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Antimicrobial activities of leaf extracts of both guava (*Psidium guajava*) and kafour (*Eucalyptus camaldulensis*) against escherichia coli

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Abstract
Guava (*Psidium guajava*) and *Eucalyptus camaldulensis* are phytotherapeutic plants used in folk medicine, either as a single plant or mixed to treat and manage various diseases like diarrhea and coughs. This study aimed to determine the antimicrobial potential of guava and *Eucalyptus camaldulensis* leaf extracts against gram-negative bacteria (*Escherichia coli*) which are some of the foodborne and spoilage bacteria, occasionally responsible for causing diarrhea. The study was carried out at the Plant Pathology Center, the University of Gezira during the period. The efficacy of these extracts was tested against this bacteria through the disc diffusion and well diffusion methods. Aqueous extract of plant material (DW) powder (50g/500ml) was used to prepare different concentrations (25, 50 and 100% the extract). Both water and antibiotic (Ampiclox) were used as control. According to the results of the antibacterial assay, the aqueous extract of the guava leaves showed inhibitory activity against the *E.coli*, with mean zones of inhibition of (11 And 14 mm) by the disc diffusion method and the well-diffusion method respectively, whereas, the aqueous extract of the *E. camaldulensis leaves* showed mean zones of inhibition of (11mm and 13mm) on both methods respectively. According to the results, no significant differences were found between the two plants at the different concentrations *(*P *value= 0.846).* The combination of (*P. guajava* and *E. camaldulensis)* at different concentrations showed relatively synergistic antimicrobial activity with inhibition zone range between (15mm and 17mm) compared to the controls that gave means of inhibitions range between (13 mm and 14mm). On the high of the obtained results, guava and *E. camaldulensis* leaf-extract might be a good candidate in the search for a natural antimicrobial agent against *E.coli*. Further studies showed to be done, to determine the antimicrobial activities against other types of bacteria and investigate other pharmacological properties of the two plants.

Keywords: *Psidium guajava, Eucalyptus camaldulensis, Escherichia coli*, the antimicrobial, zones of inhibition

Introduction
Amongst the various forms of treatment for diarrhea, the use of traditional plant remedies is common and widespread. The World Health Organization (WHO) has cataloged more than 20,000 plant species with medicinal properties providing treatments for such complaints as pneumonia, ulcers, diarrhea, bronchitis, colds, and diseases of the respiratory tract. One method, amongst the many ways in which plants are used in popular remedies, is to extract and consume essential plant oils. Essential oils are complex chemical mixtures, typically composed of more than a hundred compounds, by and large, are responsible for plant aromas. They are obtained from different parts of the plant: flowers, leaves, seeds, bark and tubers and many have medicinal properties [1]. Outbreaks of diarrhea are common in communities living in precarious conditions with poor sewerage and hygiene.
Several food types have been linked to outbreaks and act as the carriers of infectious microbes. Under such conditions, diarrhea is commonly occurred after eating contaminated fish products. Illnesses arise because the microbial causal agents, often native to the fish itself, have not been adequately controlled, or arise as a consequence of incorrect handling and or storage during industrial processing [2]. Illnesses causing gastroenteritis and diarrhea are primarily associated with enteric bacteria. Enteric bacteria are responsible for high mortality rates in numerous developing countries with as many as 50,000 people dying daily as a consequence of infection [3]. Plant remedies are increasingly being recognized by scientists as a very important low-cost alternative to industrially-produced antibiotics that are not available to all who need them because of their high price. Publishing findings on the antimicrobial activity of plant remedies is important because it raises awareness of alternative medicines which in turn drives biotechnology development.

Therefore this study aimed to evaluate the antimicrobial effects of guava and kafour tree leaf extracts on diarrhea-causing bacteria at different concentrations

Materials and Methods

Sample collection and identification

The leaves of guava*(Psidiumguajava)*, and Kafour(*Eucalyptus camaldulensis)* were obtained from the University of Gezira fields. *Escherichia coli* were obtained and investigated at the Medical Laboratory University of Gezira.

Sample preparation methods

The samples were taken as fresh leaves, then washed from sand and dust, before kept to dry at the Center of Plant Pathology, University of Gezira, for further investigation. The completely dried material was powdered and allowed for overnight extraction by distilled water, with a concentration of (25%, 50% and 100%,). Both negative controls with a concentration of 0% (distilled water) and positive control (Ampiclox) were used.

Preparation of Nutrient agar

This was a general-purpose cultured medium for bacteria. It was obtained in a dehydrated form. The constituent of the medium was beef extract, yeast extract, peptone, sodium chloride, and agar. It was prepared according to the manufactures instruction by suspending 28g in one liter distilled water. The medium was allowed to boil until it has completely dissolved. The pH of the medium was adjusted to pH 7.4±0.2 and then the medium was sterilized in an autoclave at 121 ºC (115b\in²) for 15 min [5].

Preparation of test organism

The normal agar was mixed well and poured on the sterile Petri plates. The agar media on Petri plates were allowed to sit for a few minutes. Normal agar plates were inoculated with respective bacteria (*E.coli*) and then incubated at 37 °C for overnight. Each time, a fresh bacterial culture was prepared.

The disc diffusion (Inhibition zone) method

In this method different herbal suspensions of different concentrations (25%, 50%, and 100%) were prepared and incubated overnight at room temperature(37˚c), then the media was prepared, sterilized and distributed into sterile Petri-dishes and was left to solidify at room temperature for 24 hours. After that by using sterile cotton swabs the *Escherichia coli* was inoculated in 6 Petri-dishes by full streaking, then a sterile glass fiber discs (size 6mm) were saturated with the extract of (guava*(Psidiumguajava)*, and Kafour (*E. camaldulensis*) and their combinations*)* allowed to dry and transferred on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 24 hours and the inhibition zones were measured by mm and the susceptibility is determined. Two replicates were made for each solution.

The good diffusion (Inhibition zone) method

Agar well diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA).Wells (10mm diameter and about 2 cm apart) were made in each of these plates using a sterile cork borer. A stock solution of each plant extract was prepared at a concentration of 1 mg/ml in different plant extracts. About 100 µl of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Statistical analysis

ANOVA two-way factors without replication were used in the analysis of data, and the comparison between aqueous effects was done by T-test and F-test.

Results and Discussion

Table 1: Effect of different concentrations of Guava, Kafour and their combination aqueous extract on inhibition (mm) of E.coli compared to the control using both disc and well method

Conc.	Guaa Disc well		Kafour Disc well		Guava and Kafour Disc well		control	
							Water	Ampiclox
25%	12	9	11	9	11	12	0	14
50%	16	11	16	8	16	15	0	12
100%	18	13	17	13	17	17	0	17

The results of the effects of the different concentrations of extracts of Guava on the *E.coli* inhibition zone are shown in Table 1. It was found that all concentrations of the Guava water extracts were significantly effective in inhibiting growth of *E.coli* using both methods, although, there is a great variation in the degree of inhibition between the disc and the good method as it gave 12 mm,16 mm,18 mm in the disc method and not more than 9 mm,11 mm,13mm in the good method regarding the different concentrations.

The Guava aqueous extract at the Disc method gave only 13mm at a concentration of 100% and less inhibition (9-16mm) at a concentration of (25-50%), respectively.

Table 2: Effect of different concentrations of Guava aqueous extract on inhibition(mm) of E.coli using well-diffusion method (three replications).

Concentration%	Inhibition zones(mm)		
	R1	R2	R3
25	10	7	9
50	11	12	11
100	11	13	14

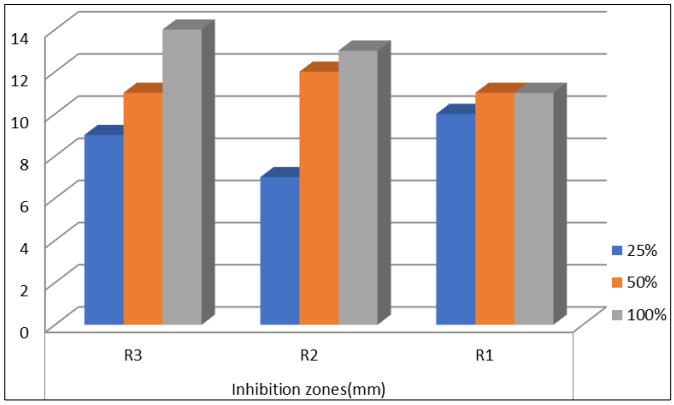

Fig 1: Effect of different concentrations of Guava aqueous extract on inhibition(mm) of *E. coli* using well-diffusion method at three replications.

The well-diffusion method gave the highest inhibition, which is 18mm at a concentration of 100% and less inhibition (12-16mm) at a concentration of (25-50%), respectively.

Table 1: Effect of different concentrations of Kafour aqueous extract on inhibition(mm) of E.coli using the disc method at three replications.

Concentration%	Inhibition zones(mm)		
	R1	R2	R3
25	13	10	10
50	16	16	15
100	17	17	18

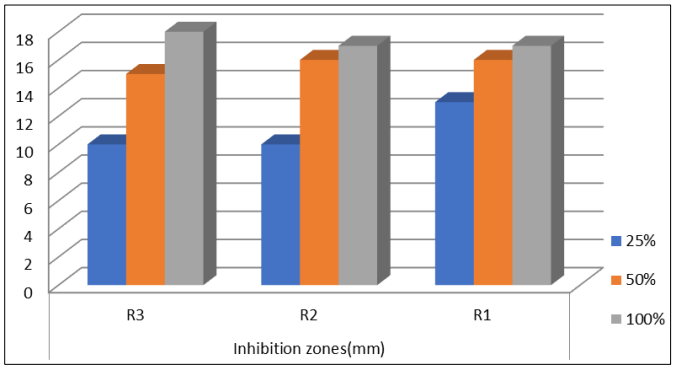

Fig 2: Effect of different concentrations of Kafour aqueous extract on inhibition(mm) of *E. coli* using disc method at three replications**.**

The disc method the Kafour aqueous extract showed an inhabitation of only (13mm) at a concentration of 100% and a lower inhibition (9-8mm) at a concentration of (25-50%), respectively.

Table 4: Effect of different concentrations of Kafour aqueous extract on inhibition (mm) of E.coli using well-diffusion method at three replications.

Concentration%	Inhibition zones(mm)		
	R1	R2	R3
25	9	7	10
50	8	9	8
100	11	15	13

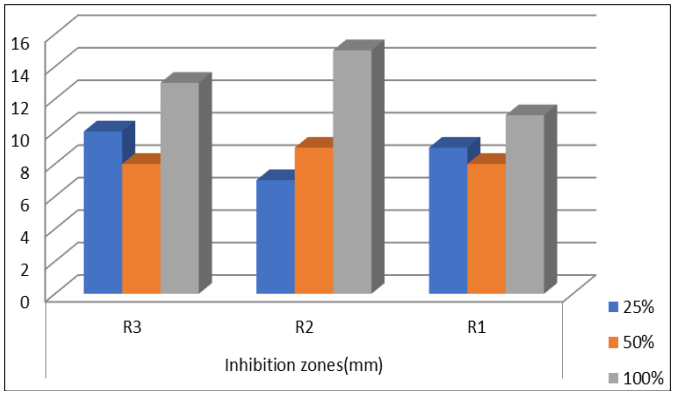

Fig 3: Effect of different concentrations of Kafour aqueous extract on inhibition (mm) of *E. coli* using well-diffusion method at three replications.

The Kafour aqueous extract at the well-diffusion method gave the highest inhibition (17mm) at a concentration of 100% and the lowest inhibition (10-16mm)at a concentration (25-50), respectively

Table 5: Effect of different combined concentrations of Kafour and Guava aqueous extract on inhibition(mm) of E.coli using Disc method at three replications.

Concentration%	Inhibition zones(mm)		
	R1	R2	R3
25	13	10	10
50	16	16	15
100	17	17	18

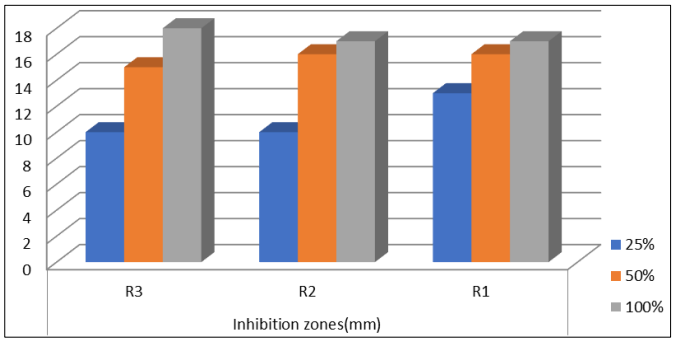

Fig 4: Effect of different combined concentrations of kafour and Guava aqueous extract on inhibition (mm) of *E.coli* using Disc method at three replications.

It is noticed that there is an increasing effect on the bacterial growth inhibition for both Guava and Kafour with the increase of the extract concentrations, reaching the maximum inhibition at (100%) concentration.

It is found that all concentrations of the combination water extracts were significantly effective in inhibiting growth of *E.coli* using both methods, although, there is a great variation in the degree of inhibition between the disc and the good method as it gave 11 mm, 16 mm, 17 mm in the disc method and not more than 12 mm, 15 mm, 17 mm in the well method regarding the different concentrations.

The combination aqueous extract using the disc method gave only 17 mm at a concentration of 100% and less inhibition (11-16mm) at a concentration of (25-50%), respectively. Statistically, there is a significant variation of the different concentrations.

Table 6: Effect of different combined concentrations of Kafour and Guava aqueous extract on inhibition(mm) of E.coli using well method at three replications

Concentration%	Inhibition zones(mm)		
	R1	R2	R3
25	11	15	11
50	15	13	16
100	17	18	15

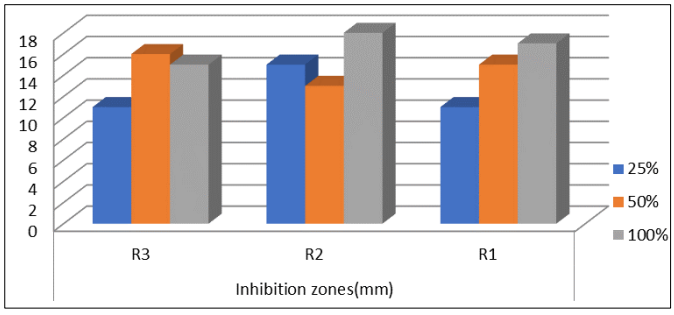

Fig 5: Effect of different combined concentrations of kafour and Guava aqueous extract on inhibition (mm) of *E.coli* using well method at three replications.

In the combination, the well-diffusion method gave the highest inhibition, which is 17 mm at a concentration of 100% and less inhibition (15-12 mm) at a concentration of (25-50%), respectively.

Table 7: Effect of different concentrations of the positive control (Antibiotic) aqueous extract on inhibition(mm) of E.coli at three replications.

Concentration%	Inhibition zones(mm)		
	R1	R2	R3
25	13	15	14
50	10	15	10
100	17	17	16

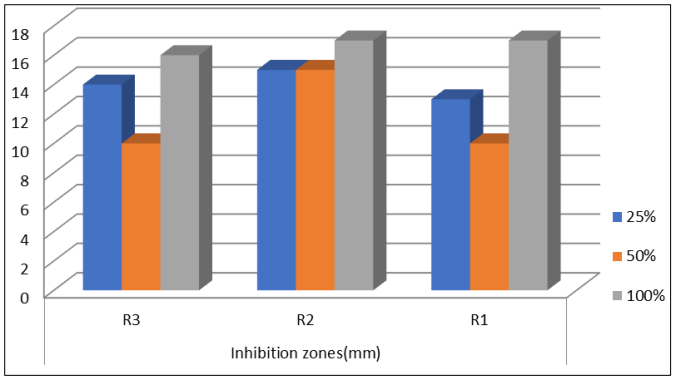

Fig 6: Effect of different concentrations of the positive control (Antibiotic) aqueous extract on inhibition (mm) of *E.coli* at three replications.

Comparison of guava mixture

The mixture was given the highest percentage inhibition when Trkir 100% (17mm), while the less inhibition of bacteria at a concentration (25-50%), namely, (10-16mm), respectively. Accordingly, the way Well more effective inhibition of the mixture of bacteria at a concentration of 100%. While the way Disc less inhibition of bacteria.

Compared with guava and antibiotic

Gave results that the effectiveness of guava and counter-air equal in the inhibition of bacteria at a concentration of 100% reaching influence While the antibiotic more effective inhibition of bacteria by Disc reaching influence (17mm) at a concentration of 100%Compared Guava (13mm) at a concentration of 100%. ratio (18mm) Well in away.

Comparison of Kafor and mixture

Results proved that the effectiveness of Kafor and antibiotic equal in the inhibition of bacteria in a manner well at a concentration of 100% and the ratio of (17mm).

The proportion of Kafor gave the effect of higher than the mixture for a way Disc process at a concentration of 100% where inhibition of bacteria ratio (13mm) either give the mixture ratio of inhibition (11mm).

Comparison between Kafor and antibiotic

Results show that the effectiveness of the antibiotic little more than Kafor in the inhibition of bacteria in a manner well as at a concentration of 100% and concentration (18mm) while the effectiveness of Kafor at a concentration of 100% more effective at inhibiting Bacteria gave the percentage of inhibition of (17mm) either eucalyptus way Disc gives highly effective to inhibit bacteria at a concentration of 100%, which is (13mm) and the counter (17mm).

Comparison between mixture and antibiotic

Where inhibition of bacteria mixture of guava and eucalyptus while the elimination of the bacteria in a manner well at a concentration of 100% and gave discouraged about the percentage (17mm), while on the way Disc at the same concentration gave (11mm) and the effect of the antibiotic through the Well at a concentration of 100% higher than the concentration shortly mixture where the ratio of (18mm). and same focus 100% way Disc the effect of the antibiotic (17mm).

Results proved that the highest concentration and more effective to inhibit bacteria concentrations of the three plants, a concentration of 100% and gave the best result.

Comparison between the Guava and Kafor and mixture and antibiotic of control

Guava compared with the control, which gave way disc.v result considering either way well high significant.

The Kafor with control gave way disc.v as a result of considering either way Well high significant.

The mixture with control gave way disc.v as a result of high considering either way Well high significant.

The antibiotic with control gave way disc.v as a result of high considering either way Well high significant are shown in Table (1). It was found that all concentrations of the herbs water extracts were significantly effective in inhibiting the growth of *E.coli* compared to the control, although, there were no significant differences between the concentrations or the different herbs. However, there was increasing effect with the increasing concentrations of the water extracts reaching its maximum at the highest concentration (100%), the inhibition zones at that concentration were (16 mm, 15 mm, 11 mm, and 9 mm), the combination of Guava and kafour ginger, ginger, and yansoon extracts, respectively.

Guava Leaves well has given the high percentage inhibition of about 18 at a concentration of 100% and less inhibition (12-16mm) at a concentration of (25-50), respectively.

Guava Disc Given the high percentage of inhibition at a concentration of 100% (13mm) and less And less inhibition (25-50%) and focus (9-16mm), respectively.

Conclusion

Plants are considered as rich sources of antibiotic treatment medications and eucalyptus leaf with its antimicrobial properties have been used in the treatment of infectious diseases formerly in ancient medicine [6].

In this research the Guava and Kafor were used to inhibition the growth of bacteria *Escherichia coli,* water was used as control. So [4] had recorded that water is a good solvent for most of the tannins, but the best solvent is a mixture of organic solvents and water.

Applying different level of extracts (25- 50 -100%) it was found that the highest inhibition is linearly correlated to the high concentration similar to the antibiotic, as supported by previous workers (Ugoh, And Nneji, 2013) it has been proposed that The extracts were more active against gram-positive microorganisms than gram-negative microorganisms.

The results obtained in this study revealed that guava has strong inhibition at a concentration of 100% and thus is more effective in retarding the growth of *E. coli* compared to Kafor, extract, combined with an antibiotic. Guava disc gives the embarking a large zone of inhibition at a concentration of 100% (18m.m) and the combination resulted in less activity than Guava alone, but this difference was statistically non-significant. In contrast [7] reported that guava leaves exhibited

a distinct resistance in some strains of bacteria involved in the present study at the concentration of 200 ug/ml. When using liprofloxacin the methanolic extract offered significant protection against *staphylococcus aureus* and *Escherichia coli*. The antibacterial potential exhibited by guava leaves extract may be contributed to the presence of flavonoids detected in the preliminary phytochemical investigations. Further study is needed to characterize the active principle. Applying different level of extracts (25- 50 -100%) it was found that the highest inhibition is linearly correlated to the high concentration similar to the antibiotic, as supported by previous workers [8] it has been proposed that the extracts were more active against gram-positive microorganisms than gram-negative microorganisms.

Eucalyptus and guava leaf extract in this study showed an inhibitory effect on the tested organisms, this finding goes in line with the findings of other authors which show that eucalyptus and guava leaf extracts have inhibitory effects on *E. coli* and Staph. aureus and topical application of eucalyptus oil clear methicillin resistance *Staphylococcus aureus* infection.

Therefore, according to the results of the antibacterial assay, the aqueous extract of the guava leaves showed inhibitory activity against the *E.coli*, with mean zones of inhibition of (11mm and 14mm)**.** According to the results, no /significant differences were found between the two plants at the different concentrations *(p-value=0.185*). The combination of (Guavaand *E. camaldulensis)* at different concentrations showed relatively strong antimicrobial activity with inhibition zone range between (15mm and 17mm) compared to the controls that gave means value inhibitions range between (13mm and 14mm). Based on the obtained results, guava and *E. camaldulensis* leaf-extract might be a good candidate in the search for a natural antimicrobial agent against *E.coli*.

Competing interests

Authors have declared that no competing interests exist.

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