Antibacterial activity of the *Acacia nilotica* gum against *Klebsiella pneumoniae* in vitro

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Abstract

This study was conducted to study the antibacterial activity of the Acacia nilotica gum aqueous solution. For this purpose the aqueous solution of the Acacia nilotica gum was prepared and studied its chemical constituent and antibacterial activity in vitro against Klebsiella pneumoniae either alone or as a combination with ciprofloxacin by using minimum inhibitory concentration (MIC) and well diffusion method. The results showed that the Arabic gum solution contain different chemical constituents; tannins, flavonoids, saponins, and terpenoids, and the (MIC) was 200mg/ml. The results also showed that the Arabic gum had weak effect against Klebsiella pneumoniae in vitro where the zones were 11.50±0.57, 10.00±1.14, 0.00±0.00 and 0.00±0.00mm at 400mg/ml 200mg/ml 100mg/ml and 50mg/ml respectively, which are significantly ($P<0.05$) less than those of ciprofloxacin that were 24.00±0.89, 18.30±0.94, 13.00±0.35 and 11.20±1.01 at 2µg, 1µg, 0.5µg and 0.25µg respectively. In concern to the combination (Arabic gum +ciprofloxacin), the resulted zones were less than those obtained from ciprofloxacin alone which were 21.00±1.03, 17.70±1.31, 12.00±0.89 and10.00±0.94 with significant difference just at doubled MIC. In conclusion, the Acacia nilotica gum aqueous solution had weak effect against Klebsiella pneumoniae in vitro with no synergistic effect with ciprofloxacin.

Keywords: *Acacia nilotica gum, Antibacterial, Klebsiella pneumoniae, Ciprofloxacin.*

Introduction

Klebsiella pneumoniae is a pathogen of the Enterobacteriaceae family that causes healthcare associated infections and has recently emerged as one of the most antibiotic-resistant organisms responsible for outbreaks in both community and healthcare settings (Taher et al., 2014). It has the ability to colonize gastrointestinal tract, nasopharynx, and the skin (Tzouvelekis et al., 2012) and also capable of causing diverse infections ranging from minor infections to very critical ones such as urinary tract infections, soft tissue infection, intra-abdominal infection, septicaemia, wound or blood infections and pneumonia (Brisse et al., 2009; Lin et al., 2016). Combating infections posed by this organism can mostly be done as a result of effective antimicrobial therapy. This involves administering of antibiotics to the infected patients. The high rate at which antibiotics are used all around the world in human therapy, animal therapy, and in livestock has given rise to the emergence of antibiotic-resistant isolates, leading to very detrimental problems in the affected individuals and in the community at large (Basode et al., 2018), as the treatment outcome of the infection would be affected by the presence of drug resistance in the infecting pathogen (Lin et al., 2016). In low-income and high-income settings, antibiotic overuse and misuse are crucial drivers of resistance, although intervention targets differ depending on income status (Murray, 2022).This however, can lead to prolonged stay in the hospital (Alga et al., 2018), cause a drastic increase in health care costs and in many cases, results in life-threatening and untreatable infections (Kumar, 2013). Antibiotic resistance is a pandemic claiming more than 750,000 deaths per year. K. pneumoniae is one of the these resistant bacteria that displayed multidrug resistance (Yousif et al., 2008; AL-Nassry 2011; Aziz and lafta, 2021), where there is increasing number of strains resistant to fluoroquinolones, third-generation cephalosporins, aminoglycosides, and even carbapenems (Penalva et al., 2019). Not surprisingly, the World Health Organization includes K. pneumoniae in the “critical” group of pathogens for which new therapeutics are urgently needed.

Plants and plants extract used for medicinal purposes and play an important role in developing countries since; they are inexpensive, effective and have a natural origin. Acacia niloticais also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia (Bargali et al.,2009), belongs to the family Fabaceae which also called Leguminosae or bean and pea family, is the third largest family in terms of agricultural and economic importance (Lewis et al., 2005). It is widely used, almost all their parts used in medication including root, bark, leaves, flower, gum, and pods (Said et al.,1997). This plant has anti-microbial, antiplasmodial, anticancer, antimutagenic and antioxidant activity and used for the treatment of cold, cough, sore throat diarrhea, dysentery, tuberculosis, piles, hepatitis C virus and burns. Polar solvent extracts showed good activity against gram negative E. coli, S. typhi and K. pneumoniae (Yadav et al., 2015). In another study the aqueous solution of the Arabic gum inhibited E. coli growth in vitro (Haider, and Abedsalih, 2022).

The seeds are very rich in phenolic constituents and also proteins. The fruits are reported to be very rich in saponins and tannins (Manzo et al., 2017). reported the extraction, purification and identification of two new compounds [ethylgalate and (+)-catechin] from the bark of A. nilotica (Ahmadu et al., 2017). The results revealed that the extract exhibited a broader spectrum of antibacterial activity and revealed potent bactericidal effect at high concentrations (up to 100 mg/ml) and bacteriostatic at lower concentrations (as low as 12.5 mg/ml) (Abdallah, 2016; Al Alawi 2018). This study aimed to investigate the antibacterial activity of the Acacia nilotica gum aqueous solution against Klebsiella pneumoniae in vitro.

Materials and Methods

Preparation of the aqueous solution of the Acacia nilotica gum

Fifty gram of Acacia nilotica gum was dissolved in 200ml distilled water for phytochemical analysis.

Phytochemical Screening

The prepared solution of the Acacia niloticais gum screened for the presence of major phytochemicals by using standard qualitative methods (Usman et al., 2009).

Minimum inhibitory concentration

The MICs of each agent (Arabic gum and ciprofloxacin)were determined in 96 well-round bottomed sterilized microplates. Serial dilutions of each antimicrobial were prepared, then 180 µL of the diluted Arabic gum solution and ciprofloxacin were pipetted into the microplate wells together with 20 µL of Klebsiella pneumoniae (1.5 x108 CFU/ml). The concentrations tested were: 400, 200, 100, 50, 25, 12.5 and 6.25 mg/ml for Arabic gum solution; 8, 4, 2,1, 0.5, 0.25 and 0.125 µg for ciprofloxacin. Microplates were incubated at 37°C for 24 hrs. Then, 10 µl of tetrazolium was added to each well. The MIC was read after two hrs of incubation as the lowest concentration of the antibacterial at which no indicator turns (changed to red color) occurred (Tsukatani et al., 2008).

Susceptibility test

The agar well diffusion method was adopted according to (Perez et al., 1990), for assessing the antibacterial activity of the prepared Acacia niloticais gum solution and standard antibacterial ciprofloxacin. 5 ml of standardized bacterial stock suspension (1.5 × 108 cfu/ml) of Klebsiella pneumoniae was mixed with 500 ml of sterile Mueller Hinton agar. 25 ml of the inoculated Mueller Hinton agar was distributed into sterile petri dishes of each. The agar was left to set for 10 minutes to allow solidifying, then a 4 wells 6 mm in diameter were made using a sterile Pasteur pipette, after that, wells were filled with 100 microliter containing 1MIC (200) mg/ml 2MIC(400)mg/ml, 0.5MIC(100)mg/ml and 0.25MIC(50)mg/ml of Acacia nilotica gum solution. The wells in the other plate were filled with 1MIC (1µg), 2MIC(2µg), 0.5MIC (0.5µg) and 0.25MIC(0.25µg) of ciprofloxacin. The wells in the last plate were filled with a combination of Acacia niloticais gum solution + ciprofloxacin as following: first well contained (400) mg/ml Acacia niloticais gum solution + (2µg) ciprofloxacin, 2nd well (200) mg/ml Acacia niloticais gum solution + (1µg) ciprofloxacin, 3rd well (100) mg/ml Acacia niloticais gum solution + (0.5µg) ciprofloxacin while the 4th well filled with (50) mg/ml Acacia niloticais gum solution + (0. 25µg) ciprofloxacin. All plates allowed to diffuse at room temperature for two hours, then incubated at 37°C for 24 hours. five replicates were carried out for each concentration of antibacterial and the activity was determined by measuring the diameter of inhibition zone around each well by millimeter against the tested organism.

Results and Discussion

Phytochemical Screening

Results of the phytochemical screening of Acacia nilotica gum aqueous solution showed that the solution contain different chemical constituents; tannins, flavonoids, saponins, and terpenoids (table 1). This result agreed with data obtained by (Najett et al., 2020) they reported that the Arabic gum contain flavonoids, saponin, tannins and terpenoids and partly with results showed its contain alkaloids, saponin, cardiac glycosides, and tannins but no flavonoids (Ali et al., 2020). So, Arabic gum presented variation in content, and this may be due to the type of extraction, method, solvent as well as the native characteristics of the plant (Vongsak et al., 2013).

Table 1. Constituents of the Acacia nilotica gum aqueous solution

Constituent	saponins	flavonoids	tannins	terpenoids	alkaloid
Result	+	+	+	+	-

Minimum inhibitory concentration

Based on the microdilution method, the MIC values of the Acacia niloticais gum solution alone against Klebsiella pneumoniae was 200mg/ml which is very high as compare with the value of ciprofloxacin which was 1µg, while the combination (Arabic gum +ciprofloxacin) has no any synergistic effect, where the MIC of ciprofloxacin stay the 200mg/ml same (1µg) when it is combined with any concentration of Arabic gum.

Susceptibility test

The antibacterial activity of the Acacia nilotica gum solution at different concentrations showed that the Arabic gum had weak effect against Klebsiella pneumoniae in vitro where the zones were 11.50±0.57, 10.00±1.14, 0.00±0.00 and 0.00±0.00mm at 400mg/ml 200mg/ml 100mg/ml and 50mg/ml respectively, which are significantly ($P<0.05$) less than those of ciprofloxacin that were 24.00±0.89, 18.30±0.94, 13.00±0.35 and 11.20±1.01 at 2µg, 1µg, 0.5µg and 0.25µg respectively. In concern to the combination (Arabic gum +ciprofloxacin), the resulted zones were less than those obtained from ciprofloxacin alone with significant difference just at doubled MIC which were 21.00±1.03, 17.70±1.31, 12.00±0.89 and10.00±0.94 (table 2; figure 1-3),

The result of this study agree partially with the data that obtained by many researchers that reported the Klebsiella pneumoniae susceptible to ciprofloxacin (Esmaeel et al., 2009; Alwan et al., 2011). Acacia nilotica have antibacterial activity against many bacteria including Klebsiella pneumoniae. Different extract of Acacia nilotica (water, acetone and methanol) have antibacterial activity against Klebsiella pneumoniae with inhibition zone ranged from 4-8.5mm (Singh et al., 2016). In another study, the inhibition zone produced by Acacia nilotica at different concentration, produces inhibition zone ranged from (8-16mm) (AL Alawi et al., 2018). The antibacterial activity may be due to the variety of groups of bioactive compounds in plant tissues as secondary metabolites, where the gum acacia include neutral sugars (rhamnose, arabinose and galactose), acids (glucuronic acid and 4-methoxyglucuronic acid) calcium, magnesium, potassium and sodium (Leung, 1980). It also contains various secondary metabolic compounds such as flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids (Majekodunmi et al., 2006). Phytochemicals exert antimicrobial activity through different mechanisms. For instance, flavonoids possess a wide range of biological activities including antimicrobial effect (Medini et al., 2014; Sukandar et al., 2014 and Andriani et al., 2019). Numerous studies stated that the antibacterial mechanisms of flavonoids are included mainly: inhibition of synthesis of nucleic acid, inhibition of cytoplasmic membrane function by influence the biofilm formation, porins, permeability, and by interaction with some crucial enzymes (Barbieri et al., 2017; Khameneh et al., 2019 and Górniak et al., 2019).

Tannins is another bioactive compound that have antibacterial activity by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides (Akiyama et al., 2001; Dharmananda, 2003). (Castillo et al., 2012) reported three hypothesis that might explain the antimicrobial mechanism of tannins: inhibition of enzyme activity by complexion with substrates of bacteria; direct action of tannins on the microorganism metabolism, through the inhibition of oxidative phosphorylation; a mechanism involving the complexion of tannins with metabolic ions, decreasing the availability of essential ions to the metabolism of the bacteria. The antibacterial activity of the Acacia nilotica gum is may be related to saponins which reported to possess antibacterial property by reducing surface tension resulting in increased permeability or leakage of cells, it resulting dicharge of intracellular compounds (Mandal et al., 2005).

Table 2: Inhibition zone produced by Acacia niloticais gum solution and ciprofloxacin and their combination against Klebsiella pneumoniae

Concern. Groups	Zone of inhibition (mm)			
	Concn1	Concn2	Concn3	Concn4
Ciprofloxacin	24.00±0.89 Aa	18.30±0.94 Ba	13.00±0.35 Ca	11.20±1.01 Ca
Combination	21.00±1.03 Ab	17.70±1.31 Ba	12.00±0.89 Ca	10.00±0.94 Ca
Arabic gum	11.50±0.57 Ac	10.00±1.14 Ab	0.00±0.00 Bb	0.00±0.00 Bb
LSD	2.46			

Means with a different small letter in the same column are significantly different (P<0.05)

Means with a different capital letter in the same row are significantly different (P<0.05)

Values: Means ± SE

N: 5

Arabic gum concentrations : 200, 400,100 and 50 mg/ml.

Ciprofloxacin concentrations: 2, 1, 0.5 and 0.25 µg.

Combination Arabic gum (mg/ml)+ ciprofloxacin (µg) : 400 + 2, 200 + 1, 100 + 0.5 and 50 + 0.25.

Figure 1: Inhibition zone produced by Acacia niloticais gum solution against Klebsiella pneumoniae, A- 400 mg/ml B- 200mg/ml C- 100mg/ml D- 50mg/ml

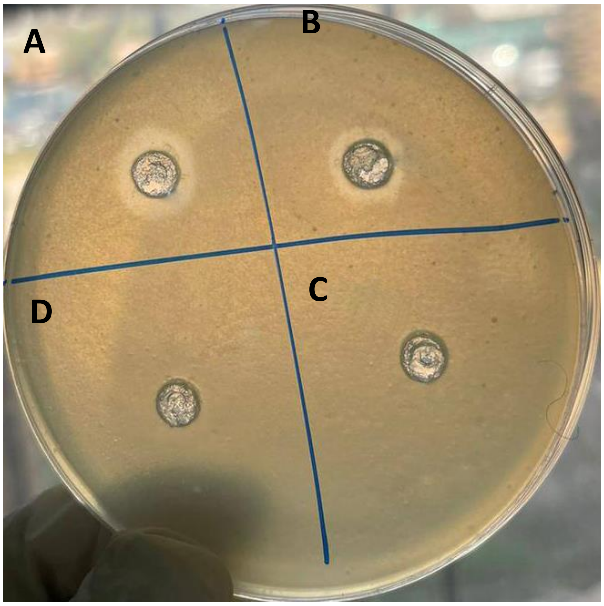


Figure 2: Inhibition zone produced by ciprofloxacin against Klebsiella pneumoniae, A- 2µg B- 1µg C- 0.5µg D- 0.25µg

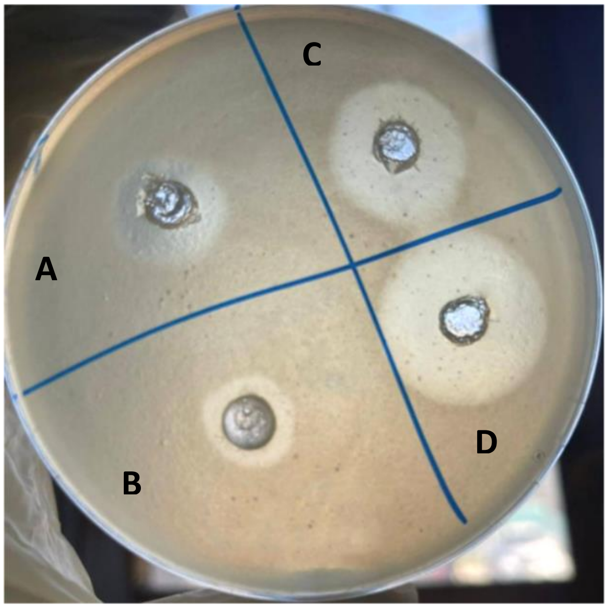
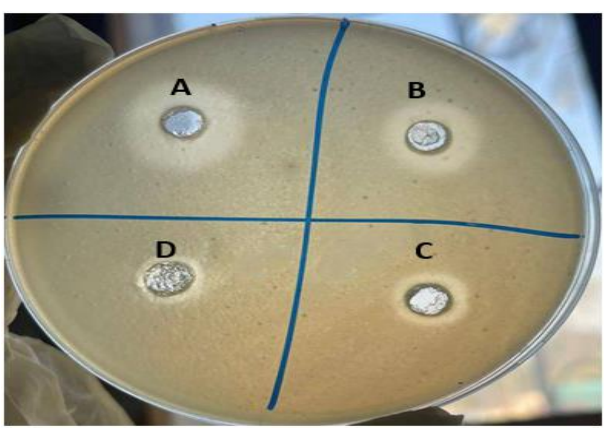


Figure 3: Inhibition zone produced by combination (Acacia niloticais gum solution + ciprofloxacin) against Klebsiella pneumoniae

A- (400) mg/ml Acacia niloticais gum solution + (2µg) ciprofloxacin B- (200) mg/ml Acacia niloticais gum solution + (1µg) ciprofloxacin C- (100) mg/ml Acacia niloticais gum solution + (0.5µg) ciprofloxacin D-(50) mg/ml Acacia niloticais gum solution + (0. 25µg) ciprofloxacin.



Conclusions

According to the obtained results, Acacia niloticais gum solution had weak antibacterial activity against Klebsiella pneumoniae as compared with the ciprofloxacin, as well as there was no synergistic effect for their combination.

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