Bacterial Growth Inhibitory Effect of *Ceratonia siliqua* L. Plant Extracts Alone and in Combination with Some Antimicrobial Agents

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Abstract: Objectives: To evaluate the antibacterial activity of the total extracts, methanolic and water extract of leaves with stems and the pods of *Ceratonia siliqua* L. each alone and in combination with some antimicrobials on some pathogens. As the wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. Many studies showed that the efficacy of antimicrobials can be improved by combining them with crude plant extracts.

Materials and Methods: the antibacterial activity of the total extracts, methanolic and water extract of leaves with stems and the pods of *Ceratonia siliqua* L. each alone and in combination with some antimicrobials was evaluated using well-diffusion method. Cytotoxicity of the total methanolic extract against Huh-7 liver and A-495 lung cancer cell lines was assessed using SRB method.

Results: Well diffusion method demonstrates an in-vitro antibacterial activity of the tested extracts against tested microorganisms. Combination of the tested extracts with antimicrobials increased the activity of the tested antimicrobials. A dose dependant effect on both Huh-7 liver and A-495 lung cancer cells was observed.

Conclusion: Our results revealed the importance of plant extracts when associated with antibiotics to control resistant bacteria that become a threat to human health. In addition, the tested plant extracts can be exposed for further investigation to be used as hepatoprotective agent.

Keywords: Ceratonia siliqua L plant extract, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, combination, antimicrobial agents.

INTRODUCTION

The bacterial resistance to the known antibacterial agents had become a serious global problem for instance, bacterial infections are responsible for 90% of infections found in health care services and 70% of the bacterial infections were resistant to at least one antibiotic [1]. Resistance mechanisms may include the production of drug inactivating enzymes, efflux pumps and target-site or outer membrane modifications. Resistance to multiple drugs is usually the result of the combination of different mechanisms in single isolate or the action of a single potent resistance mechanisms [2]. As a result, new antibacterial agents or combinations are desperately needed. *Ceratonia siliqua* L. is a leguminous evergreen tree which is native to the Mediterranean region it belongs to family Fabaceae and to the Caesalpinioideae sub-family [3]. It is called carob, algarroba, locust bean, locust tree, St. John's bread and in Arabic is kharroub [4, 5]. The major phytochemicals detected in *Ceratonia siliqua* L. are polyphenols including condensed and hydrolysable tannins, phenolic acids, flavonoids, and flavonoidal glycosids suggesting a potential antibacterial and cytotoxic activities [6-13]. Carob pods are a traditional part of the diet in the Mediterranean region and carob syrup is a popular drink in many countries including Egypt [14]. Historically carob pods have been used as animal and human food. The carob powder formed from the pods after removal of the seeds is often used as a chocolate or cocoa substitute. The seed endosperm contains galactomannans which called Carob gum or Locust bean gum (LBG) or E411. Locust bean gum is commonly used as a dietary fiber, thickening agent, foaming agent, emulsifier, stabilizer and as drug delivery agent [15] making it very important in the biopharmaceutical field [16]. The leaves and fruits of *Ceratonia siliqua* L. are used to cure various diseases [14].

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MATERIALS AND METHODS

Plant Materials

The leaves and stems of *Ceratonia siliqua* L. were collected from El-Zohria garden, Cairo, Egypt in February 2008. A voucher sample (Mn-Ph-Cog-004) was deposited in the Herbarium of The Pharmacognosy Department Faculty of Pharmacy Minia University. The plant was kindly identified by Dr. Mamdoh Shokry, director of El-Zohria garden. The pods of *Ceratonia siliqua* L. (Carob pods) were purchased from a local store.

Preparation of the Extracts

The air dried powdered leaves with stems (60 g) and pods (20 g) of *Ceratonia siliqua* L. were macerated separately in methanol and the extracts were then concentrated each separately under reduced pressure to yield a viscous gummy material viz. total methanolic extract of leaves with stems (TML) (6.2 g) and total methanolic extract of pods (TMP) (2.3 g), respectively. The aqueous extracts were prepared by maceration of powdered leaves with stems (20 g) and pods (20 g) separately in water then concentrated independently under reduced pressure to give total aqueous extract of leaves with stems (TAL) (2.2 g) and total aqueous extract of pods (TAP) (2.4 g), respectively.

Preparation of the Fractions of the Methanolic Extract of Leaves with Stems and Isolation of the Single Compounds

The methanolic extract of leaves with stems (5 g) was suspended in the least amount of distilled water and partitioned with successive portions of petroleum ether and chloroform. The extracts were concentrated under reduced pressure to afford petroleum ether fraction (1.5 g) and chloroform fraction (0.5 g). The aqueous mother liquor was concentrated under reduced pressure and the residue (2.7 g) was suspended in least amount of distilled water then subjected to diaion HP-20 column and eluted with water, 50%methanol, and methanol successively. The aqueous fraction was rejected and the other two fractions were concentrated each separately under reduced pressure to afford methanol fraction (0.6 g) and 50% methanol fraction (1.1 g). All extracts and fractions were weighed and dissolved in the DMF to obtain the desired concentrations. The four fractions were subjected to further chromatographic purification steps to yield compounds **1–9**

Structure Elucidation of the Compounds 1–9

Structure elucidation of the isolated compounds **1–9** was carried out using several spectral techniques; UV, 1H –NMR, 13C -NMR and mass spectroscopy and detailed data were recently submitted by elsewhere. The structures of compounds **1–9** were as follows (Figure **1**): Nonadecanol (**1**), lupeol (**2**), 5,7- dihydroxy-8,4`-dimethoxy isoflavone (**3**), (2S)-7,4`-dihydroxyflavanone (liquiritigenin) (**4**), 7,4`- dihydroxy-3`-methoxy flavone (geraldone) (**5**), gallic acid methyl ester (**6**), 7,4`-dihydroxyisoflavone (genistein) (**7**), quercetin-3-O-α-L-rhamnopyranoside (quericitrin) (**8**), myricetin-3-O-α-L-rhamnopyranoside (myricitrin) (**9**).

Bacterial Strains

Micro-organisms used in this study include, *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa.* All bacterial strains used were clinical isolates obtained from Microbiology Department, Faculty of Pharmacy, Minia University.

Evaluation of Antibacterial Activity Using Well Diffusion Technique

Microorganism (0.5 ml) of 1×10^6 CFU/ml (0.5Mcfarland turbidity) were plated in sterile petri dishes then 20 ml of sterile, molten and cooled (45°C) Muller Hinton agar media was added to all petri dishes. The plates then were rotated slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched using a sterile cork borer.

Each sample (5mg/ml) was applied as triplicate. The plates were allowed to stand for one hour for prediffusion of the extract to occur then incubated overnight at 37°C. All plates were examined and zones of inhibition were recorded [17-19]. This method was adopted for all microbiological experiments in this study.

Determination of the Minimum Inhibitory Concentration (MIC)

Two fold serial dilutions were performed on tested extracts, fractions and antibiotics. The initial concentration was 5mg/ml for extracts and fractions, 64 μg /ml for ampicillin, 32 μg /ml for gentamicin, 128 μg /ml for amikacin, and 8 μg /ml for clindamycin. Equal volumes of the extracts, fractions and antibiotics were applied separately to each well in three replicates using

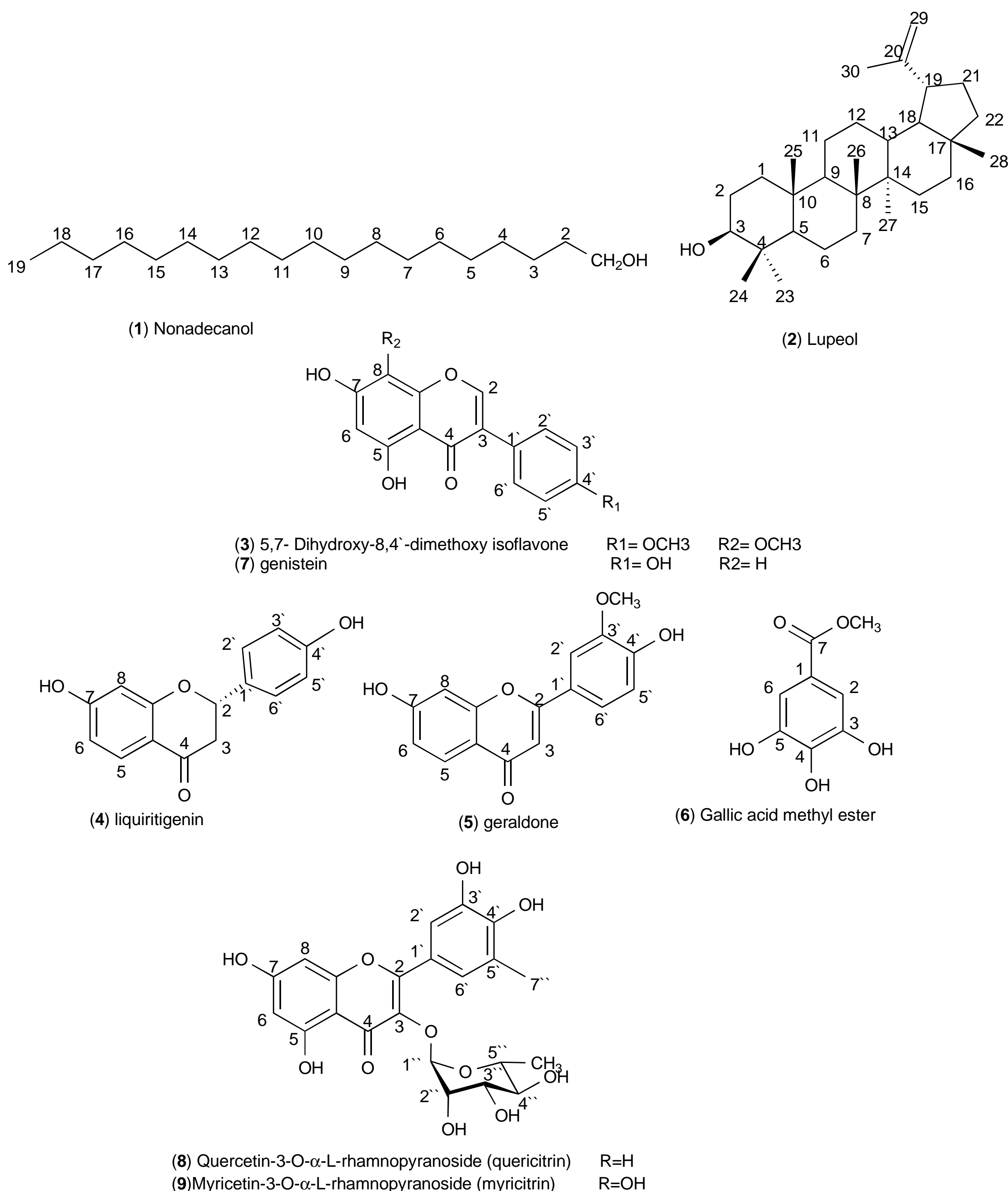

Figure 1: Structures of the isolated compounds **1-9**.

a micropipette [19]. All plates were incubated overnight at 37°C, then collected and zones of inhibition that developed were measured. The average of the zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value [19].

Determination of the Relative Inhibition Zones

Fifty µl of extracts and fractions (5 mg/ml) and of antibiotics Ampicillin (10 µg/ml), Clindamycin (2 µg/ml),

Gentamicin (10 µg/ml), Amikacin (30 µg/ml) were applied separately to each well. The plates were incubated overnight at 37°C. The diameter of inhibition zone was measured in mm (excluding cup size) and compared with negative control (solvent without extract) and positive control (standard antibiotics). The relative inhibition zone was recorded as the antibacterial activity of the extract. The percentage of relative inhibition zone diameter was calculated according to the equation [18].

$$\%\ of\ relative\ inhibition = \frac{IZD\ of\ sample - IZD\ of\ negative\ control}{IZD\ of\ positive\ control} \times 100$$

Where IZD is inhibition zone diameter.

This value would indicate the antibacterial potency of the extracts and fractions by comparing with commonly used antibacterial agents [18].

Determination of the Combined Activity of the Extracts and Fractions with Antibiotics

The antibacterial activity was measured using well diffusion method. 30 µl of *Ceratonia siliqua* L. extracts (5mg/ml) or antibiotic [Ampicillin (10 µg/ml), Clindamycin (2 µg/ml), Gentamicin (10 µg/ml), Amikacin (30 µg/ml)] and incase of combination 30 µl of each was added to each well. Every extract and fraction was combined with all standard antibiotics for each bacterial strain. The inhibition zone of the antibiotic alone (without combination) was considered as control. Replicates of each plate were done. Then the plates were incubated overnight at 37°C. The antibacterial activity was assessed by measuring the inhibition zone diameter (mm) around the well. The average of three replicates for each extract, antibiotic and combination was calculated [20, 21].

Cytotoxicity Assessment Against Huh-7 Liver and A-495 Lung Cancer Cell Lines

Cell Culture

Human hepatoma cell line (Huh-7) and human lung cancer cell line (A-495) were grown in DMEM (Dulbecco's Modified Eagle's Medium) with high glucose level 0.45% and supplemented with 10% heat inactivated FBS (fetal bovine serum), 100 units/ml of penicillin and 100 mg/ml of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO2. The cells were maintained as “monolayer culture” by serial subculturing.

Sulforhodamine B (SRB) Cytotoxicity Assay

Principle

Cytotoxicity testing is based on one or more mammalian cell lines being grown under conditions where they are actively growing and undergoing mitotic division. Cells are cultured in a microtitre well plate and the rate of multiplication and growth is measured indirectly by formation of a colour, the intensity of which is directly proportional to the number of cells present [22]. A variety of experiments can be used and the most basic is to compare the rate of proliferation of a cancer cell line in the presence and absence of the test substance, usually after a specified time such as Sulforhodamine B assay test. SRB assay [23] is used for cell density determination, based on the measurement of cellular protein content.

Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000-2000 cells/well in RPMI-1640 supplemented medium. After 24 hours, cells were incubated for 72 hours with various concentrations of the tested compounds. Following 72 hours treatment, the cells were fixed with 10% trichloroacetic acid for 1 hour at 4 °C. Wells were stained for 10 minutes. at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 hours and the dye was solubilized with Tris-HCl for 5 minute on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (model 680, Bio rad).

Data Analysis

The dose response curve of compounds was analyzed using Emax model.

$$\%\ \text{Cell viability} = (100 - \text{R}) \times \left(1 - \frac{[\text{D}]^{\text{m}}}{\text{K}_{\text{d}}^{\text{m}} + [\text{D}]^{\text{m}}}\right) + \text{R}$$

Where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, Kd is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC50 was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., Kd = IC50 when R=0 and Emax =100-R) [24].

Statistical Analysis of the Data

Results of the microbiological experiments were expressed as means ± S.E.M. One-Way ANOVA was

Table 1: Minimum Inhibitory Concentration (MIC) of Antibiotics and the Plant Extracts Against the Tested Microorganisms (µg/ml)

Tested organisms	Ampicillin	Clindamycin	Gentamicin	Amikacin	TML	TMP	TAL	TAP	Pet. eth. Fr.	Chloroform fr.	Methanol fr.	50% Methanol fr.
S. aureus	0.0625 S	0.09 S	16 R	14 S	NIZ	NIZ	516.8	NIZ	NIZ	400.2	485.39	493.25
E. coli	12.8 I	NT	3.26 S	8.39 S	87.09	200.9	182.4	180.9	15.29	119.626	44.27	139.2
K. pneumoniae	8 S	NT	3.36 S	1 S	81.06	179.3	180.6	157.6	13.45	99.28	63.33	127.9
Ps. aeruginosa	NT	NT	3.47 S	13.7 S	75.5	24.745	40.3	210.5	60.4	52.6	166.724	108.14

NIZ: inhibition zone equal to the diameter of the well so that their MICs were not calculated.
NT: Not Tested.
S = susceptible, R= resistant, I= intermediate.

used to compare the mean between each control/Antibiotic- extract treated group. *p* values less than 0.05 were considered significant. Differences were done using SPSS, 17 statistical software (SPSS Inc., Chicago, IL).

RESULTS

Determination of the Minimum Inhibitory Concentration

The MICs of the extracts, the fractions and the antibiotics varied between 0.0625µg/ml and 516.8µg/ml. The lowest MIC value for Carob preparations was exhibited by the chloroform fraction against *S. aureus* (400.2 µg/ml), the petroleum ether fraction against *E. coli* and *K. pneumoniae* (15.29 & 13.45 µg/ml, respectively) and the total methanolic extract of pods against *Ps. aeruginosa* (24.745µg/ml) (Table **1**). The results of antibiotics were interpreted according to CLSI [25].

Determination of the Relative Inhibition Zones

The antimicrobial activity of the tested extracts and fractions relative to antibiotics for *S. aureus* was illustrated in Table **2**. It showed that 50% methanol fraction had the highest activity compared to other tested extracts as its activity represents 71.8% of ampicillin, 62.1% of clindamycin, 153% of gentamicin and 135.3% of amikacin activity. Also, the chloroform fraction showed activity higher than gentamicin (106.6%) and (94.1%) amikacin activity. On the other hand, the total methanolic extract of the leaves and stems and the total aqueous extract of pods showed the lowest activity against *S. aureus* in comparison with the tested antimicrobials. Concerning *E.coli* and *K. pneumoniae,* Table **2** showed that all tested extracts and fractions except the petroleum ether fraction had higher activity than ampicillin and the total methanolic extract of the leaves and stems was the most active among all the tested samples. On the other hand, gentamicin was more active than the tested extracts. For amikacin, the total aqueous extract of the leaves and stems, the chloroform fraction and the 50% methanol fraction were found to have the same antibacterial activity. The petroleum ether fraction was found to have the lowest activity in comparison to antimicrobial agents and tested extracts but the petroleum ether fraction had the highest antibacterial activity (250% of gentamicin and 333% of amikacin) against *Ps. aeruginosa* followed by the 50% methanol and methanol fractions.

Determination of the Combined Activity of the Extracts and Fractions with Antibiotics

In general, the zones of inhibition in antibiotic/plant extract plates were in the range of 1- 39 mm wider than the zones of inhibition in the control plates (containing antibiotics without the plant extract) depending on the species of bacteria. The effect of combination of extracts and fractions with antibacterial agents tested on *S. aureus* isolate (Table **3**) showed that all extracts had the ability to increase the effect of ampicillin. On the other hand, all the total extracts and the 50% methanol fraction increased the activity of clindamycin. A significant increase in the activity of gentamicin and amikacin against *S. aureus* was shown by the total methanolic extract of pods, total aqueous extract of leaves and the chloroform fraction. For *E. coli,* Table **3** showed that *E. coli* was resistant to ampicillin but its activity increased by combination with the four fractions. A significant increase in ampicillin activity was shown by 50% methanol fraction. The table

Table 2: Antimicrobial Activity Profile Indicated in Percentage of Relative Inhibition Zone Diameter to Antimicrobials Used for the Tested Microorganisms

	Samples	Ampicillin	Gentamicin	Amikacin	Clindamycin
S. aureus	TML	37.5	80	70.6	32.4
	TMP	3	6	5	2
	TAL	34.3	73.3	64	29.7
	TAP	3	6	5	2
	Pet. ether fr.	21.9	46.6	41.1	18.9
	Chloroform fr.	50	106.6	94.1	43.2
	Methanol fr.	46.8	100	88.2	40.5
	50% methanol fr.	71.8	153	135.3	62.1
E. coli & K. pneumoniae	TML	333	47.6	11.1	NT
	TMP	266	38	88.8	
	TAL	300	42.8	100	
	TAP	233	33.3	77.7	
	Pet. ether fr.	33.3	4.7	11.1	
	Chloroform fr.	300	42.8	100	
	Methanol fr.	266	38	88.8	
	50% methanol fr.	300	42.8	100	
P . aeruginosa	TML	NT	25	33.3	NT
	TMP		25	33.3	
	TAL		25	33.3	
	TAP		25	33.3	
	Pet. ether fr.		250	333	
	Chloroform fr.		75	100	
	Methanol fr.		150	200	
	50% methanol fr.		175	233	

TML: total methanolic extract of leaves, TMP: total methanolic extract of pods, TAL: total aqueous extract of leaves, TAP: total aqueous extract of pods, Pet. ether fr.: Petroleum ether fraction, Nt: not tested.

Table 3: Effect of the Tested Antibiotics Alone and their Combinations with the Tested Extracts on *S. aureus and E. coli*

	Samples	Inhibition zones (mm)± S.E.M			
		Ampicillin (10 μg/ml)	Clindamycin (2 μg/ml)	Gentamicin (10 μg/ml)	Amikacin (30 μg/ml)
S. aureus	Control	40±1.15 (S)	15.66±0.88 (I)	24.33±1.2 (S)	32±1.15 (S)
	TML	51±0.57*	52.33±1.2*	32±1.15*	34.66±0.66*
	TMP	55.6±1.2*	52±1.15*	40.66±0.66*	35.33±0.33*
	TAL	57±0.66*	45.33±1.7*	33.66±0.88*	37.33±0.66*
	TAP	59±0.33*	42.66±1.4*	37.33±1.2*	41.66±1.2*
	Pet. ether fr	44.3±1.2*	44.66±0.88*	27±1.5	32.33±0.33
	Chloroform fr.	53.66±0.88*	40.33±0.88*	37.66±0.66*	34.66±0.66*
	Methanol fr.	55±0.0*	44.66±1.4*	34.66±1.4*	30.33±0.33
	50% methanol fr.	61.66±0.66*	54.33±1.2*	35±0.0*	33±1
E. coli	Control	11±0.57 (R)	NT	21±0.57 (S)	14 ± 1.1 (R)
	TML	13.66±0.33*	NT	18.33±0.66	17 ± 0.8*
	TMP	10.66±0.66	NT	17±1	20 ± 0.0*
	TAL	11±1	NT	22±1.15	21.66±0.6*
	TAP	11.6±0.88	NT	20±0.0	17.3±0.66*
	Pet. ether fr	16.33±0.33*	NT	21.66±0.66	22±1.15*
	Chloroform fr.	17.33±0.33*	NT	20±0.0	20±0.0*
	Methanol fr.	18.33±0.33*	NT	21.33±0.88	17±0.0*
	50% methanol fr.	18.33±0.66*	NT	26.66±0.66*	24±14.5*

S = susceptible, R= resistant, I= intermediate.

*P-value, $P < 0.05$: significant. Control: Antibiotic alone.

also showed that *E. coli* was resistant to amikacin but upon combination, the activity of amikacin increased with all tested extracts and fractions to the susceptible range. For *Klebsiella pneumoniae,* Table **4** showed that 50% methanol fraction caused a significant increase in the activity of ampicillin, gentamicin and amikacin. *K. pneumoniae* showed resistance against ampicillin and combination with petroleum ether, methanol and 50% methanol fractions increased the activity of ampicillin to the susceptible range. Also, Table **4** showed that the aqueous and methanolic extracts of pods in addition to the methanol and 50% methanol fractions caused a significant increase in the activity of gentamicin and amikacin *against Pseudomonas aeruginosa*. They also increased susceptibility of *Ps. aeruginosa* from intermediate to gentamicin and resistant to amikacin to the susceptible range (Figure **2**).

Cytotoxicity

The total methanolic extract of *Ceratonia siliqua* L. leaves and stems demonstrated dose dependant

Table 4: Effect of the Tested Antibiotics Alone and their Combinations with the Tested Extracts on *K. pneumoniae* and *Ps. aeruginosa*

	Samples	Inhibition zones (mm)± S.E.M		
		Ampicillin (10 μg/ml)	Gentamicin (10 μg/ml)	Amikacin (30 μg/ml)
K. pneumoniae	Control	10.33±0.33 (R)	23±1 (S)	23.33±0.66 (S)
	TML	11±0.57	25.33±0.88	24±1.15
	TMP	11.66±0.33	21.66±0.88	24.66±1.4
	TAL	15.66±0.66*	23.33±1.2	23.06±0.05
	TAP	13.33±0.66*	25.66±0.66	23.33±1.2
	Pet. ether fr	17.66±1.3*	22±1.15	28.66±0.88*
	Chloroform fr.	11.33±0.88	30±1.15*	22.33±0.88
	Methanol fr.	21±0.577*	26±1	27.66±1.2*
	50% methanol fr.	25.66±0.66*	29.33±1.4*	28.33±0.66*
Ps. aeruginosa	Control	NT	20±0.0 (S)	21.66±0.66 (S)
	TML	NT	25.66±0.66*	20±0.0
	TMP	NT	30±0.0*	28.6±0.88*
	TAL	NT	28.66±1.4*	27±0.0*
	TAP	NT	32±1.15*	29.6±0.66*
	Pet. ether fr	NT	32.33±0.33*	23±1.5
	Chloroform fr.	NT	34±1.15*	28±1.15*
	Methanol fr.	NT	27.66±0.88*	27.3±0.33*
	50% methanol fr.	NT	32±1.15*	32±1.15*

S = susceptible, R= resistant, I= intermediate.
*P-value, P < 0.05: significant. Control: Antibiotic alone.

Zones of Inhibitions of the Tested Antibiotics According to CLIS.

microorganisms	Ampicillin (10 μg/ml)			Clindamycin (2 μg/ml)			Gentamicin (10 μg/ml)			Amikacin (30 μg/ml)		
S. aureus	S	I	R	S	I	R	S	I	R	S	I	R
	≥ 29	-	≤ 28	≥ 21	15-20	≤ 14	≥ 15	13-14	≤ 12	≥ 17	15-16	≤ 14
E. coli & K. pneumoniae	≥ 17	14-16	≤ 13	NT	NT	NT	≥ 15	13-14	≤12	≥ 17	15-16	≤14
P. aeruginosa	NT	NT	NT	NT	NT	NT	≥ 15	13-14	≤12	≥ 17	15-16	≤14

S = susceptible, R= resistant, I= intermediate.

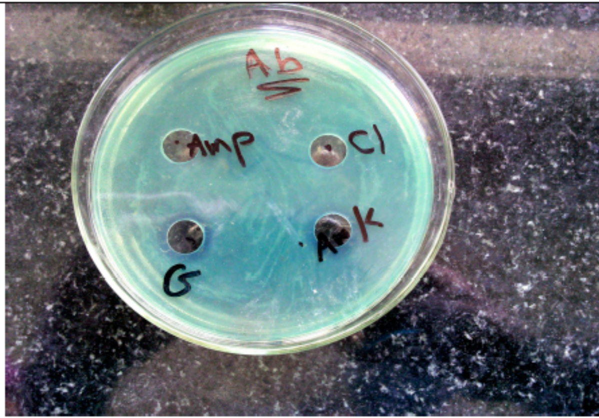

Fig. 2a: Zones of inhibition produced by the tested antibiotics alone

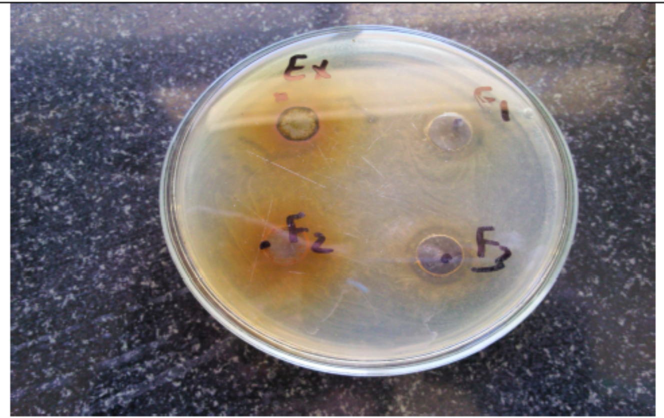

Fig.2b: Zones of inhibition produced by plant extract and the different fractions F1- F3

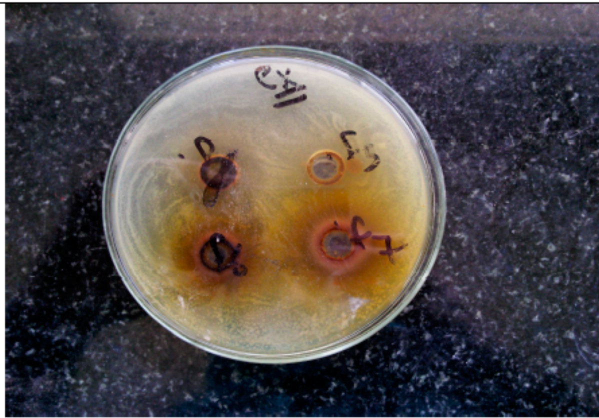

Fig2c: Zones of inhibition produced by plant extract and the different fractions F4-F7.

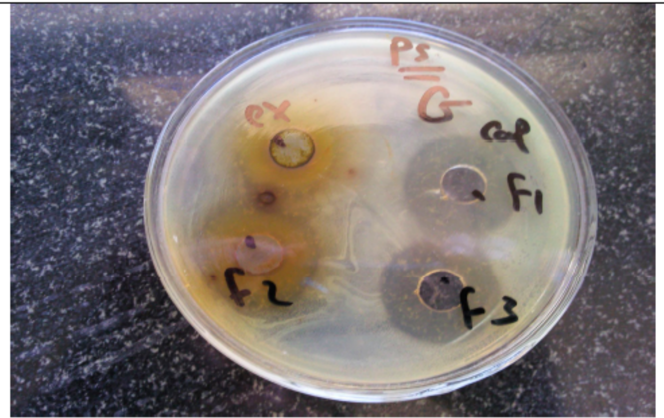

Fig2d: Zones of inhibitions increased by Gentamicin/ plant extract & F1-F3 combination

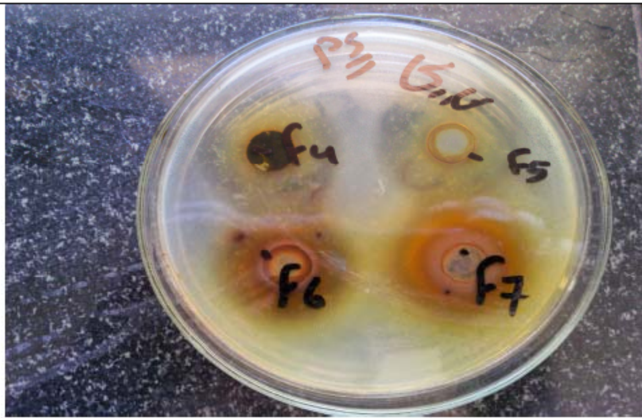

Fig2e: Zones of inhibitions increased by Gentamicin/ plant extract & F4-F7 combination.

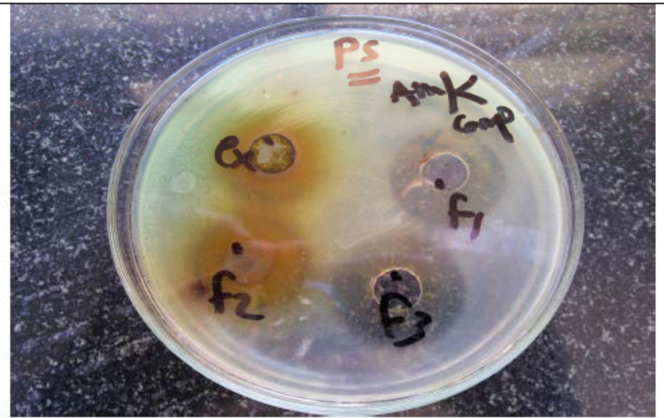

Fig2d: Zones of inhibitions increased by Amikacin/ plant extract & F1-F3 combination

(Figure 2). Continued.

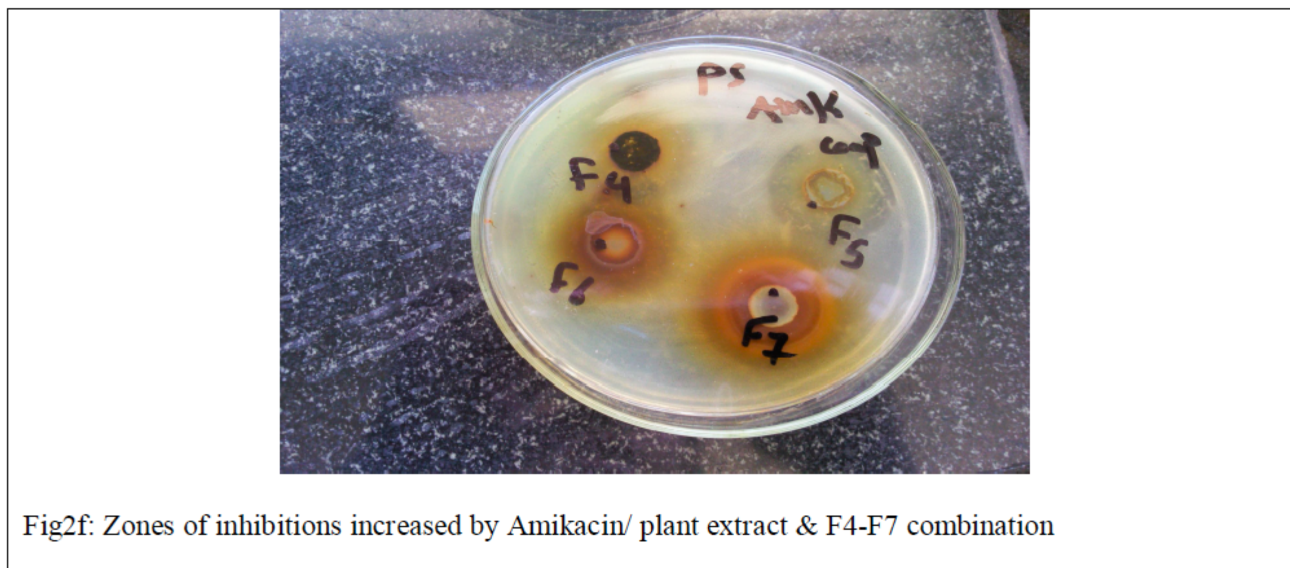

Fig2f: Zones of inhibitions increased by Amikacin/ plant extract & F4-F7 combination

Ex: TML F1: TMP F2: TAL F3: TAP F4:pet.eth fr. F5: Cloroform fr. F6: MeOH fr. F7:50%MeOH fr. , Amp: ampicillin, Cl: clindamycin, Amk: amikacin, G : gentamicin

Figure 2: Antimicrobial activity of the tested antibiotics and the tested plant extracts each alone and in combination against the tested *Pseudomonas aeruginosa*.

Table 5: The Results of IC_{50} of *Ceratonia siliqua* L. Extract Against Cell Lines

Cell line	IC_{50} (µg/ml)	R (resistance)
A-495 lung cancer	170	24
Huh-7 liver cancer	30	0

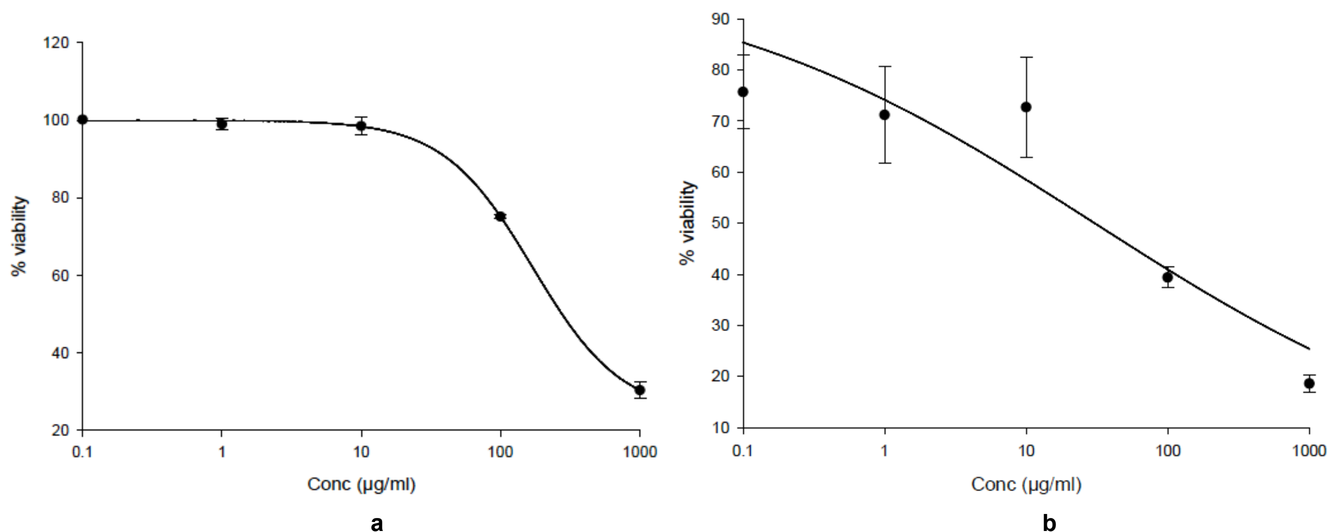

Figure 3: a: The results of cytotoxicity against A-495 lung cancer cell line.

b: The results of cytotoxicity against Huh-7 liver cancer cell line.

cytotoxicity on both Huh-7 liver and A-495 lung cancer cells with IC50 of 30µg/ml and 170 µg /ml, respectively assessed by the inhibitory effects in SRB assay (Table **5**, Figure **3a** & **3b**).

DISCUSSION

Currently, due to the dramatic failures of synthetic antibiotics to overcome the developing resistant pathogens, medicinal plants emerge as alternative source for new natural antimicrobial agents [26]. It is known that phytochemical compounds of medicinal plants such as alkaloids, flavonoids, phenols, glycosides, saponins, sterols etc. have curative properties [27]. The strong antibacterial activity of *Ceratonia siliqua* L. preparations and synergistic effect with antibiotics may be attributed to its content of flavonoids and tannins. Flavonoids and phenolic compounds are present in different quantities in most vascular plants [28]. They are a subject of medical research, have pharmacological benefits, including antioxidant, anti-inflammatory, antiallergic, hepatoprotective, antiviral, antimicrobial and anticarcinogenic activities [29-31]. Quercetin, apigenin and (-)-epigallocatechin were reported to inhibit DNA and RNA synthesis. On the same time, quercetin, flavanones and catechins were reported to have inhibitory activity on cytoplasmic membrane function. This could illustrate the synergism exhibited by *Ceratonia siliqua* L. extracts and fractions with antibiotics [1]. Naringin, apigenin and rutin are Carob flavonoids had reported antibacterial activity [32]. Our study showed that 50% methanol fraction had the highest activity compared to other tested extracts as its activity represents 71.8% of ampicillin, 62.1% of clindamycin, 153% of gentamicin and 135.3% of amikacin activity and the petroleum ether fraction had the highest antibacterial activity (250% of gentamicin and 333% of amikacin) against *Ps. aeruginosa.* It was found also that *Ceratonia siliqua* showed an increase in the antimicrobial activity of the tested antimicrobials against the tested microorganisms as the zones of inhibition in antibiotic/plant extract plates were in the range of 1-39 mm wider than the zones of inhibition in the control plates (containing antibiotics without the plant extract) depending on the species of bacteria which in agreement with the results obtained by Bijen and Tuba and Ben Hsouna *et al.*, who reported that methanol extract of *Ceratonia siliqua* showed strong action on *Staphylococcus* aureus [33], *Enterococcus* and*, Escherichia coli* [13].

The cytotoxicity results were considered promising for total extracts. The higher activity on Huh-7 liver cancer cells with no resistance (R=0) revealed the specificity of this extract towards the mentioned cell line. We could relate this to the presence of high aggregation of polar compounds as flavonoids, tannins and phenolic acids which were known for its hepatoprotective and cytotoxic activities [31]. Hepato-cellular Carcinoma is the most frequent primary liver cancer, the 5th commonest neoplasm in the world and the 3rd cause of cancer related deaths. From all the above mentioned we can recommend further investigations on the use of the extract of *Ceratonia siliqua* L. leaves and stems or its constituents as heptatoprotective agents.

CONCLUSION

Our results showed that plant extracts increase the therapeutic activity of the tested antimicrobials against the tested microorganisms.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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