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Evaluation of the antibacterial effect of a combination of the ethanolic extracts of *Citrus limon* peelsand *Eucalyptus globulus* leaves against *Streptococcus pneumoniae*

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Abstract
Pneumococcus, also known as *Streptococcus pneumoniae*, is the cause of several serious human illnesses, including pneumonia, bronchitis, bacterial meningitis, sepsis, otitis media, and corneal ulcers. Some of these illnesses cause morbidity and mortality globally. *Streptococcus pneumoniae* is currently responsible for most of pneumonia infections globally. ith prevalence ranging from 15% to 53.7%. This study therefore, evaluated the antibacterial activity of a combination of *Citrus limon* peels and *Eucalyptus globulus* leaves against *Streptococcus pneumoniae*. The combined extracts exhibited a moderate antibacterial activity against *Streptococcus pneumoniae*, which was determined by agar well diffusion method. The combination however exhibited a higher mean and standard error of the mean zone of inhibition 41±2 mm, 35±0.5773 mm, 21±1.5275 mm, compared to the single extracts, even when it contained only half of the concentration of each extract. The MIC of the combination of the extracts of *Citrus limon* peels and *Eucalyptus globulus* leaves extracts was 0.488mg/ml, *Citrus limon* was 1.953 mg/ml and was 1.953 mg/ml. This study revealed no bactericidal action of the extract against the test organism (*Streptococcus pneumoniae*). The FIC of the extract was 0.4998 which was less than FIC index which is 0.5, this indicated that there was synergistic antibacterial effect of the extract combination.

Keywords: *Eucalyptus globulus*, *streptococcus pneumonia*, *Citrus limon*

Introduction
The bacteria *Streptococcus pneumoniae* is frequently responsible for a number of disorders, including meningitis, otitis media, sepsis, and community-acquired pneumonia. (Feldman & Anderson, 2020) [3]. All of these diseases are "dead ends" in the life cycle of the organism, hence the bacterial components that cause invasive diseases also need to be adaptive in order to invade and proliferate (Weiser, Ferreira, & Paton, 2018) [44]. The most prevalent invasive isolate is *Streptococcus pneumoniae*, and at least one-third of hospital deaths in neonates and one-quarter of mortality in children under one are caused by community-acquired bacteraemia. (Olarte & Jackson, 2021) [4]. *H. influenzae* was the most frequent cause of bacterial meningitis until the *Haemophilus influenzae* type b (Hib) vaccination was developed. These days, it is *Streptococcus pneumoniae* (Abdullahi, Nyiro, Lewa, Slack, & Scott, 2008) [10].

The most frequent cause of community-acquired pneumonia (CAP), *Streptococcus pneumoniae*, has shown a sharp rise in antimicrobial resistance (Peyrani *et al*., 2019) [7]. Pneumococci strains with penicillin resistance were first identified in South Africa and Spain in the late 1970s (Musher *et al*., 2022) [5]. By the beginning of the 1990s, penicillin-resistant *Streptococcus pneumoniae* clones had spread quickly throughout Europe and the world (Oyedum, 2022) [6]. Along with penicillin resistance, resistance to macrolides and other antibiotic classes also increased (Oyedum, 2022) [6]. Meningitis, otitis media, and lower respiratory tract infections have all been associated with treatment failures brought on by pneumococci that are resistant to antibiotics (Lynch & Zhanel, 2009) [30].

Citrus limon (L.) Burm. f. is a tree from the Rutaceae family with evergreen foliage and tasty yellow fruits (Turan & Mammadov, 2021) [9].

C. limon has several different names in different languages, including lemon in English, Zitrone in German, le citron in French, limón in Spanish, and nngméng in Chinese. *C. limon* essential oil was used as sugar in conventional Roman medicine to treat coughs (Gyawali *et al*., 2021) [8]. The juice is traditionally used to treat scurvy, sore throats, fevers, rheumatism, high blood pressure, and chest pain in addition to being high in vitamin C, which helps prevent infections (Ekiert, Szopa, & Klimek-Szczykutowicz, 2020) [21]. The fact that *Citrus limon*'s ethanolic extracts exhibited a wide range of antibacterial activity and efficacy comparable to that of synthetic antioxidants highlights the plant's medicinal value as a potential source for drug development in the face of the clear lack of efficient and safe antibacterial medications and supports its use in traditional medicine (Otang & Afolayan, 2016) [35].

The Tasmanian blue gum (*Eucalyptus globulus* Labill), a native tree of southern Victoria and Tasmania in eastern Australia, is one of more than 800 species in the Myrtaceae family of angiosperms (Cerasoli, Caldeira, Pereira, & Cau, 2016) [17]. Since the beginning of time, *Eucalyptus globulus* has been used to cure various common illnesses, including wounds, bleeding gums, gonorrhea, asthma, bronchial affections, fever, pharyngitis, and stomatitis (Gurcharn, Irshad, & Jatinder, 2017) [24].

Materials and Methods

An experimental study was carried out at Kampala International University Microbiology and Pharmacognosy laboratories where MIC, MBC and FIC of ethanolic extracts of *Eucalyptus globulus* and *Citrus limon* against *Streptococcus pneumoniae* was assessed.

Study Area

The leaves of *Eucalyptus globulus*were collected from trees in Bwejiraje village, bushenyi district. Fresh *Citrus limon* shall be bought from local farmers in Bushenyi district. Extraction of the plant material was carried out in the KIU Pharmacognosy laboratory and the microbial testing was carried out in KIU Microbiology laboratories. The period of study was between September and October of 2023.

Preparation of the Plant Extracts

Plant collection and identification

The leaves of *Eucalyptus globulus* were collected from trees in Bwejiraje village, Bushenyi district and were identified by a botanist.

Fresh *Citrus limon* were bought from local farmers in Bushenyi district and were identified by a botanist.

Drying and pulverizations

The *Eucalyptus globulus* leaves were shade dried under room temperature of 37 degree to prevent degradation of the phytochemicals. They were dried for two weeks with daily weight measurements until a constant weight was obtained. The dried leaves were crushed in a mortar using a pestle and sieved to the appropriate particle size. A reduced particle size improved mixing with the solvent and storage.

The *Citrus limon* peels were cut into pieces and dried under shade to prevent degradation of the phytochemicals. They were dried for two weeks with daily weight measurements until a constant weight is obtained. The dried peels were crushed in a mortar using a pestle and sieved to the appropriate particle size.

Plant extraction

200 g of *Eucalyptus globulus* powder were weighed and mixed with 1000 ml of ethanol (96%) in a ratio of 1:5. And were left to stand for 72 hours with occasional shaking for at least one hour daily. After 72 hours, the mixture was filtered using a funnel and Whatman No. 1 filter paper to obtain a marc (residue) and filtrate. Then a beaker was weighed and the filtrate was poured into the beaker and was kept under a water bath at 40 °C until all the ethanol solvent was evaporated. After evaporation of the ethanol solvent, the beaker plus the extract were weighed and the weight obtained was used to obtain the net weight of the extract by subtracting off the weight of the beaker that was determined before evaporation. The extract was then refrigerated.

200 g of *Citrus limon* powder were weighed and mixed with 1000ml of ethanol (96%) in a ratio of 1:5. And were left to stand for 72 hours with occasional shaking for at least one hour daily. After 72 hours, the mixture was filtered using a funnel and Whatman No. 1 filter paper to obtain a marc (residue) and filtrate. Then a beaker was weighed and the filtrate was poured into the beaker and was kept under a water bath at 40 °C until all the ethanol solvent was evaporated. After evaporation of the ethanol solvent, the beaker plus the extract were weighed and the weight obtained was used to obtain the net weight of the extract by subtracting off the weight of the beaker that was determined before evaporation. The extract was then refrigerated (Gurusiddappa, Christeena, Bharat, & Shankramma, 17 May 2023) [1].

Determination of Percentage Yield

The given formula was used to calculate the percentage yield of the extract that was obtained using different solvents.

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the extract recovered}}{\text{Weight of dry powder}} \times 100$$

Preparation of the Mueller Hinton agar

3.8 g of Mueller Hinton agar were dissolved in 100ml of distilled water and heated with agitation to boil for one minute until the components was fully dissolve. Sheep blood was added after the solution had been autoclaved for 15 minutes at 121 °C and cooled to roughly 45 °C. After that, it was evenly placed into petri dishes at a depth of 4 mm, and it was incubated for 24 hours at 37 °C.

Preparation of the *Streptococcus pneumoniae* isolate

The test microorganism used in this study was a clinical isolate of *Streptococcus pneumoniae* that was obtained from microbiology laboratory of Kampala International University, it was the sub-cultured on blood agar to obtain the greenish alpha hemolytic pure colonies.

Antibacterial susceptibility of extracts of *Citrus limon*, *Eucalyptus globulus* extract and combination of the plant extracts.

Antibacterial activity of extracts was tested using agar well diffusion method. The agar plate surface was inoculated by thin streaking of inoculum of *Streptococcus pneumoniae* uniformly over the entire agar surface then five holes with a diameter of 6mm was punched aseptically with a sterile cork borer in 6 agar plates.

And a volume of 50 µl of *Citrus limon*, *Eucalyptus globulus* extract and combination of the plant extracts at concentration of 1000 mg/ml, 500 mmg/ml, 250 mg/ml were introduced into the wells and 50 µl of 200 µg/ml of positive control of ampiclox (a combination of amoxicillin and cloxicillin) and 50 µl of 0.85% of negative control (normal saline) were introduced as well in wells. This was done in triplicates, in order to validate the results obtained. Then agar plates were incubated under suitable conditions at 37 °C for 24 hours. Resulting zone of inhibition was measured using a meter rule.

Determining of Minimum Inhibitory Concentrations

The minimum inhibitory concentration (MIC) is the antimicrobial agent's concentration at which a bacterium cannot grow. Micro broth dilution method was used (Helio, Rodrigo, Jennifer, Gerald, & Robert, 2019).

The test bacteria were standardized to an equivalent of 0.5 MC Farland standard. 100 µl from the standardized bacteria shall be transferred into 9.9 ml of sterile water. A stock concentration of each plant extract at 500 mg/mL were prepared. Microbroth dilution method was done by firstly adding 50 µL of preprepared brain heart infusion broth into each of the wells of the micro titre plate. A twofold serial dilution of the stock of each plant extract was made by transferring 50 µL of the plant extract into the first well of the micro titre plate containing BHI. Contents of first well was mixed using a clean pipette tip and 50 µL of the contents picked and transferred into well 2. The above procedure was repeated to the last well. Discard 50 µL from the well. 50 µL of the prepared bacterial suspension was added into each of the wells that contain the serially diluted plant extract.

The above procedure was done for the *Citrus limon*, *Eucalyptus globulus* extract and combination of the plant extracts in a ratio. The above procedure was also be done for my positive control where amoxicillin was used. Incubated the wells at 37 ºC for 24 hrs. To each of the wells, 30 µL of 0.0015% resazurin was added and then further incubated for 2 hr. Each well plate was examined for any colour change from blue to pink which was indicative of presence of bacterial growth that causes reduction of resazurin compound. Henceforth, the well that contained the lowest concentration and remained blue was considered as the MIC of the extract. The above experiment was done in triplicates, in order to validate the results obtained. (To prove accuracy of the results obtained).

Determining of the Minimum Bactericidal Concentration

The wells that remained blue or no growth will be sub cultured on Mueller Hinton agar aseptically using a wire loop and incubated at 37 ºC for 24 hrs. The wells whose contents allow less than 0.1% of the original inoculum of 1.5×10^{8} cfuml^{-1} to grow indicates the MBC.

3Determining of the Fractional Inhibitory Concentration

FIC will be determined for each extract by dividing the MIC of the extracts when used in combination with the MIC of each extract when used alone. It is the test to estimate the interaction between two or more drugs intended to be used in combination. It is determined by the equation below. The FIC is used to classify the interaction as either synergistic (≤0.50), additive (0.50-1), indifferent (1-4), or antagonistic (>4

$$FIC = \frac{\text{MIC of drug A in combination with drug B}}{\text{MIC of drug A alone}}$$

Ethical Considerations

The used culture, inoculum and plates will be disposed of in a way that does not cause harm to the environment and minimizes the risk of infection of healthy individuals. They will be autoclaved at 121 degrees Celsius for 20 minutes and then disposed.

Statistical Analysis

Statistical significance was established using one way analysis of variance (ANOVA) and data were reported as mean ± standard deviation. IBM Statistical package version 20 (SPSS-20) and Stata corp STAT (V15) was used.

Results

The antibacterial activity of the ethanolic extracts of *Citrus limon* and *Eucalyptus globulus against* clinical isolate of *Streptococcus pneumonia.*

Table 1: Percentage yield of ethanolic *Citrus limon* peels extract

Extracts	Weight of powder residue (g)	Weight of Extract (g)	Percentage Yield Extract
Ethanol 96%	132.2	14.17	11%

Table 2: Percentage yield of ethanolic *Eucalyptus globulus* extracts

Extracts	Weight of powder residue (G)	Weight of extract (G)	Percentage yield extract
Ethanol 96%	180	13.49	7.5%

Antibacterial susceptibility of extracts of *Citrus limon*, *Eucalyptus globulus* extract and combination of their plant extracts

This contains the results of the zones of inhibition of *Citrus limon*, *Eucalyptus globulus* extract and the combination of their plant extracts. The zones of inhibition were measured using a ruler.

Table 3: Zones of inhibition of *Citrus limon*

	Zones of inhibition				
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**	**Positive control**	**Negative control**
Experiment (Mm)	20	18	10	12	0
Replicate 1 (mm)	20	17	9	12	0
Replicate 2 (mm)	21	18	11	12	0

Table 4. Zones of inhibition *of Eucalyptus globulus*

	Zones of inhibition				
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**	**Positive control**	**Negative control**
Experiment (mm)	17	16	11	12	0
Replicate 1 (mm)	18	16	10	12	0
Replicate 2 (mm)	18	17	11	12	0

Table 5: Zones of inhibition of the combination of the extracts of *Citrus limon and Eucalyptus globulus*

	Zones of inhibition				
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**	**Positive control**	**Negative control**
Experiment (mm)	41	36	21	12	0
Replicate 1 (mm)	39	35	20	12	0
Replicate 2 (mm)	43	36	23	12	0

Descriptive analysis

The tables below present the descriptive statistics of the data used in the study for the activities of *Eucalyptus globulus*, *Citrus limon* and the combination of the two against *Streptococcus pneumoniae* and its different characteristics.

Table 6: Antimicrobial activity of *Eucalyptus globulus* against *Streptococcus pneumoniae*

Zones of inhibition			
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**
Mean	17.66667	16.33333	10.66667
Median	18	16	11
Minimum	17	16	10
Maximum	18	17	11
STd Dev	0.57735	0.57735	0.57735
Skewness	-1.732051	1.732051	-1.732051

Table 7: Antimicrobial activity of *Citrus limon* against *Streptococcus pneumoniae*

Zones of inhibition			
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**
Mean	20.33333	17.66667	10
Median	20	18	10
Minimum	20	17	9
Maximum	21	18	11
Std Dev	0.471405	0.471405	0.816497
Skewness	1,732051	-1,732051	0

Table 8: Antimicrobial activity of the combination of *Eucalyptus globulus* and *Citrus limon* against *Streptococcus pneumoniae*

Zones of inhibition			
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**
Mean	41	35.66667	21.33333
Median	41	36	21
Minimum	43	36	23
Maximum	39	35	20
Std Dev	2	0.57735	1.527525
Skewness	0	-1.732051	0.93522

Determination of the Increase in Fold Area

Increase in fold area is a statistical test for the characterization of combination effects. Increase in fold area was calculated by the equation below.

$$A1 = \frac{Y - X}{X}$$

$$A2 = \frac{Y - Z}{Z}$$

$$A = A1 + A2$$

Where A is the increase in the fold area
A1 is the increase in fold area with respect to *Eucalyptus globulus*
A2 is the increase in fold area with respect to *Citrus limon*
Y is the zone of inhibition of the combination of *Eucalyptus globulus* and *Citrus limon*
X is the zone of inhibition of *Eucalyptus globulus*
Z is the zone of inhibition *Citrus limon*

A. If A1 or A2 < 0 and A1 + A2> 0, there is antagonism, and this antagonism is caused by one of the two substances.
B. If A1 and A2<0 the two substances are categorically antagonistic
C. If A1 and A2>0 and A1 + A2<2, there is an additive effect
D. If A1 and A2>0 and A1 + A2>2, there is a synergistic effect

Table 9: The Increase in Fold Area

Concentration	**A_1**	**A_2**	**A**
1000 mg/ml	1.016393443	1.320754717	2.33714816
500 mg/ml	1.018867925	1.183673469	2.202541394
250 mg/ml	1.133333333	1	2.133333333

Since the values of A1 and A2 are all greater than or equal to one under the concentrations of 1000 mg/ml, 500 mg/ml and 250 mg/ml, and the value of A is greater than 2 under all concentrations (1000 mg/ml, 500 mg/ml, 250 mg/ml), Then we can conclude that there is synergy in the experimented results.

The measures of variation and measures of variability were obtained and by standard deviation we can observe that there were no wide variations among the data evidenced by the relatively small deviations within the data set across all the levels of the experiment.

From the graph above, we can observe that at low concentrations (250mg/ml) of *Citrus limon* and *Eucalyptus globulus*, the activity of the two was below the positive control implying that there was normal fold area reaction but an in higher concentrations, the fold area widens beyond the positive controls implying greater activity between *Citrus limon* and *Eucalyptus globulus* against *streptococcus pneumonia* in larger concentrations.

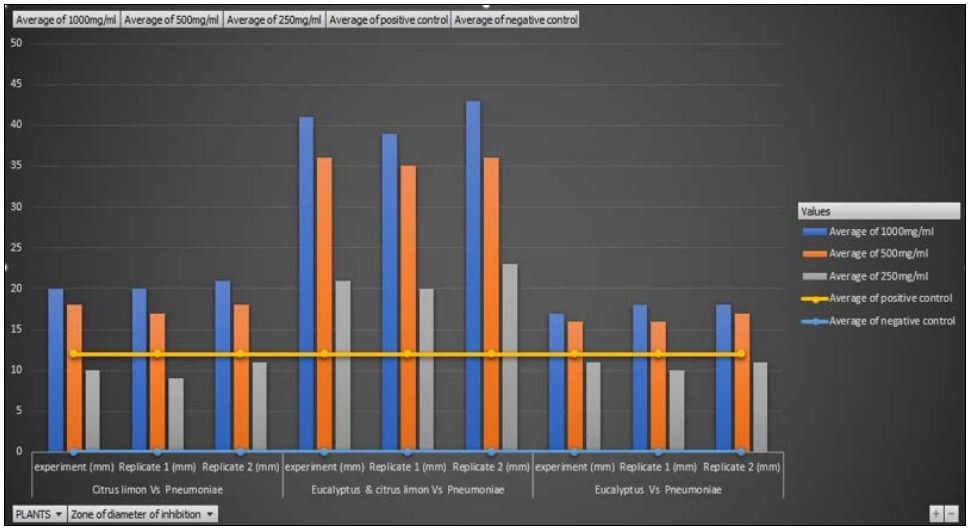

Fig 1: Comparison of mean inhibition zone diameters of *Citrus limon, Eucalyptus globulus* extracts and their combination of the different concentrations against *Streptococcus pneumoniae*

It is also observed that the combination effect of *Citrus limon* and *Eucalyptus globulus* is larger and more effective than the individual reactions of *Citrus limon* and *Eucalyptus globulus* at all levels of concentrations with the lowest concentration (250 mg/ml) of the combination being above the positive control.

Table 10: Concentrations of *Eucalyptus globulus* and *Citrus limon*in the Combintion

Total concentration of extract in the combination	Concentration of *Eucalyptus globulus*	Concentration of *Citrus limon*
1:1 =1000 mg/ml	500 mg/ml	500 mg/ml
1:2 =500 mg/ml	250 mg/ml	250 mg/ml
1:4 =250 mg/ml	125 mg/ml	125 mg/ml

The table above shows that for each concentration of the combination, there was half the concentration of each of the plant extracts. This means that the results received were due to a combination of the two extracts and not a result of an excess of the extract with superior on inferior antibacterial effect.

Table 11: Test for Diameter of inhibition against Positive Controls

Mean	32.6667
Standard Deviation	8.902247
Standard Error	2.967416
t-statistic	6.9645
P (T < t)	0.9999

Ho: Mean zone of inhibition = Positive control
Ha: Mean zone of inhibition ≠ Positive control

Since the t-statistic = 6.9645 is greater than the significance level of 0.05, we reject the null hypothesis and conclude that the mean diameter of inhibition is actually greater than the positive controls indicating that the results acquired are not just basic.

Minimum inhibitory concentration of *Citrus limon* and *Eucalyptus globulus* extracts and their combinations

Table 12: MIC for the combination of *Citrus limon* and *Eucalyptus globulus* extracts

Experiment	Replicate 1	Replicate 2	Mean
0.488 mg/ml	0.488 mg/ml	0.488 mg/ml	0.488 mg/ml

Table 13: MIC for the *Eucalyptus globulus* extracts

Experiment	Replicate 1	Replicate 2	Mean
1.953 mg/ml	1.953 mg/ml	1.953 mg/ml	1.953 mg/ml

Table 14: MIC for the *Citrus limon* extracts

Experiment	Replicate 1	Replicate 2	Mean
1.953 mg/ml	1.953 mg/ml	1.953 mg/ml	1.953 mg/ml

Determination of FIC (Fractional Inhibitory Concentration)
FIC value was determined using the standard formula:

$$FIC = \frac{\text{MIC of the extract in combination}}{\text{MICof the extract a lone.}}$$

Using the above formula
FIC of *Citrus limon* extract = 0.2499
FIC of *Eucalyptus globulus*=0.2499
FIC index = FIC of *Citrus limon* extract + FIC of *Eucalyptus globulus*

FIC index = 0.2499+ 0.2499 = 0.4998

Table 15: FIC (Fractional Inhibitory Concentration)

FIC *Citrus limon*	**0.2499**
FIC *Eucalyptus globulus*	0.2499
FIC INDEX	0.4998 < 0.5

From further calculations of FIC index, it was also discovered that the FIC index also indicated synergy where by the acquired FIC value (0.4998) < 0.5. Therefore, the combination of the two extracts exhibited synergistic antibacterial effect.

The results were classified following synergy (FIC ≤ 0.5), addition (0.5 ≤ FIC > 1), indifference (1 ≤ FIC > 4), and antagonism (FIC > 4).

Discussion

Percentage yield of ethanolic extract of *Citrus limon* peel

Several studies have reported the percentage yield of *Citrus limon*. For example; the solvent extract of the lemon peel using various solvents produced different outcomes in each of the tests conducted in this study. The solvent extract of the lemon peel using various solvents produced different outcomes in each of the tests conducted in this study. The greatest percentage yield was ethanol (5.4%) (Gurusiddappa, Christeena, Bharat, & Shankramma, 17 May 2023) [1].

In this study, the percentage yield of the ethanolic extract of *Citrus limon peel* was found to be 11%. This value indicates the amount of the extract obtained from the dried plant material. The value of the percentage yield obtained in this study was higher compared to the ones reported in other previous studies, this indicated that the method of extraction was more efficient. This may be attributed to the different extraction used.

Percentage yield of ethanolic extract of *Eucalyptus globulus*

In this study, the percentage yield of the ethanolic extract of *Eucalyptus globulus* was found to be 7.5%. This value indicates the amount of the extract obtained from the dried plant material. The value of the percentage yield obtained in this study was higher compared to the ones reported in other previous study done by (NZ Immaroh *et al*., 2021) that reported a percentage yield of 2.0% (hydro-distillation), 2.2% (solvent extraction), 2.6% (the extraction by using ultrasound) and 3.6% (supercritical carbon dioxide) hence their method of extraction was more efficient. The difference in the percentage yield is greatly affected by the extraction methods which in turn affects the biological composition and yield. Time for the extraction process affected the yield. In some extraction methods, the longer of extraction time applied, the higher the yield would be (NZ Immaroh *et al*., 2021). This study employed maceration method of extraction different from that done by (NZ Immaroh *et al*., 2021) that employed multiple extraction methods like hydro-distillation, solvent extraction, extraction by using ultrasound and supercritical carbon dioxide extraction methods.

Antibacterial susceptibility of extracts of *Citrus limon*, *Eucalyptus globulus* extract and combination of their plant extracts

The results obtained agreed with those of past research as they indicated that *Citrus limon* ethanolic extracts have an antibacterial effect. Numerous studies have demonstrated the antibacterial, antioxidant, and anticancer properties of citrus lemons. The chemicals that give citrus lemons their antibacterial properties include flavonoids, carotenoids, limonoid, tannin, and terpenoid. (Mustafa J. Shuaib, Taher I. Shailabi, Elham O. Borwis, & Akram S. Muhammed, 2021) [34]. Similarly, for *Eucalyptus globulus*, the results agreed with those obtained from past research as the ethanolic extracts exhibited mean zones of inhibition of 20.33333±0.471, 17.66667±0471, 10±0.816 mm at the different concentrations of 1000 mg/ml, 500 mg/ml/ 250 mg/ml respectively, comparable to those obtained against similar microorganisms. (Assad, *et al*., 2021) [12]. This activity could be attributed to the phytochemical constituents revealed by using High Performance Liquid Chromatography (HPLC), Kaempferol, Quercetin and Myrecetin and Piperitone (17.77%), 1-8 cineol (17.62%) and Sabinol (2.57%) revealed by Mass spectrum (Assad, *et al*., 2021) [12].

The combination however exhibited a higher mean zone of inhibition 41±2 mm, 35±0.5773 mm, 21±1.5275 mm, compared to the single extracts, even when it contained only half of the concentration of each extract. This would allow a reduced dose of the two extracts, reducing exposure to toxicities and guarantee improved efficacy. The positive control (amoxicillin) had a mean inhibition zone diameter of 12±0.5773 mm, while the negative control (normal saline 0.85%) showed no inhibition. The mean zone of inhibition of the combination at the different concentration was higher than the mean zone of inhibition of the positive control. Results from the study also show potential for solving problems of the apparent emergence of a drug-resistant strain of *Streptococcus pneumoniae* infectious pathogen.

Minimum inhibitory concentration

The study investigated the minimum inhibitory concentration (MIC) of the ethanolic extract of *Eucalyptus globulus*leaves, *Citrus limon* peels and the combination of the extracts against *Streptococcus pneumoniae*. The results showed the MIC of the *Eucalyptus globulus* was 1.953 mg/ml, which is lower than 16mg/ml obtained by (Salari M. H., 2006) [41]. The antibacterial activity of *Eucalyptus globulus* may be attributed to Euca- lyptal (1,8-cineole), the active compound present in the leaves of the plant (Salari M. H., 2006) [41]. The difference in the MIC results could be attributed to different strains of bacteria and the methods of extraction of the used. The MIC of *Citrus limon* was 1.953 mg/ml which is contrary to 0.25-0.5 mg/ml reported by (Pascale, Sabine Leroy, & Sureeporn Suriyaprom, 2022).

The combination *Citrus limon* and *Eucalyptus globulus* revealed an MIC of 0.488mg/ml which is in line with the study done by (Ramona Iseppi, Martina Mariani, & Carla Condò,, 2021).

Minimum Bactericidal concentration

This study revealed no bactericidal action of the extract against the test organism (*Streptococcus pneumoniae*) which was line with the MIC results where an inhibitory effect of the extract against the test organism was visibly seen.

Conclusion

Citrus limon exhibited a stronger antibacterial effect than *Eucalyptus globulus* against *Streptococcus pneumoniae.*

The combination of *Eucalyptus globulus* and *Citrus limon was* synergistic against *Streptococcus pneumoniae.*

Conflict of Interest

Not available

Financial Support

Not available

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