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Invitro Determination Effectiveness of *Acacia senegal* and Juice of *Actinidia deliciosa* with Estimate Synergistic Effect Towards Some Pathogenic Bacteria of Human

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ABSTRACT

Antimicrobial resistance is a real threat to human health in worldwide. One of these medicinal plants that are commonly used in addition to its source as food is also used as a treatment for some cases. Gum Arabic (GA) consider it protection against various bacterial infections. Kiwi fruit Commonly known as the, *Actinidia deliciosa* valuable medicinal properties such as its anti-inflammatory, anti-bacterial and anti-asthmatic efficacy. Gum Arabic in different concentrations (10,20,30 and 50)% also *Actinidia deliciosa* in concentrations (25,50,75 and 100)% were used to determine the effect and inhibition activity against 4 species of bacteria were isolated from the different sites of the human body's infection included Urinary tract Infection (UTI) ,skin infections (pus), and from stool (diarrhea), these bacteria were (*Staphylococcus aureus* ,*Salmonella sp*. , *Klebsiella pneumonia* and *E. coli*) and compared with inhibitory effect of some antibiotic discs (Trimethoprim/Sulfamethaxazole (25 µg), Ciprofloxacin 10 µg, Vancomycin (30µg), Norfloxacin (10 µg), and Lincomycin (15µg). *Salmonella sp.* most affected by gum Arabic with an inhibition rate of 10, while with kiwi fruits the inhibition rate was 8 compared to the used antibiotics, which showed resistance to it. The Minimum Inhibitory concentration (MIC) of gum Arabic against all bacterial species was at 10%, while MIC of kiwi fruit solution was at 25%. Minimum Inhibitory Concentration (MIC) of gum Arabic against bacterial isolates was 10% and 25% of kiwi fruit.30% of gum Arabic showed highest inhibition activity toward *Klebsiella pneumonia*e and *E. coli* at 15 and 10 mm but 50% of it showed highest activity toward *Salmonella sp* and *Staphylococcus aureus* at 12 and 16mm, 75% and 100% of Kiwi fruit juice was more effective against *Klebsiella pneumonia*e and *E. coli* at same measure inhibition zone at 9mm but *Salmonella sp* and *Staphylococcus aureus* were more affected by 75% Kiwi fruit juice at 11 and 12 mm respectively compared with other concentrations. The synergism effect of Gum Arabic (10 µg/ml) with Trimethoprim (25 µg/ml) was more effected against *E. coli*, the inhibition zone was at 12mm and *Staphylococcus aureus* was at 30mm, *E. coli* was resistant to Gum Arabic (10 µg/ml) and Triethoprime (25µg/ml) each of one alone, but *Staphylococcus aureus* bacteria the inhibition zone was at 9mm when treated with Gum Arabic (10 µg/ml) and resistant to Triethoprime (25µg/ml). in the same time *Klebsiella pneumonia*e and *Salmonella sp*. not affected with this synergism and Triethoprime (25µg/ml) aone, but they affected with Gum Arabic *Acacia Senegal* (10 µg/ml), the inhibition zones were 11 and 10 mm respectively.

Keywords- Gum Arabic (*Acacia Senegal*), Kiwi fruit *(Actinidia deliciosa*) juice, bacterial species and Trimethoprime.

I. INTRODUCTION

The increasing incidence of pathogens, especially drug resistance, raises an urgent need to identify and isolate new biologically active compounds from medicinal plants through the use of modern standardized analytical procedures. Some medicinal and plant-derived compounds may provide new and direct pathways to pathogenic bacteria (Vaou *et al*.,2021) . Antimicrobial resistance is a real threat to human health in worldwide. The high cost of finding a new antibiotic, which starts from the discovery stage of the antibiotic until it is marketed, is very high with a low return on investment, in addition to the slowdown in the development of new antibiotics, significantly since the time of the golden discovery era during the 1950s' of the

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twentieth century. One of these medicinal plants that are commonly used in addition to its source as food is also used as a treatment for some cases is gum Arabic (GA), which is considered a traditional herbal medicine that stems from the Willdenow Acacia (L.) tree, and consists of a mixture of complex sugars and glycoproteins (Agrawal, 2018). And some use it in daily applications to treat many diseases and consider it protection against various bacterial infections. The details of his mechanism behind these observations are still vague and unclear in detail. A. Senegal is known as gum Arabic, which is in the form of dried secretions rich in fibers that have a high solubility, especially in water. This substance comes out from cracks formed in the bark of branches and stems (Magnini *et al*., 2020). The various parts of this species A. senegal are used in boiled or boiled form, and can also be eaten dry. Where this type was used traditionally among some peoples to treat coughs, colds, dysentery, diarrhea, gonorrhea, phlegm, urinary tract disorders and sore throat. (Chassagne *etal*.,2021 and Baien *etal*.,2020)

Kiwi fruit Commonly known as the, *Actinidia deliciosa* is an excellent Vitamin C supplement. It is also rich in antioxidant compounds such as oxy-carotenoid, lutein, fatty acids, alpha-linolenic acid, and omega-3, thus stimulating the anti-inflammatory property. It also has well-known and valuable medicinal properties such as its anti-inflammatory, anti-bacterial and anti-asthmatic efficacy. Kiwi fruit has many medicinal properties, and this can be attributed to the presence of many bioactive compounds in it (Harsh *et al*., 2016 and Preeti *etal*.,2021).

II. MATERIALS AND METHODS

The experiment was done in biology department/ Microbiology laboratories - Sciences college in University of Tikrit.

2-1: Bacterial samples collection:

4 species of bacteria were isolated from the different sites of the human body's infection included Urinary tract Infection (UTI), skin infections (pus), and from stool (diarrhea), bacterial species were (*Staphylococcus aureus, Salmonella sp., Klebsiella pneumonia* and *E. coli*).

2-2: Identification of bacterial isolates:

The phenotypic characteristics diagnosis was approved to identify all bacterial isolates of visible bacterial colonies like Shape with arrangement, Consistency and Color, on the different culture plate on the different media.

2-3: Microscopic examination:

Bacterial isolates were classified under a microscope according to note the shapes and clusters. Also, depending on the receptivity of bacteria to the gram stain to classify them as positive or negative for the stain (Alfred, 2005).

2-4: Biochemical tests:

The necessary and specific biochemical tests were conducted to diagnose each bacterial isolate, (Alfred, 2005 and Betty *et al*., 2007). API kit also was used to confirm the diagnosis of bacterial isolates.

2-5: Extracts preparation:

1-Gum Arabic (*Acacia Senegal*):

Gum Arabic powder was used, manufactured by (Nature Gums)/Sudan packed by sidratul corporation SdnBhd/Malaysia. The aqueous extract was prepared by soaked 50 grams of Gum Arabic powder in 100 ml of distillated water, and then sterilized by filtration by using Millipore filter 0.45 µm, this represents the concentration of 50% of extract, then other concentrations 10, 20 and 30% were prepared from it. (Hindi et al., 2013) This represents a concentration of 50% of the extract, then other concentrations were prepared from it, which were used to measure its antibacterial activity for some bacterial species.

2-Kiwi fruit (*Actinidia deliciosa*):

The kiwi fruits (*Actinidia deliciosa*) were washed and cleaned and then skin and seeds was peeled off, fruit was cut into small pieces. The blender was used to make the fruit juice, and it was filtered through several layers of sterile gauze, then the juice was sterilized by Millipore filter 0.45 µm to get sterilized juice 100% (Kodasi *et al*., 2023). then from this solution the concentrations 25, 50 and 75% were prepared.

2-6: Estimation of the Minimum Inhibitory concentration (MIC) method of Extracts:

The bacterial suspensions were prepared for each bacterial isolate, and the suspension was at the age 24 hrs. after incubated at 37 Cº for 24 hrs. In comparison with tubes of standard 0.5 McFarland solution, the turbidity of tubes containing bacterial suspensions selected for this study were compared before cultured on Muller-Hinton agar media, the bacterial isolates then cultured on Muller-Hinton agar media, the culture were spread on media by using sterilized cotton swabs, then appropriate 4 wells were done for each bacterial isolate to conduct the test for both extract solutions (Gum Arabic and Kiwi juice) and by used the sterilized crock poorer, then about 50 µl of extract solution in the different concentrations in each well were added, and then every cultured plate was incubated at 37Cº for 24 hrs (Nayyef and Thalij, 2020).

2-7: Antibiotics sensitivity test

To conduct an antibiotic sensitivity test for the bacterial isolates selected in this study, a group of antibiotics was selected as shown in Table (1), where the antibiotic discs were placed on the surface of Mueller-Hinton agar medium by using sterile forceps, after spreading the bacterial inoculum by spreading on the medium. The cultured plates were then incubated for 24 hours at 37 °C, then the inhibition zone was measured by using a ruler and in millimeters (Betty *et al*., 2007).

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Table 1: Antibiotic discs

Antibiotics name	Symbol	Concentration µg/ml	Synthetic company
Ciprofloxacin	CIP	5µg	**Bioanalyse/ Turkey**
Norfloxacin	NOR	10 µg	
Vancomycin	VA	30 µg	
Lincomycin	L	15 µg	
Trimethoprime/Sulfamethaxazole	SXT	25 µg	

2-8: Prepare the trimethoprim antibiotic solution and synergistic with gum Arabic:

At first the Trimethoprime antibiotic 25 µg/ml solution was prepared by dissolving 0.0025 g of pure anti-trimethoprim powder in 100 ml of sterile distilled water and then it sterilized by using a Millipore filter to obtain trimethoprim antibiotic solution with a concentration 25 µg/ml. Then, equal proportions of the sterile antibiotic solution 25 µg/ml were mixed with the sterile gum Arabic solution as well, at a concentration of 20 µg/ml To obtain an equivalent solution, a mixture of the two materials is ready to perform a sensitivity test against bacterial isolates.

III. RESULTS AND DISCUSSION

Table 2: The inhibition activity of gum Arabic compared with some antibiotics towards bacterial isolates

Bacterial isolates inhibition zones				
Gum Arabic	*Klebsiellae pneumoniae*	*E. coli*	*Salmonella sp.*	*Staphylococcus aureus*
	9	7	10	13
Kiwi fruit juice	7	7	8	8
Antibiotics inhibition zones				
NOR (10 µg)	18.5	27	0	18.5
Cip 10 µg	26.5	33	0	26.5
SXT (25 µg)	0	0	0	0
L (5 µg)	0	0	0	0
VA (30 µg)	0	0	0	0

In table (2) were investigate the mean inhibition activity of kiwi juice and Gum Arabic on some bacterial isolates. The inhibition means of these two compounds solutions differed according to the bacterial species, *Staphylococcus aureus* recorded a highest rate of inhibition by gum Arabic solution at 13 and kiwi juice at 8 among all selected bacterial species, while *E. coli* showed lowest affect with them at 7. Other bacterial species varied between the means of these inhibitory means, as they were affected by *Klebsiella pneumonia* (9 and 7), *Salmonella sp*. (10 and 8). The antibiotics (Norfloxacin 10 µg and Ciprofloxacin 10 µg) on average, it was more effective on bacterial isolates compared to gum Arabic Kiwi juice, same effect at 18.5 and 26.5 respectively on *Klebsiella pneumonia* and *Staphylococcus aureus*, 27 and 33 on *Salmonella sp*. but in same time this bacterium was resistant to these antibiotics. All bacterial isolates in table () showed affected with Gum Arabic and Kiwi juice more than (sulfamethaxazole (25 µg, Lincomycin 5 µg and Vancomycin30 µg) which did not show any inhibitory effect on the bacterial isolates, on the contrary, the isolates were resistant to them.

Table 3: The Minimum inhibitory concentration MIC (mm) of Gum Arabic and Kiwi fruit juice toward the bacterial isolates.

Bacterial species	**MIC (µg/ml) with different concentrations of Gum Arabic**				**Kiwi fruit juice**			
	10%	20%	30%	50%	25%	50%	75%	100%
Klebsiella pneumoniae	+	+	-	+	-	+	+	+
E. coli	+	+	-	+	-	+	+	+
Salmonella sp.	+	+	+	+	-	+	+	+
Staphylococcus aureus	+	+	+	+	-	+	+	+

The results recorded in the table 3 show the minimum inhibitory concentration MIC of gum Arabic and Kiwi juice. The MIC of Gum Arabic on all bacterial isolates were at 10%, while the MIC of Kiwi juice were effect on bacterial isolates at concentration 50%.

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Table 4: The Gum Arabic activity and Kiwi juice in different concentrations against some bacterial isolates

	Gum Arabic				Kiwi fruit juice			
	10%	20%	30%	50%	25%	50%	75%	100%
Klebsiella pneumoniae	10.5	11	15	0	0	8	9	9
E. coli	9	10	10	0	0	8	9	9
Salmonella sp.	10	11	11	12	0	9.5	11	10
Staphylococcus aureus	8.5	12.5	13	16	0	10.5	12	10

From the table (4) the results showed the different effect of Gum Arabic activity and Kiwi juice (*Actinidia deliciosa)* with different concentrations on bacterial isolates. The concentration 10% of Gum Arabic was effect on all bacterial isolates *Klebsiella pneumonia*e, *E. coli*, *Salmonella sp.* and *Staphylococcus aureus* at 10.5, 9, 10 and 8.5 mm. The concentrations 20% and 30% of Gum Arabic was an effect on bacterial species *Klebsiella pneumonia*e at inhibition zone 11 and 15 mm respectively, while inhibition diameters of *E. coli* were 10 mm equally, While in contrast it was for the effect of kiwi juice on *Klebsiella pneumonia*e and *E. coli* in concentrations 50% the inhibition zones were at 8 mm equally and 9mm for each concentrations 75 and 100%.

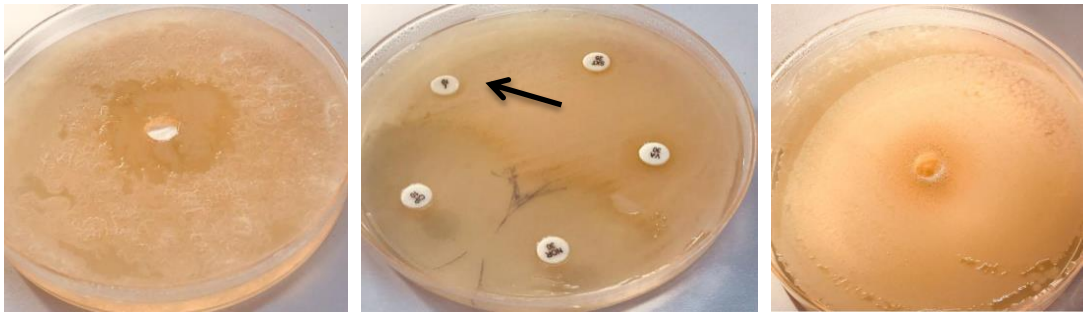
Salmonella sp. showed affected with 20% and 30% of Gum Arabic at same inhibition zones 11 mm, the same inhibition zone diameter also with 75% of kiwi juice, but in concentrations 50 and 100% of Kiwi juice were effect on *Salmonella sp.* with inhibition zone 9.5 and 10 mm.

Staphylococcus aureus showed affected with different concentrations 20, 30 and s% at 12.5, 13 and 16mm, while with 50, 75 and 100% of Kiwi juice the inhibition zones were at 10.5, 12 and 10 mm.
on the other hand, the lowest concentration of kiwi juice at 25% had no effect on bacterial isolates. On the contrary, all isolates were resistant to it.

Table 5: Synergism effect of Gum Arabic with Trimethoprim on some bacterial isolates

Bacterial species	Gum Arabic (10 µg/ml)	Trimethoprime (25µg/ml)	Gum Arabic (10 µg/ml) with Trimethoprim (25 µg/ml)
Klebsiella pneumoniae	11	0	0
E. coli	0	0	12
Salmonella sp.	10	0	0
Staphylococcus aureus	9	0	30

From the results recorded in Table 5, which shows a comparison of the effect on bacterial isolates when using Gum Arabic 10 µg/ml alone or the Trimethoprime/ sulfamethaxazole25µg/ml with the synergistic effect of gum Arabic with anti-trimethoprim, where at the same time gum Arabic will replace sulfamethaxazole, meaning that it will be a synergistic action, as it was observed that there is a variation in the effect on all bacterial isolates. *Klebsiella pneumonia*e, *Salmonella sp.* and *Staphylococcus aureus* were resistant to Trimethoprime/ sulfamethaxazole25µg/ml compared with Gum Arabic10 µg/ml which had the effect of inhibitory diameters at 11, 10 and 9mm respectively, Except for *E. coli*, it was resistant to both Gum Arabic 10 µg/ml and trimethoprim the Trimethoprime/ sulfamethaxazole25µg/ml. The inhibition diameters differed when the Trimethoprim 25 µg/ml synergized with gum Arabic10 µg/m, where the *E. coli* bacteria showed an effect on this compound and an inhibitory diameter 12mm, and 30mm for *Staphylococcus aureus*, while *Klebsiella pneumonia* and *Salmonella sp.* were resist to it.



(A) (B) (C)

Fig (1): Inhibition zone of S.aureus when (A) use Gum Arabic (10 µg/ml) with Trimethoprim (25 µg/ml) (B) Use antibiotic Trimethoprime/sulphamethaxazole (C) use Trimethoprime (25µg/ml) only

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IV. DISCUSSION

According to a study conducted by (Daffalla, 2018) on the gum Arabic of Acacia Senegal (GA), where he noticed the aqueous extract has antimicrobial activity, as it that contains effective substances that have an inhibitory role for microorganisms, and according to phytochemical examination GA consist of variety number of bioactive and biomolecules as antimicrobial activity like saponins, alkaloids, few amounts of tannins and some cardiac glycosides. Study of (Pinelli *et al*., 2013 and Kichaoi *et al*., 2015) about the antioxidant activity of kiwifruit also shown antimicrobial effect towards some human pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes*.

The results of this study were consistent with a study presented by (Fisher K, Phillips, 2008, Alim et al., 2019) were founded the gram-positive bacteria special S. aureus more vulnerable to the kiwifruit polyphenols compared with gram-negative bacteria Specifically, bacteria *E. coli*, and the inhibition zones of gram-positive were larger than gram negative bacteria and this due to polyphenols. Gram-negative bacteria have hydrophilic outer membrane consist lipopolysaccharide molecules, which act as barrier to the hydrophobic compounds this barrier decrease but not block diffusion of the hydrophobic compounds (Khromykh *et al*., 2022).

Although studies are very limited on the effect of kiwi (Actinidia *deliciosa*) as an antimicrobial especially against *S. aureus, E. coli, K. pneumonia, P. aeruginosa, and C. albicans (*Sudha *et al*., 2019). In another study conducted by the researchers, (Mishra *et al*., 2010), about evaluating the effectiveness of some fruits rich in vitamin C, such as *Vitis vinifera, Emblica officinalis, Hylocereus undatus, Syzygium cumini and Actinidia deliciosa* toward some pathogenic bacterial species and concluded that *Actinidia deliciosa* showed the maximum antimicrobial activity against *K. pneumonia* followed by S. *aureus, P.aeruginosa, E. coli, and C. albicans.*

The results of *Acacia Senegal* activity towards these bacteria was Compatible with study of (Bnuyan *et al*., 2015) who found in their study about gum Arabic against some pathogenic microorganisms which included gram positive and gram negative bacteria such as *Staphylococcus aureus, S. epidermidis, Streptococcus pneumoniae, Pseudomonas aeroginosa, Proteus merabilis, Enterobacter, Acinetobacter, , Serratia , Klebsiella pneumoniae, Salmonella typhi* and *E. coli* in addition to fungi like *Candida albicans* by using agar well diffusion method have antimicrobial activity, the aqueous extract of Gum Arabic was able to offer antimicrobial activity and the inhibition diameters against these microorganisms was about20 mm, Therefore, it can be used as an antimicrobial source to treat various medical cases that caused by these pathogenic species.

The antimicrobial effect is due to its rich terpene content as well as secondary metabolites such as tannins and flavonoids in addition to other compounds, which were monitored in their anti-microbial activity (Seigler, 2003; - Lawrence et al., 2015; Aloqbi, 2020)

Also, a part of these active substances, in addition to other additional bioactive compounds, had an anti-bacterial effect, but in varying proportions in kiwi fruits such as Phenolic acids, Anthocyanins, Coumarins, Tannins and Organic acids. Antibacterial activity of kiwi fruit can be attributed due to the presence of manifold of bioactive compounds such as Phenolic acids, Coumarins, Anthocyanins, Organic acids, and Tannins. Specifically, the Coumarins and Phenolic compounds have the ability to inactivate the enzymes of bacterial cell that are responsible for bacterial substances in to host cells also tannins is a key component of the kiwi fruit exhibit antimicrobial activity, by inhibition the extracellular enzymes of microbes and thus leading to deprivation the substrates which required for growth of microbes. Tannins inhibiting oxidative phosphorylation and this affects metabolism of microbes (Kayser and Kolodziej., 1999).

It is known that kiwi fruits are rich in vitamin C, which works to rid cells of free radicals and other reactive types of oxygen, which work it reduces inflammation, and thus acts as a powerful antioxidant (Tada and Miura, 2019; Preeti *et al*., 2021). Arabic gum an anionic polysaccharide and it can be used as a carrier successfully because of its unique physical and biological properties such as its biocompatibility, charge-based character and biodegradability (Aliabbasi et al., 2022).

Sulfamethaxazole inhibited bacterial dihydropteroate synthase enzyme. Trimethoprim is competitive with the enzyme dihydrofolate reductase, which leads to its inhibition, which stops the production and conversion of tetrahydrofolate from folic acid to its active form. tetrahydrofolate is an important component for purines synthesizing that required for production of DNA and protein (Eyler and Shvets, 2019). Several studies have showed the effectiveness of antimicrobial gum Arabic and its increased effectiveness when associated with other antimicrobial compounds.

Because it enhances drug efficacy and absorption specifically absorption of amoxicillin antibiotic (Al-Jubori *et al*., 2023). In a study by El-Tayeb *et al*., they found that when measuring the concentration and compared between two groups, One group took gum Arabic two hours after taking amoxicillin while the other that took the antibiotic simultaneously with gum Arabic and they were found the peak to be significantly decrease in group that took Gum Arabic alone. The active compounds in gum Arabic, such as flavonoids and polyphenols, can inhibit the efflux pumps in bacteria, which is one of the resistance mechanisms, allowing antibiotics to remain for as long as possible within the bacteria for the purpose of interacting with the desired target as well as disruption of the bacterial cell membrane. The polyphenolic compounds have bacteriostatic effects such as gallic acid and coumarins, , In addition,

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flavonoids contribute to the precipitation of proteins, a non-specific interaction which lowest the bacterial metabolism(Zhou *et al*., 2015; Qiu et al., 2014) depleting the motive force and electron of bacterial proton flow and thus reducing the amount of available biochemical energy Which can contribute to inducing the non-reproductive state of bacterial metabolism to act as the inhibitors of enzymatic reversible (Santajit *et al*., 2016; Nitsch-Velásquez, 2020). Extensive studies have conducted isolation and characterization of the kiwifruit flesh phenolic components of by the thin-layer chromatography, ultra high performance of liquid chromatography and the high performance liquid chromatography (Sun-Waterhouse *et al*., 2009).

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