THE ANTIMICROBIAL EFFECTIVENESS OF THE MIXED EXTRACTIONS OF ALLIUM SATIVUM AND SYZGIUM AROMATICUM AGAINST PATHOGENIC MICROBES TRIGGERING INFLAMMATION IN ASTHMA AND SINUSITIS PATIENTS

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Abstract— Ethanol extraction of *Allium sativum* and *Syzgiumaromaticum* were tested to evaluate their antimicrobial activities, as well as, the potential of use of both of them at different ratios, against inflammation triggering pathogens isolated from asthma and sinusitis patients. The extract of each plant has prepared, whereas the mixed extractions prepared respectively based on the ratio of 1:1, 1:2, and 2:1, prior to perform the antimicrobial tests against isolated microorganisms. The antimicrobial survival test showed that both extracts have significant effectiveness against all microbial isolates, whereas, the effectiveness of using mixed extractions was slightly lower compared to the use of a single extract. This study found no signs of improvement or enhancement on the antimicrobial effectiveness of the mixed extractions of both *Allium sativum* and *Syzgiumaromaticum* compared to the effectiveness of each extract alone against the tested microbial species.

Keywords— *Allium sativum*, *Syzgiumaromaticum*, Antimicrobial Inhibition, Asthma, Sinusitis.

I. INTRODUCTION

Asthma is a hyperactivity of the bronchial tree with paroxysmal narrowing of the small airways which may reverse spontaneously or after treatment. It is increasingly common in many countries but it is relatively rare cause of death [1]. Sinusitis is an inflammation of the mucosa layer of the facial sinuses and nasal passages that may occur due to a viral, bacterial and fungal infection, as well as, it may also results from an allergic or autoimmune reaction [2,3].Asthma and sinusitis can be correlated due to the inflammation triggered by certain pathogens. Many previous studies mentioned to the relationship between asthma and sinusitis against microorganisms infections and referred that to the treatment of sinusitis with certain antibiotics that may have effects and/or complications with asthma disease [4,5]. There are several species of microorganisms have been reported to trigger both asthma and sinusitis symptoms [5]. For example, a relationship between specific types of asthma and some fungal infections, caused by *Aspergillus Fumigatus* (allergic bronchopulmunory aspergillosis), has been identified for many years ago [4]. During past few decades, the worldwide showed an increase in the incidence of microbial infections that resistance to many synthetic medications and antibiotics used in medicinal practice [6], moreover, certain medications used to treat some of serious microbial infections considered highly expensive, especially in the developing countries, therefore, researchers start to look into natural resources and herbal remedies as alternative antifungal and antibacterial agents that could effectively fight against infections. *Allium sativum* (Garlic) is probably one of the earliest known medicinal plants, used since ancient times to cure different medicinal conditions [7]. It is well known throughout history as a safe, natural, remedy for variety of ailments, such as snake bites, parasitic infections, abdominal pains, rheumatism, and hemorrhoids. Garlic can be used also to lower blood pressure and cholesterol levels, fight infections, for gastro-intestinal disorders, prevents cancer, and recommended as antimicrobial, anti-inflammatory, antithrombotic, and antitumor [8-11]. Desired medicinal results of garlic are obtained when bulbs are chewed and swallowed or mixed with food and eaten [10]. *Syzgiumaromaticum*, widely known as clove, is an aromatic dried flower bud, normally used in the Indian and Indonesian cuisine, native to the small islands of Maluku in Eastern Indonesia, known as the "Spice Islands", though it is also grown in India, Malaysia, and Sri Lanka [12]. The aroma of the cloves is pleasant yet spicy, can be used to make drawers and closets smell nice, and it tastes good in certain dishes, it has also some medicinal purposes as well. Clove had been one of the popular herbs in Europe during seventh and eighth century [13]. Therefore, this study has conducted to evaluate the antimicrobial activities of the mixed extractions of garlic *(Allium sativum)* and clove *(Syzgiumaromaticum)*, at different ratios, against isolated microorganisms triggering inflammation in asthma and sinusitis patients.

II. DETAILS EXPERIMENTAL

2.1. Patients Samples

A survey questioner has been conducted among university students to determine those are asthmatic and sinusitis patients. We selected 15 students who are confirmed with asthma [14], 15 students who are confirmed with sinusitis [15] and 10 healthy students

served as control. All volunteers should not take any kind of antibiotic for at least one week prior the experiment.

2.2. Microbes Isolation and Identification

Nasal swab have taken from all the participants, using sterile swab from both nostrils. Swabs kept in 10ml of nutrient broth and incubated at 37^{o}C for 24 hours. Microbes' isolation and identification (with proper biochemical tests and gram staining) being executed, and then sub-cultured to isolate a pure growth of each microbe onto nutrient agar and blood agar for viabilities testing. Among all our volunteers' samples, we obtained five common species of microorganisms, been identified and isolated, includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Corynebacterium sp.* and *Aspergillus fumigatus*. Stock cultures were maintained on nutrient agar slant at 4°C and then sub-cultured in peptone water at 37°C prior to each microbial test.

2.3. Microorganism Suspension Preparation

A single colony isolated from nutrient agar and transferred into 10ml of sterile normal saline for preparation of microbial suspension. The turbidity of the microbe suspension adjusted with standard 0.5 McFarland solution till it reaches 0.08 O.D, and then measured using spectrophotometer at 625 wavelength. Vancomycin and Amphotericine B were used as positive controls, they diluted with 5% of dimethyl sulfoxide solution (DMSO) till reached 0.10mg/ml, and the solution then stored at a room temperature, while 5% DMSO solution used alone as a negative control. Both positive and negative controls observations were compared with sample plants extractions.

2.4. Plant Extracts

Thebulbs of the garlic (*Allium sativum*) were cut into small pieces and crushed. Then soaked in ethanol for three weeks, and filtered with filter papers using filter funnel. The filtratewas evaporated at a reduced pressure of 45^{o}C until it turns into a syrupy residue[16]. Dried clove (*Syzgiumaromaticum*) budS finely ground, and soaked into 500ml absolute ethanol, then filtered with filter paper using filter funnel. The filtrate concentrated using rotary vacuum evaporator at 50^{o}C [17]. A0.5g of each crude of *Allium sativum*and *Syzgiumaromaticum*were diluted in 10ml of 0.5% DMSO solution to produce a final concentration of 50mg/ml of each extract.

2.5. Antimicrobial Assays

The survival (sensitivity) test has determined using well-diffusion method. Prepared microbial suspension of each species tested on Mueller-Hinton agar plate, and diameter of the zone of inhibition obtained and recorded. The Minimum Inhibition Concentration (MIC) determined using serial dilutions method by preparing seires of 10 different concentrations of the tested extraction, in addition, positive and negative controls. All test tubes incubated at 37^{o}C for 24 hours and then microbial growth examined by measuring the turbidity absorbance at optical density of 625nm wavelength of spectrophotometer.

2.6. Statistical Assays

Statistical Assays assessed using SPSS (v.17). The results have expressed as Mean±SEM. The significance of differences were confirmed by one-way ANOVA and multiple Dunnett t-test. Pearson Correlation Coefficient (r) calculated to measure the strength of association between the two variables used, whereas $P<0.05$ considered statistically significant, and $P<0.001$ considered highly significant differences.

III. RESULTS AND DISCUSSION

Five commomn species of microorganisms were successfully isolated and identified from all the participants, these includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Corynebacterium sp.* and *Aspergillus fumigatus*. The isloted microbial species from each group illustrated as a percentages in Fig.1.

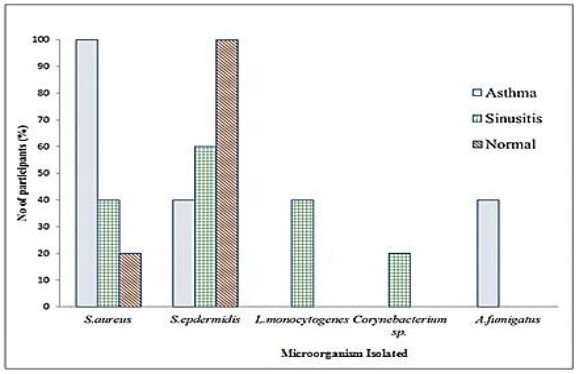


Fig.1. Microorganism Species Isolated from the Three Groups Studied (%)

The Pearson Correlation Analysis showed weak correlation ($P<0.05$) between asthma and sinusitis in term of microbial species isolation (Table 1).

Table 1: Correlation Analysis between Asthma and Sinusitis Patients

Variable	r-value	p-value
Asthma	0.139*	0.793
Sinusitis		

** weak correlation*

The antimicrobial sensitivity test of the ethanol extracts of *Allium sativum*(AS) and *Syzgiumaromaticum* (SA), in addition, the mixed

extractions (of both AS:SA) using different ratios (v/v), 1:1, 1:2 and 2:1 respectively, againstall the isolated microbes were assessed compared to positive and negative controls (Fig.2).

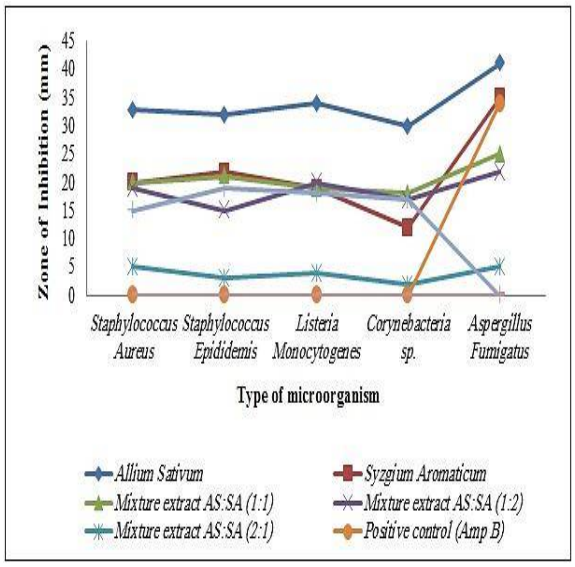


Fig.2.Sensitivity Test (Zone of Inhibition) of the Different Extracts against Isolated Microorganisms

Results showed the highest inhibition zone occurs against *Aspergillus fumigatus* using the extract of *Allium sativum* compared to other extractions. All the results are tabulated in and processed statistically, as shown in Table 2.

Table 2: Antimicrobial Activities of *Allium sativum* (AS) and *Syzgiumaromaticum* (SA) Extractions AgainstTestedMicrobes

	S. aureus	*S. epidermidis*	*L. Monocytogenes*	*A. fumigatus*	*Corynebacterium sp.*
Replicates	6	6	6	6	6
AS	33±2.65*	32±8.33*	34±2.08*	41±0.58*	30±2.52*
SA	20±3.61	22±6.43	19±2.31	35±1.09	12±2.31
AS:SA 1:1	20±3.54	21±5.45	19±2.81	25±3.64	18±2.87
AS:SA 1:2	19±2.35	15±5.63	20±5.84	22±4.56	17±3.54
AS:SA 2:1	5±2.21	3±3.25	4±1.54	5±5.45	2±1.71
+ve Control	15±2.65	19±20.0	18±2.31	34±0.58	17±2.10
-ve Control	0	0	0	0	0

** Significant ($p<0.05$)*

The Minimum Inhibitory Concentration (MIC) of both plants and the mixed extractions were tested against the isolated microorganisms (Fig.3).

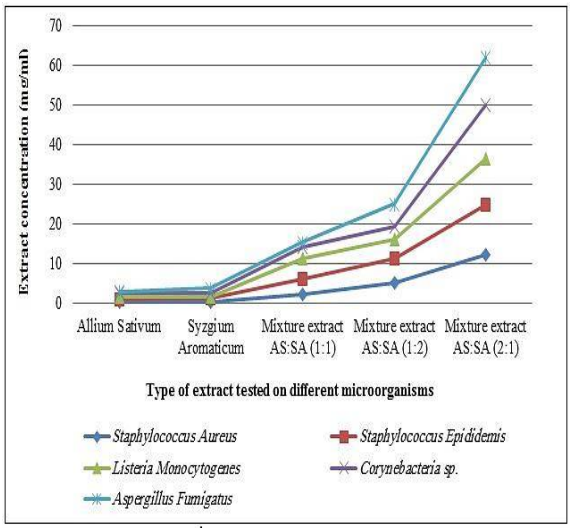


Fig.3.MIC Values of Different Ethanol Extracts against Isolated Microorganisms

Results showed the lowest MIC value were seen by *Allium sativum* extract (0.3) against *Staphylococcus aureus*. All the results are tabulated in and processed statistically, as shown in Table 3.

Table 3: MIC Values of *Allium sativum* (AS) and *Syzgiumaromaticum* (SA) Extractions AgainstTested Microbes

MIC value (mg/ml)(Mean±SEM)					
Name of organism	AS	SA	AS:SA 1:1	AS:SA 1:2	AS:SA 2:1
S. aureus	0.3	0.5	2.4	5.2	12.5
S. epidermidis	0.8	0.8	3.8	6.3	12.5
L. monocytogenes	0.8	0.5	5.3	4.9	11.5
A. fumigatus	0.5	1.3	1.0	5.6	12.0
Corynebacterium sp.	0.8	0.8	3.0	3.1	13.5

Both of the plants, garlic (*Allium sativum*) and clove (*Syzgium aromaticum*), are well known for their antimicrobial activities against variety of pathogenic microorganisms. Previous researches have reported the effectiveness of each, garlic and clove in inhibiting growth of several microbes that are causing different illness and complications. But this present study could be the first to look after the inhibitory effectiveness of using a mixture of these two extracts on the pathogenic microbes of the upper respiratory

tract, those are usually triggering the incidence of asthma attack and/or sinusitis. Our results indicated that the inhibitory effectiveness of the garlic (as antibacterial and antifungal agent) was significantly higher ($P<0.05$) compared to clove and also even the positive controls used (synthetic commercial antibiotics).

Several studies have mentioned that garliccontains high quantities of "Allicin", which is a pure, bioactive and the most powerful medicinal compound that has different biological properties to be used as antimicrobial and antifungal agent, and it is highly recommended to be used for treatment of bacterial and fungal infections [18-21].

On other hand, Chinese traditional medicine made extensive use of glove (*Syzgiumaromaticum*), especially its flowers and oil, while in India it was used by the traditional Ayurveda healers since ancient times to treat respiratory and digestive ailments [22], and many other benefits than stated above, it is one of the best natural anticancer agent [23,24], antifungal agent, anti-inflammatory, and a great antioxidant agent [25]. This indicated that clove has antibacterial and antifungal inhibitory effects, but yet in both cases, still not as high as garlic, even mixed extractions of clove and garlic (SA:AS = 1:1, 1:2, 2:1) still didn't show any significant differences compared to the use of garlic extract alone.

CONCLUSIONS

Ethanol extract of garlic (*Allium sativum*) is showing highly antimicrobial effectiveness against pathogenic microbes triggering inflammation and causing serious complications in asthma and sinusitis patients compared to clove (*Syzgiumaromaticum*). The mixed extract of garlic and clove did not improve the effectiveness against these microbes. Garlic ethanol extract is showing significantly higher antibacterial and antifungalproperties ($P<0.05$) compared to the positive control (the commercial antibiotic) against bacteria and fungi species tested.

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