistant mutants, minimizing toxicity and obtaining the synergistic activity. Besides, it could be used as an alternative to monotherapy for patients with invasive infections that are difficult to treat, such as those due to multi-resistant species and for those who fail to respond to the standard treatment [8]. In this combination study, the additive, antagonism, indifferent and synergism effects could be observed. Synergism has a phenomenon in which two different compounds are combined to enhance their individual activity. If the combination results in worsening effect, it is called antagonism. Effect which is less than synergistic but not antagonistic is termed as additive or indifference [15].

In this study, *Cymbopogon* sp. essential oils in combination exhibited a different percentage antimicrobial agent with inhibitory effect towards foodborne and pathogenic bacterial of additive, antagonism, indifferent and synergism effects towards different strains of bacterial as summarized in Table 1. A good combination indicated a high percentage of synergistic effect. However, there is no

TABLE I
COMBINATION EFFECTS OF *C. flexuosus* AND *C. nardus* ESSENTIAL OILS

[a] **Combination of CF+CN (v/v + v/v)**	**Relationship between *C. flexuosus* and *C. nardus***					
	[b] **ST**	[c] **EC**	[d] **SM**	[e] **SE**	[f] **BS**	[g] **SA**
P + P	[h] I	[i] A	I	A	A	I
P + 1/10	I	A	I	[j] AD	A	I
P + 1/20	I	A	A	AD	A	I
P + 1/40	I	A	A	AD	AD	I
P + 1/50	I	A	A	AD	AD	I
1/10 + P	A	AD	I	A	A	I
1/10 + 1/10	A	AD	AD	AD	A	I
1/10 + 1/20	A	AD	A	AD	A	I
1/10 + 1/40	A	AD	A	AD	AD	I
1/10 + 1/50	A	AD	A	AD	AD	I
1/20 + P	A	AD	I	A	A	I
1/20 + 1/10	A	AD	A	AD	A	I
1/20 + 1/20	A	AD	A	AD	A	AD
1/20 + 1/40	A	AD	A	AD	AD	AD
1/20 + 1/50	A	AD	A	AD	AD	AD
1/40 + P	A	AD	I	A	A	I
1/40 + 1/10	A	AD	AD	AD	A	I
1/40 + 1/20	I	AD	A	AD	A	AD
1/40 + 1/40	I	AD	A	AD	AD	S
1/40 + 1/50	I	AD	A	AD	AD	S
1/50 + P	A	AD	I	A	A	I
1/50 + 1/10	A	[k] S	AD	AD	A	I
1/50 + 1/20	I	S	A	AD	A	AD
1/50 + 1/40	I	S	A	S	I	S
1/50 + 1/50	I	S	A	S	I	S
Combinations of CF+CN (%)	A = 56% I = 44%	AD =64% A = 20%	A = 64% I = 16% AD =12%	AD =72% A = 20% S = 8%	A = 60% AD =32% I = 8%	AD =64% I = 20% S = 16%
Overall Combinations of CF+CN (%)	AD = 40.67%; A = 28.67%; I = 16.00%; S = 14.66%					

[a] Combination CF+CN (v/v + v/v) = Combination of *C. flexuosus* and *C. nardus* essential oils. [b] ST = *S. typhimurium*. [c] EC = *E. coli*. [d] SM = *S. marcescens*. [e] SE = *S. epidermidis*. [f] BS = *B. subtilis*. [g] SA = *S. aureus*. [h] I = Indifferent. [i] A = Antagonistic. [j] AD = Additive. [k] S = Synergistic.

specific combination ratio since the effects are depended on the concentration of each treatment and bacterial test. Generally, *C. flexuosus* and *C. nardus* essential oils in combination exhibited more additive effect (40.67%) rather than antagonism (28.67%), indifference (16.00%) and synergistic (14.66%) effects. The combination of *Cymbopogon* sp. essential oils showed a high inhibitory activity against *S. aureus* with 16% synergistic, 64% additive and 20% indifferent effects. *S. aureus* causes a variety of suppurative (pus-forming) infections in humans. It causes superficial skin lesions such as boils, styes and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis [16]. Thus, the combination therapy of *Cymbopogon* sp. essential oils might be used to overcome the ailments or infections by *S. aureus*.

However, *Cymbopogon* sp. essential oils in combination indicated a low inhibitory activity towards *S. typhimirium* with 56% antagonistic and 44% indifferent effects. Meanwhile, the combination treatment towards the other four bacterial strains exhibited intermediate activity with high percentage of antagonistic or without synergistic effects. In this study, most of the additive and synergism effects occur on Gram-positive bacteria, while antagonism effects occur on Gram-negative bacteria. It showed that the *Cymbopogon* sp. essential oils in combination are more effective towards Gram-positive bacteria and less effective against Gram-negative bacteria. This study agrees with the findings by [17] in which reported that the combination of two different plant extracts may be effective for the treatment of infections by Gram-positive organisms and may not be effective for the treatment of infections by Gram-negative organisms.

IV. Conclusions

Cymbopogon flexuosus and *C. nardus* essential oils in combination were revealed inhibitory activity against six tested bacterial strains with different effects include synergistic, additive, indifferent and antagonistic. The *Cymbopogon* sp. essential oils in combination exhibited high synergistic effect against *S. aureus*. This finding suggests that essential oils from *Cymbopogon* sp. showed a potential inhibitory activity against *S. aureus* and can be further used for clinical treatment. Therefore, there is a need for a study on post-antibiotic effect (PAE) and possible impact of PAE in the clinical situation.

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