GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(*Published By*: Global Institute for Research & Education)
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In vitro Antibacterial Potential of *Rosa Damascena* and *Terminalia Chebula* against Bacterial Peritonitis

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1. Abstract

Twenty two bacterial isolates were collected from hundred peritoneal samples taken from patients that were admitted to Tanta university hospital, Egypt. All the investigated isolates were identified morphologically and biochemically. The twenty two isolates were subjected to *in vitro* evaluation for antibiotic sensitivity test using antibiotics from different classes. *Rosa damascena* and *Terminalia chebula* were extracted using different solvents (Methanol and acetone) and were investigated for antibacterial activity against the 22 bacterial isolates. The sensitivity was determined using agar well diffusion method and the inhibition zones were compared with the standard drug gentamicin. The extracts showed a wide spectrum of inhibition against the tested isolates. Acetone extracts of the two plants were proven to have the strongest antibacterial activity than the methanol extracts. The minimum inhibitory concentrations of extracts were determined. GC-MS and FT-IR analyses were carried out for the *T. chebula* acetone extract as the best antibacterial agent.

Key words: *Antibacterial Activity, Rosa damascena, Terminalia chebula*

2. Introduction

Bacterial infections are present at admission or develop during hospitalization in about 30% of patients with cirrhosis (Fernandez *et al*., 2002). A large proportion of these patients have ascites. Patients with cirrhosis have increased risk to develop bacterial infection, sepsis, sepsis induced organ failure and death (Gustot *et al*., 2009). The mortality of infected patients with cirrhosis reaches 38% (Arvaniti *et al*., 2010). Cirrhotic patients are 2 times more likely to die from sepsis than individuals without cirrhosis (Foreman *et al*., 2003). Hospital mortality of cirrhotic patients with septic shock may exceed 70% (Plessier *et al*., 2003). Bacterial peritonitis and urinary infections are the most frequent infections followed by pneumonia (Fernandez and Gustot, 2012; Gustot and Moreau, 2015).

The continuous spread of multidrug-resistant pathogens has become a threat to public health and a major concern for infection control practitioners worldwide (Borowitz and Naser, 2011; El-Shouny *et al*., 2015). Conventional antibiotics usually provide effective therapy for bacterial infections. However, these bacteria have become resistant to one or more antibiotics and the population of Multidrug Resistant (MDR) bacteria is increasing (Stewart and Costerton, 2001; Hauser *et al*., 2016). Therefore, there is a need to search for substances from other sources with proven antibacterial activity. Consequently, this has led to the search for more efficient antibacterial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (Moreillion *et al*., 2005; Akeela *et al*., 2014).

Rosa damascena commonly known as the Damask rose or sometimes as the Rose of Castile, it is mainly cultivated worldwide for the production of rose essential oil used in perfumery and to make rose water. The flower petals are also edible. They may be used to flavor food, as a garnish and as an herbal tea (Libster, 2002; Verma *et al*., 2011). The flowers of the plant are also rich sources of flavonoids (Velioglu and Mazza, 1991). In addition to perfuming effect, the plant has been traditionally used for medical purposes from a long time ago. In ancient medical books, *Rosa damascena* has been suggested for treatment of menstrual bleeding, treatment of abdominal pain and digestive problems (AveSina, 1990), as well as chest pain and as an anti-inflammatory. Recent studies suggest a wide variety of therapeutic effects for *Rosa damascena* (Ozkan *et al*., 2004; Ulusoy *et al*., 2009; Shokouhinejad *et al*., 2010; Nyeem *et al*., 2016).

Terminalia chebula is a medicinal plant belonging to family Combretaceae. It is commonly called as black myrobalan. The fruits of *T. chebula* are commonly used in treatment of various ailments such as allergy, vomiting, urinary tract infections, cardiac diseases, digestive problems, bleeding, cancer, skin disorders and diabetes mellitus (Chatttopadhyay and Bhattacharyya, 2007). It also possesses antioxidant activity and free radical scavenging property. Antimicrobial activities of *T. chebula* have also been reported in many research publications (Vonshak *et al*., 2003; Kim *et al*., 2006; Singh and Kumar 2013; Hamed and EL-Kamali, 2016).

The aim of this work is to evaluate the efficacy of two herbal extracts (*R. damascena* and *T. chebula*) against drug resistant bacteria causing bacterial peritonitis as alternative remedy.

3. Materials and Methods

3.1 Plant Materials

The plants used in this study were obtained purchased from the local market of Matroh, Egypt. The identification of the two plants was carried out in Herbarium, Botany department, Faculty of Science, Tanta University, Egypt.

Table 1: Plants used to evaluate antibacterial activity.

Family name	Latin name	English name	Parts of plant used
Rosaceae	*Rosa damascena*	Damask rose	Flower
Combretaceae	*Terminalia chebula*	Chebulic fruit	Fruit

3.2 Preparation of plant extracts

Dried plants were cut into small pieces using a sharp knife. They were ground into powder using a blender; 20 g of each plant powder were taken for extraction. The extraction was done by using acetone and methanol (200 ml) separately for 48 hours at room temperature and filtered using Whatman"s No1filter paper (9 cm). The filtrate obtained was concentrated at 35°C in a rotary evaporator to obtain the crude extract. The crude extracts were kept at 4°C until further uses (Djeussi *et al.,* 2013).

3.3 Tested bacteria

Pure cultures of 22 tested bacterial isolates used in the study were obtained from cultivation of 100 medical specimens of peritoneal fluid of patients admitted to Tanta university hospital, Egypt. These specimens were taken from the Central Lab. of the hospital. All specimens were cultivated in nutrient broth overnight at 37°C for 24 hours, then sub cultured in nutrient agar and blood agar for another 24 hours. All the investigated isolates were identified morphologically and biochemically according to Bergey's Manual (Garrity, 2001). The bacterial isolates were maintained on nutrient agar slants and stored at 4ºC prior to use.

3.4 Antibiotics susceptibility assay of isolated bacteria

Antibiotics susceptibility was assessed using the disc diffusion method for all bacterial isolates as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2014) using thirty selected antibiotic discs (Bioanalyse, Turkey) from different classes of antibiotics. These antibiotics (µg/disc) were amoxicillin (25), ampicillin (10), oxacillin (1), pipracillin (100), amikacin(30), gentamicin (10), kanamycin (30), neomycin (30), streptomycin (10), tobramycin (10), ceftazidime (30), ceftriaxone (30), ceftizoxime (30), nalidixic acid (30), ciprofloxacin (5), norfloxacin (10), cefoperazone (75), cefotaxime (30), imipenem (10), meropenem (10), colistin sulphate (10), aztreonam (10), chloramphenicol (30), vancomycin (30), tetracycline (30), ampicillin/sulbactam (10/10), amoxicillin/clavulanic acid (20/10), pipracillin/tazobactam (100/10), trimethoprim/sulphamethoxazole (1.25/23.75) and cefoperazone/sulbactam (75/30).

Melted and cooled Müller Hinton agar medium was poured in sterile Petri dishes and swabbed with 100 µl from nutrient broth culture of bacterial isolates incubated overnight at 37°C for 24 hours under aseptic conditions. Antibiotics discs were placed on the surface of the inoculated plates with sterile forceps and pressed gently to ensure good contact with the surface of the medium. Following overnight incubation at 37± 0.2°C for 16 to 18 hours, zone of inhibition (mm) for each antibiotic disc was measured and values were interpreted as sensitive or resistant for each antibiotic by referring to performance standards for antimicrobial susceptibility testing (CSLI 2014).

3.5 Antibacterial activity assay of plant extracts

The twenty two isolates were screened for their susceptibility to plant extracts using the agar well diffusion method to determine the inhibition zone diameter that plant extracts were made into suspensions using DMSO (100 mg/ml). Each bacterial isolate was sub cultured overnight in nutrient broth, then adjusted to obtain turbidity equal to 10^6 CFU/ml using the turbid meter, 100 µl of each broth cultures was inoculated into three well dried plates of nutrient agar (replicate) and was spread homogeneously using sterile glass rod and left to dry for 15 min. Wells of 8 mm diameter were made in nutrient agar surface using sterile borer. 50 µl of each extract suspension was inserted into the wells by automatic pipette, the plates were incubated at 37°C for 24 hr. after incubation time the inhibition zones diameter in mm were measured, these inhibition zones were compared with negative control (50 µl DEMSO) and a positive control (Gentamicin antibiotic) (Atata, 2003; Bonjar, 2004).

3.6 Determination of minimum inhibitory concentration (MIC)

To measure the MIC values of the best extracts that showed the highest antibacterial activity against the tested bacteria, various concentrations of the plant extracts 12.5, 25, 50, 100, 200, 400 mg/ml in DEMSO were assayed against the tested bacteria. The minimum inhibitory concentration (MIC) of plant extract was determined using the agar diffusion method. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth (Perez *et al*., 1990; Gislene *et al*., 2000).

3.7 Identification of the most sensitive bacterial isolate by using the Biomerieux VITEK® 2 system

Tested bacterium mostly affected by the plant extract was cultured on the appropriate liquid nutritional medium and was incubated overnight at 37°C. Then the cultures were centrifuged at 3000 rpm for 20 min, washed with sterile saline solution and the turbidity of the bacterial suspensions was adjusted with a densitometer to match that of a McFarland 0.5 standard in 0.45% sterile sodium chloride solution, then the VITEK 2 cards were filled with bacterial suspension and manually loaded into the VITEK 2 system.

3.8 Spectral studies of the extracts have strong antibacterial activity

GC-MS Spectroscopy

Gas Chromatography - mass spectroscopy (GC-MS) spectrum analysis of acetone extract of *Terminalia chebula* was investigated. By using GC-MS the qualitative and quantitative evaluation of components of the studied extract could be determined (Douglas, 2012).

GC-MS analysis of acetone extract was examined in Claurs 580/560S. Perkin Elmer Company in the Central Lab, Tanta University, Egypt. Work was done with column 30.0 m x 250 µm. Rtx-5MS (cross bond 5% diphenyl 95% dimethylpolysiloxane), equipped with heated FID. The GC conditions were employed using Helium as carrier gas at a constant flow rate of 0.8 ml/min., initial temperature was 100°C for 2 min and was programmed to reach 250°C at a rate of 5°C/min. hold 0 min, ramp 15°C/min to 300°C, hold 25 min, Injection temperature at 270°C, Volume=1 µl, Injection mode was Split=20:1, Solvent Delay=5.00 min, Transfer Temp=250°C, Source Temp=280°C and Scan was at 50 to 600Da. The chromatogram obtained from gas chromatography was then analyzed in mass spectrometry to get the mass of

all fractions. The identification of phytochemical components was achieved through retention time and mass spectrometry by comparing the mass spectra of unknown peaks with those stored in Wiley 9 GC-MS library.

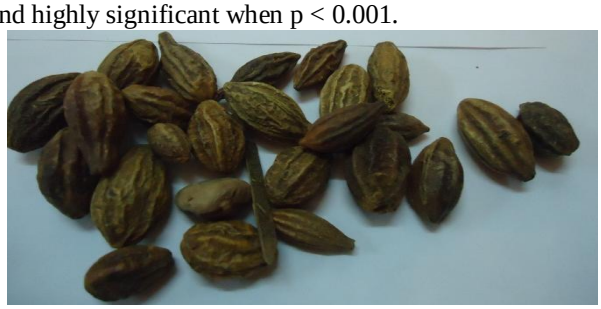
FT-IR Spectroscopy

Fourier transform infrared spectroscopy (FT-IR spectrum) analysis of acetone extract of *Terminalia chebula* was investigated for its characteristic functional groups. This analysis was used to determine the safety of these extracts by determines the function groups and to be sure from the absence of toxic groups like cyano group (C≡N) and acetylenic group (Nouh, 2016). This FT-IR spectrum analysis was determined using Jasco FT/IR-6100 spectrophotometer in the Central Lab, Tanta University, Egypt.

3.9 Statistical Analysis

All experiments were performed in triplicate. The data were recorded as mean ± standard deviation (SD). All statistical analyses including ANOVA were carried out by using the SPSS software, version 20 (SPSS Inc., Chicago, IL, USA). Differences were considered significant when $p < 0.05$ and highly significant when $p < 0.001$.

Rosa damascena



Terminalia chebula

4. Results and Discussion

According to the antibiotic sensitivity of the isolated bacteria, the resistance percentage ranged from 4.5 % to 100 %; that one isolate only was resistant to cefoperazone/sulbactam 75/30 µg and imipenem 10 µg (4.5 % resistance of isolates). However, all the twenty two isolates were resistant to ceftazidime 30 µg (100 % resistance of isolates). The best antibiotics were cefoperazone/sulbactam (sulperazone) and imipenem (tienam). Due to their high cost, gentamicin was selected as a positive control to compare its antibacterial activity with antibacterial activities of plant extracts used in this study. Gentamicin is a broad spectrum antibiotic and its cost is very low for any patient. Also resistance percentage of gentamicin against the tested bacteria was 31.8 % where seven isolates were resistant to this drug (Table 2).

Table 2: Antibiotic resistance percentage of the isolated bacteria.

Antibiotics	Symbol	Conc. (µg /disc)	Resistant isolates	
			No.	%
Amikacin	AK	30	5	22.7
Amoxicillin	AX	25	14	63.6
Amoxicillin/Clavulanic acid	AMC	20/10	10	45.5
Ampicillin	AM	10	19	86.4
Ampicillin/ Sulbactam	SAM	10/10	16	72.7
Aztreonam	ATM	10	19	86.4
Cefoperazone	CEP	75	16	72.7
Cefoperazone/Sulbactam	CES	75/30	1	4.5
Cefotaxime	CTX	30	17	77.3
Ceftazidime	CAZ	30	22	100.0
Ceftizoxime	ZOX	30	20	90.9
Ceftriaxon	CRO	30	21	95.5
Chloramphenicol	C	30	8	36.4
Ciprofloxacin	CIP	5	10	45.5
Colistin Sulphate	CT	10	18	81.8
Gentamicin	CN	10	7	31.8
Imipenem	IPM	10	1	4.5
Kanamycin	K	30	10	45.5
Meropenem	MEM	10	4	18.2
Nalidixic Acid	NA	30	15	68.2
Neomycin	N	30	11	50.0
Norfloxacin	NOR	10	9	40.9
Oxacillin	OX	1	18	81.8
Pipracillin	PRL	100	17	77.3
Pipracillin/Tazobactam	TPZ	100/10	3	13.6
Streptomycin	S	10	13	59.1
Tetracycline	TE	30	16	72.7
Tobramycin	TOB	10	10	45.5
Trimethoprim/Sulphamethoxazole	SXT	1.25/23.75	15	68.2
Vancomycin	VA	30	16	72.7

Total number of isolates = 22 isolates.

The study was performed to investigate the antibacterial properties of two plant extracts against the tested bacterial isolates. The results revealed that damask rose (*Rosa damascena*) extracts have considerable inhibitory effect against the tested bacteria. Both methanol and acetone extracts showed inhibitory effect on eleven isolates. The acetone extract showed a mean of inhibition zones of the sensitive isolates of 15.73 mm greater than methanol extract which recorded a mean of inhibition zones of the sensitive isolates of 14.27 mm. The acetone extract inhibited the the isolates number 7, 27, 34, 43, 48, 50, 75, 77, 84, 87 and 88. The maximum inhibition zone by acetone extract was observed on isolates number 7 and 88 (18 mm). El-Shouny *et al*. (2014) stated that chamomile acetone extract showed highest inhibition zones of 27 mm against the tested *staphylococcus aureus* and 18 mm against *candida albicans.*

Table 3: Antibacterial activity of damask rose extracts 100 mg/ml using different solvents by well diffusion method.

Clinical bacterial isolates		Sp. No.	Damask rose extracts		Gentamicin 10 µg
			Zone of inhibition (mm)		
			Methanol	Acetone	
Gram +ve bacteria	***Bacillus clausii***	75	11±0.57	17±1.0	18±1.52
	Bacillus clausii	88	18±2.0	18±2.0	22±1.52
	Staphylococcus aureus	50	12±0.57	17±1.15	20±2.08
	Staphylococcus aureus	77	13±0.57	14±0.57	21±2.08
	Staphylococcus aureus	84	15±0.57	14±0.57	22±1.52
	Staphylococcus aureus	87	11±0.57	15±1.0	23±1.15
Gram -ve bacteria	***Enterobacter cloacae***	7	19±1.0	18±1.0	20±1.52
	Escherichia coli	27	17±1.0	17±0.57	18±0.57
	Escherichia coli	43	12±0.57	12±0.57	12±0.0
	Salmonella typhi	34	14±0.57	15±0.57	15±1.0
	Shigella dysenteriae	48	15±0.57	16±1.15	13±1.15
sensitive isolates			**11**	**11**	**15**
Mean of inhibition zone for sensitive isolates			**14.27**	**15.73**	**18.54**
Total mean			**7.13**	**7.86**	
P. value			< 0.001**	< 0.001**	< 0.001**

Values are mean inhibition zone (mm) ± S.D of three replicates, *significant at $P \leq 0.05$ **highly significant at $P \leq 0.001$ using one way analysis of variance (ANOVA), 0 = no zone of inhibition, control= 0, Sp. No.: specimen's number of peritoneal fluid, total number of isolates= 22.

Similar results were obtained by Shohayeb *et al*. (2014) who evaluated the antimicrobial activity of *R. damascena* essential oil and different extracts of petals against three Gram-positive bacteria, seven Gram-negative bacteria, one acid-fast bacterium and three fungi. Rose oil and all tested rose fractions exerted broad spectrum antibacterial activity against all tested bacteria and fungi. Also Mahboubi *et al*. (2011) confirm the antibacterial activity of the *Rosa damascena* oil that the oil showed antimicrobial activity against a large number of microorganisms especially against *Proteus vulgaris* and *Klebsiella pneumoniae*.Also the study revealed that chebulic extracts (*Terminalia chebula*) have considerable antibacterial activity against the tested bacteria. Methanol and acetone extracts showed inhibitory effect on fourteen isolates. The acetone extract recorded a mean of inhibition zones of the sensitive isolates of 14.78 mm slightly greater than methanol extract which showed a mean of inhibition zones of the sensitive isolates of 14.57 mm. The acetone extract affected the isolates number 7, 9, 27, 31, 46, 48, 50, 58, 75, 76, 77, 84, 87 and 88. The maximum inhibition by acetone extract was observed on isolate number 7 (21 mm).

Table 4: Antibacterial activity of chebulic extracts 100 mg/ml using different solvents by well diffusion method.

Clinical bacterial isolates		Sp. No.	Chebulic extracts		Gentamicin 10 µg
			Zone of inhibition (mm)		
			Methanol	Acetone	
Gram +ve bacteria	***Bacillus clausii***	75	16±1.15	18±1.15	18±1.52
	Bacillus clausii	88	16±1.52	16±2.08	22±1.52
	Staphylococcus aureus	50	14±0.57	12±0.57	20±2.08
	Staphylococcus aureus	77	15±1.15	15±0.57	21±2.08
	Staphylococcus aureus	84	14±0.57	15±0.57	22±1.52
	Staphylococcus aureus	87	14±1.15	13±0.57	23±1.15

Clinical bacterial isolates		Sp. No.	Chebulic extracts		Gentamicin 10 µg
			Zone of inhibition (mm)		
			Methanol	Acetone	
Gram -ve bacteria	*Enterobacter cloacae*	7	19±1.52	21±1.52	20±1.52
	Escherichia coli	27	17±1.52	18±0.57	18±0.57
	Escherichia coli	46	10±0.57	10±0.0	15±0.57
	Escherichia coli	58	12±0.57	16±1.15	8±0.57
	Klebsiella pneumoniae	31	15±0.57	13±0.57	14±0.57
	Proteus mirabilis	9	13±0.57	15±0.57	14±0.57
	Proteus mirabilis	76	14±0.57	13±0.57	13±0.57
	Shigella dysenteriae	48	15±0.57	12±0.57	13±1.15
No. of sensitive isolates			14	14	15
Mean of inhibition zone of sensitive isolates			14.57	14.78	17.2
Total mean			9.27	9.40	
P. value			< 0.001**	< 0.001**	< 0.001**

Values are mean inhibition zone (mm) ± S.D of three replicates, *significant at $P \leq 0.05$ **highly significant at $P \leq 0.001$ using one way analysis of variance (ANOVA), 0 = no zone of inhibition, control = 0. , Sp. No.: specimen's number of peritoneal fluid, total number of isolates= 22.

The extract of *T. chebula* fruits was studied by Kannan *et al.* (2009) and Bag *et al.* (2012) and they found that ethanol extract of *T. chebula* fruits demonstrated a strong antimicrobial activity against all the tested isolates. Thus *T. chebula* fruit extract was highly effective against *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853. Phytochemical analysis of extract of *T. chebula* was made by Bag *et al.* (2012) and revealed the presence of high concentration of phenolics and low concentration of flavonoids and terpenoids.

To compare the antibacterial activity of *Rosa damascena* extracts with that of *Terminalia chebula* extracts, total mean for inhibition zones of all extracts were determined. The total means for inhibition zones of acetone and methanol extracts of *R. damascene* were 7.86 and 7.13 mm, respectively. The total means for inhibition zones of acetone and methanol extracts of *T. chebula* were 9.40 and 9.27 mm, respectively. So it was clear that the acetone extract of *T. chebula* was the best extract and have the highest antibacterial activity. So it was important to determine its minimum inhibitory concentrations (MIC) against the sensitive isolates and identify the most sensitive isolate to acetone extract of *T. chebula*, then study its composition by GC-MS spectrum analysis and study its safety by Infra-red spectrum analysis to investigate its characteristic functional groups.

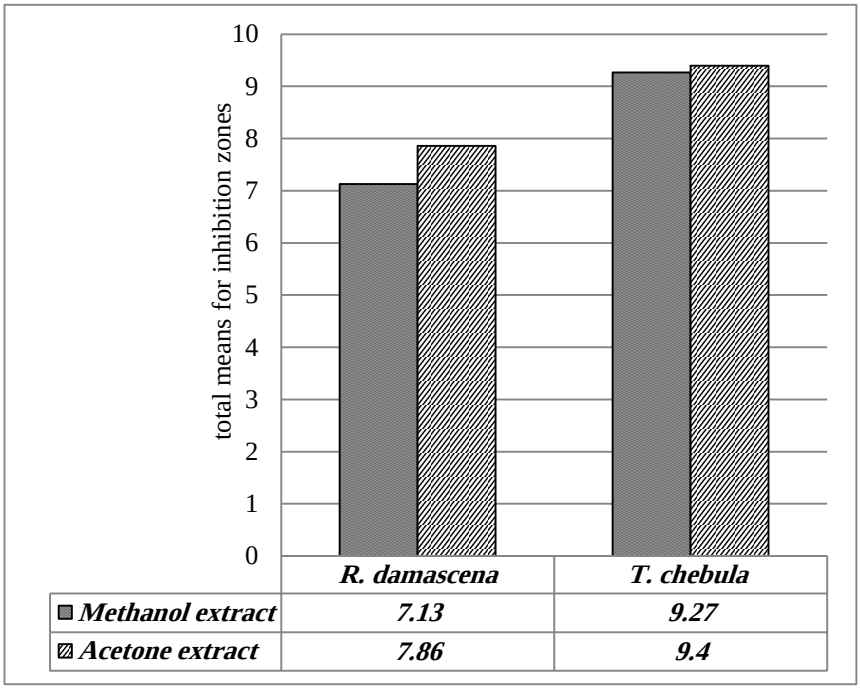


Fig 1: Difference between plant extracts according to their antibacterial activities.

To measure the minimum inhibitory concentrations (MIC) value of acetone extract of *T. chebula*, various concentrations of acetone extract 12.5, 25, 50, 100, 200 and 400 mg/ml were prepared in DMSO and were assayed against the sensitive fourteen bacterial isolates. Table (5) showed that the antibacterial activity increasing by increasing

the chebulic extract concentration from 12.5 to 400 mg/ml. The results revealed variability in the inhibitory concentration of *T. chebula* extract that the MICs of acetone extract of *T. chebula* against tested bacterial isolates ranged from 12.5 to 50 mg/ml.

Table 5: MIC of acetone extract of *T. chebula* against the tested isolates.

Clinical bacterial isolates			Zone of inhibition (mm)					
			Conc. of acetone extract (mg/ml)					
			12.5	25	50	100	200	400
Gram +ve bacteria	*Bacillus clausii*	75	11±1.0	14±0.57	16±0.57	19±0.57	20±2.08	22±2.08
	Bacillus clausii	88	10±0.57	13±0.57	15±1.0	18±1.52	21±1.52	22±2.0
	Staphylococcus aureus	50	0±0.0	0±0.0	10±0.0	12±0.57	14±0.57	16±1.0
	Staphylococcus aureus	77	0±0.0	10±0.57	12±0.57	15±0.57	18±1.52	20±2.0
	Staphylococcus aureus	84	0±0.0	0±0.0	11±0.57	14±0.57	16±0.57	19±1.52
	Staphylococcus aureus	87	0±0.0	0±0.0	10±0.0	13±0.57	15±0.57	18±1.15
Gram -ve bacteria	*Enterobacter cloacae*	7	0±0.0	13±0.57	15±0.57	18±1.15	21±2.0	23±1.15
	Escherichia coli	27	0±0.0	11±1.15	14±0.57	17±1.52	19±1.52	20±2.08
	Escherichia coli	46	0±0.0	0±0.0	0±0.0	10±0.57	12±0.0	14±1.0
	Escherichia coli	58	0±0.0	11±0.57	13±0.57	15±1.0	17±1.15	18±1.52
	Klebsiella pneumoniae	31	0±0.0	0±0.0	10±0.57	12±0.57	13±0.57	15±0.57
	Proteus mirabilis	9	0±0.0	0±0.0	12±1.0	14±0.57	16±0.57	17±0.57
	Proteus mirabilis	76	0±0.0	0±0.0	0±0.0	12±0.0	13±0.57	15±1.0
	Shigella dysenteriae	48	0±0.0	0±0.0	0±0.0	11±0.57	13±0.57	15±0.57
Total mean			1.38	4.95	9.40	14.17	16.26	18.64

Values are mean inhibition zone (mm) ± S.D of three replicates, 0 = no zone of inhibition, control = 0, Sp. No.: specimen's number of peritoneal fluid, total number of sensitive isolates to *Terminalia chebula*=14.

The isolate number (7) was selected as a representative to MDR bacteria and sensitive to the plants extracts were confirmed phenotypically using VITEK 2 system. This system revealed that the selected isolate number (7) were *Enterobacter cloacae* with 99% probability percentage (Table 6).

Table 6: Confirmed identification of isolate number (7) by VITEK 2.

Identification information			Card: GN (Gram negative)								
Selected organism			*Enterobacter cloacae*								
probability percentage: 99%			**Confidence:** Excellent identification								
Bionumber: 0627735553573010											
Biochemical details											
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-
7	dCEL	+	9	BGAL	+	10	H_2S	-	11	BNAG	+
12	AGLT	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+
21	BXYL	+	22	BAlap	-	23	proA	+	26	LIP	-
27	PLE	+	29	TYrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+
37	MNT	+	39	5KG	-	40	ILATK	+	41	AGLU	-
42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	+
46	GlYA	+	47	ODC	+	48	LDC	-	53	IHISa	-
56	CMT	-	57	BGUR	-	58	O129R	+	59	GGAA	-
61	IMLTa	-	62	ELLM	-	64	ILATa	-			

APPA: Ala-Phe-Pro-Arylamidase; ADO: Adonitol; PyrA: L-Pyrrolydonyl-Arylamidase; IARL: L-Arabitol; dCEL: D-Cellobiose; BGAL: Beta-Galactosidase; H_2S: H_2S Production; BNAG: Beta-N-Acetyl-Glucosaminidase; AGLTp: Glutamyl Arylamidase pNa; dGLU: D-Glucose; GGT: Gamma-Glutamyl-Tranferase; OFF: Fermentation/Glucose ; BGLU: Beta-Glucosidase; dMAL: D-Maltose; dMAN: D-Manitol; dMNE: D-Mannose; BXYL: Beta-Xylosidase; BAlap: Beta alanine Arylamidase pNa; proA : L-Proline Arylamidase; LIP: Lipase; PLE: Palatinose; TYrA: Tyrosine Arylamidase; URE: Urease dSOR: D-Sorbitol; SAC: Saccharose/Sucrose; dTAG: D-Tagatose; dTRE: D-Trehalose; CIT: Citrate-sodium; MNT: Malonate; 5KG: 5-Keto-D-Gluconate; ILATK: L-Lactate Alkalinisation; AGLU: Alpha-Glucosidase; SUCT: Succinate Alkalisation; NAGA: Beta-N-Acetyl-Galactosaminidase; AGAL: Alpha-Galactosidase; PHOS: Phosphatase; GlyA: Glycine Arylamidase; ODC: Ornithine Decarboxylase; LDC: Lysine Decarboxylase IHISa: L-Histidine Assimilation; CMT: Coumarate; BGUR: Beta-Glucuronidase O129R: 2,4-Diamino-6,7-

Diisopropylpteridine Resistance; GGAA: Glu-Gly-Arg-Arylamidase; IMLTa: L-Malate Assimilation; ELLM: ELLMAN; ILATa: L-Lactate Assimilation.

GC-MS analysis of acetone extract of *Terminalia chebula*

The results of GC-MS analysis of the acetone extract of *T. chebula* confirmed the presence of 15 peaks of different compounds with retention times of 6.06, 6.52, 7.03, 9.63, 9.91, 10.97, 11.14, 12.48, 14.89, 16.51, 16.76, 22.77, 25.45, 27.30 and 32.68 minutes. The ten leading compounds were illustrated in table 7 that active principle, area of the peak concentration (%), retention time (RT) are presented in the table. According to this GC-MS, the major components of *T. chebula* acetone extract were 3,5-Dimethyl-3-heptene (1.34%), 1,6-anhydro-á-D-Glucopyranose (1.39%), Diethyl Phthalate (1.46%), 1-Cyclopentyl-2,2-dimethyl-1-propanol (1.78%), Succinic acid, 3-hex-4-ynyl 3-methylbutyl ester (1.94%), taurine (10.1 %) and 5-Hydroxymethylfurfural (11.1 %).

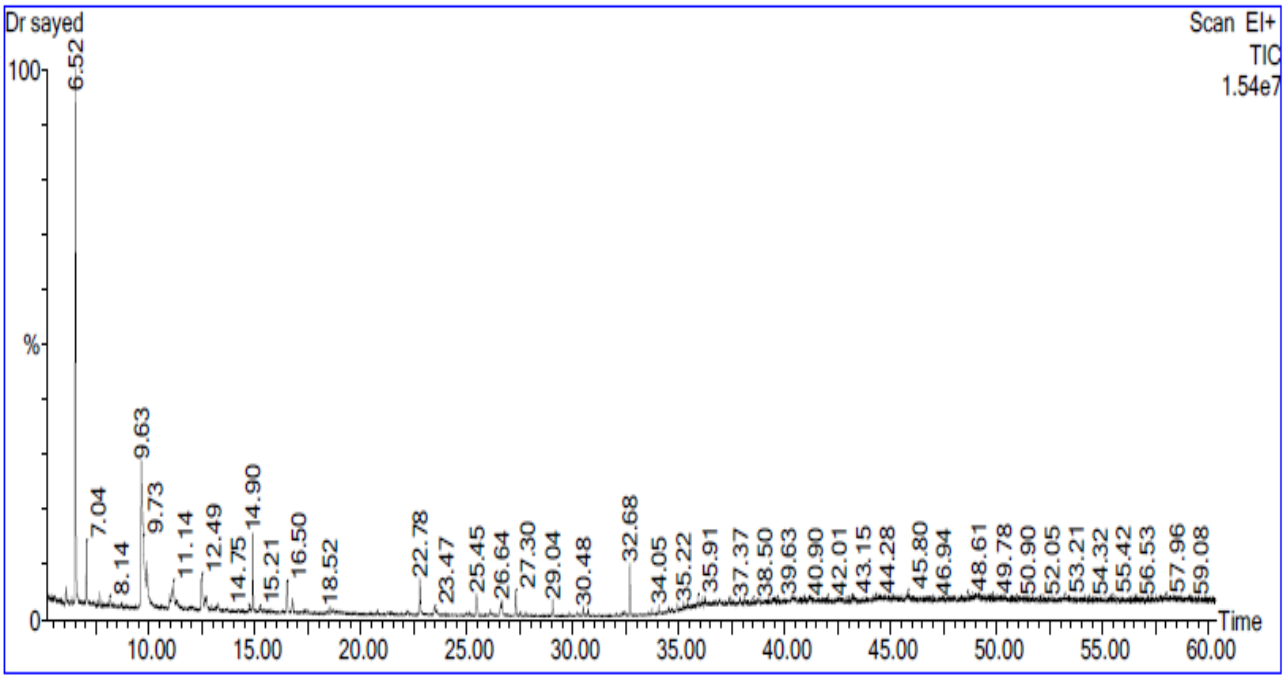


Fig 2: GC-MS chromatogram of *Terminalia chebula* acetone extract.

Table 7: GC-MS chromatogram of *Terminalia chebula* acetone extract

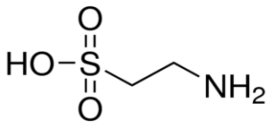
Peak	RT	Area	Area %	Compound name.
2	6.524	592,930.1	11.107	5-Hydroxymethylfurfural
3	7.039	71,514.1	1.340	3,5-Dimethyl-3-heptene
4	9.630	540,243.8	10.120	Taurine
5	9.910	104,009.5	1.948	Succinic acid, 3-hex-4-ynyl 3-methylbutyl ester
7	11.141	95,080.5	1.781	1-Cyclopentyl-2,2-dimethyl-1-propanol
8	12.486	74, 404.1	1.394	1,6-anhydro-á-D-Glucopyranose
9	14.897	78,129.4	1.464	Diethyl Phthalate
10	16.518	57,115.7	1.070	Methamphetamine
12	22.775	49,762.6	0.932	D-Arabinose
15	32.684	50,786.4	0.951	Phthalic acid, 4-methylhept-3-yl octadecyl ester

Singh *et al.* (2008) studied the antioxidant and antimicrobial activities of essential oil and various oleoresins of *Elettaria cardamomum* (seeds and pods). They reported that essential oil and various oleoresins of *E. cardamomum* have broad spectrum antibacterial activity against various fungal and bacterial isolates tested and they used GC-MS analysis to determine the main components of *E. cardamomum* and found that 5-hydroxymethylfurfural (28.9%) was the most abundant compound in the ethanol extract.

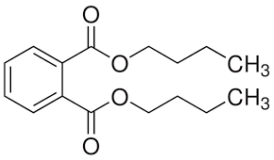
Islambulchilar *et al.* (2011) reported that the taurine was as a major intracellular free β-amino acid and it is known to be an endogenous antioxidant. So potentially the co-therapy of taurine and gentamicin would reduce the adverse effects of the antibiotic. In this concern, El-Shouny *et al.* (2016) reported that antibiotics combination with rosmary methanol extract had synergistic inhibitory effect on multi drug resistant bacteria.

Velanganni *et al*. (2011) studied the phytochemical screening and antimicrobial activity of the stem of *Mallotus philippensis* and reported that Diethyl phthalate has antimicrobial activity and used medicinally for the preparation of many consumer formulations. The diethyl phthalate was found to be present as major constituents of ethanol extract of *Mallotus philippensis* with highest peak area of 94.47 %.

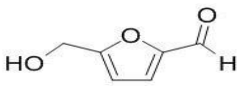
Taurine



Diethyl Phthalate



5-Hydroxymethylfurfural



FT-IR analysis of acetone extract of *Terminalia chebula*

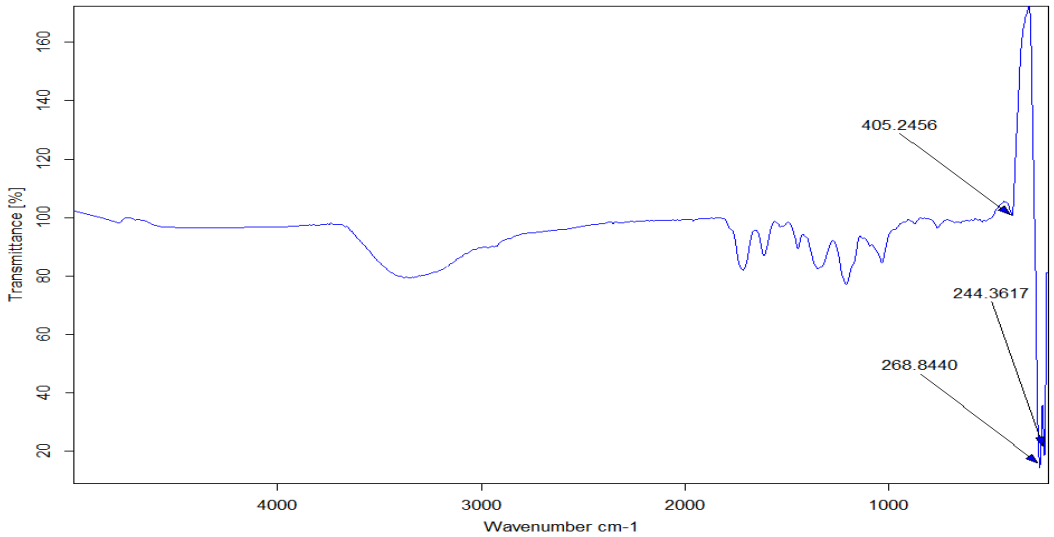


Fig 3: FT-IR chromatogram of *Terminalia chebula* acetone extract.

Table 8: Functional group profile of *Terminalia chebula* acetone extract by FT-IR analysis.

Functional group	Range of wave number (cm^{-1})	Presence in *T. chebula*
Hydroxyl group (OH)	3300-3600	Present
Amino group (NH_2)	3200-3400	Absent
Aliphatic saturated hydrocarbon chain (CH_3,CH_2,CH)	2850-2980	Absent
Aldehyde group (CHO)	2850-2900	Absent
Carbonyl group (C=O)	1700-1750	Present
Aliphatic unsaturated hydrocarbon chain (C=C)	1500-1600	Present
Aromatic hydrocarbon ring	3100-3200	Absent
cyano group (C≡N)	2000-2250	Absent
acetylenic group (C≡C)	2000-2250	Absent

The safety of acetone extract of *T. chebula* in the present study was determined through FT-IR analysis that FT-IR study was carried out to analyse the functional groups present in the extract. As shown in figure 3 and table 6, there are no peaks for toxic cyanide group (2000-2250 cm^{-1}) and no peaks for acetylene group (2000-2250 cm^{-1}). This technique of FT-IR was used in many studies to evaluate the safety of extracts and analyzed for toxicological effect. Salimon *et al.* (2012) used FT-IR analysis to analyze Rubber (*Hevea brasiliensis*) seed oil and studied the presence of toxic groups like cyanide group.

5. Conclusion

The results of present study reveals that all the tested plant extracts used in this study have potent antibacterial activity and could potentially be used as a source of natural antibacterial for treating drug resistant bacteria. Out of all extracts, the acetone extract of *T. chebula* fruits was the most active antibacterial agent.

6. Recommendation

Further studies are required for isolation and purification of the active ingredients of the plant responsible for these inhibitory effects for the tested bacteria and to better understand the mechanism of such actions.

7. Acknowledgements

The authors are thankful to staff members of the "Central Lab. of Tanta University hospital, Egypt" for providing the medical specimens of peritoneal fluid.

8. References

Alan, R. Hauser, Joan Mecsas, Donald, T. Moir (2016). Beyond Antibiotics: New Therapeutic Approaches for Bacterial Infections. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 2016 http://sumo.ly/jS2A via @QxMD

Anwesa Bag, Subir Kumar Bhattacharyya, Nishith Kumar Pal, Rabi Ranjan Chattopadhyay, (2012). *In vitro* antimicrobial potential of *Terminalia chebula* fruit extracts against multidrug–resistant uropathogens. *Asian Pacific Journal of Tropical Biomedicine*, 2(3), pp. S1883–S1887.

Arvaniti V., D'Amcio G., Fede G., Manousou P., Tsochatzis E., Pleguezuelo M. (2010). Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology*, 139(4), pp. 1246–1256.

Atata, R., Sani, A. and Ajewole, S.M. (2003). Effect of stem back extracts of *Enantia chloranta* on some clinical isolates. *Biokemistri*, 15 (2), pp. 84-92.

Ave Sina, M. (1990). Law in medicine. Translated by Sharafkhandy, A., Tehran, Soroush. Persian. PP: 129-131.

Borowitz, S. and Naser, S. (2011). Gut pathogens. *The Official Journal of International Society for Genomic and Evolutionary Microbiology*, 17 (15): pp. 1927-2062.

Chatttopadhyay, R.R., Bhattacharyya, S.K. (2007). *Terminalia chebula*: An update. *Pharmacogn Rev*., 1, pp. 151–156.

CLSI (2014). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement, CLSI. Vol. 34 No. 1.

Doriane E. Djeussi, Jaures A.K. Noumedem, Jackson A. Seukep, Aime G. Fankam, Igor K. Voukeng, Simplice B. Tankeo, Antoine H.L. Nkuete and Victor Kuete (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complementary and Alternative Medicine*, 13(164), http://www.biomedcentral.com/1472-6882/13/164.

Douglas F. (2012). GC/MS analysis. *Scientific Testimony Online Journal*. Scientific Testimony Available from http://www.scientific.org/tutorials/articles/gcms.html.

El-Shouny W.A., Abd El-Zaher E. H. F., Khalil M.A., Abd El-Salam O., (2014) Antimicrobial activity of chamomile acetone extract against some experimentally-induced skin infections in mice. *International Journal of Environmental Research*, 2, pp. 58-70.

El-Shouny W.A., Allam N. G., Khalil M.A., Hegazy A M. (2016). Anti-bacterial response of combination between antibiotics and some plant extracts against multidrug resistant bacteria. *Advances in Biological Research*, 10 (1), pp. 51-57

El-Shouny W.A., Samir S., Nouh S. (2015). Drug resistance and virulence potential of *Pseudomonas aeruginosa* isolated from ocular infections. *Egyptian Journal of Botany and Microbiology*, vol (II), pp. 529-544.

Fernandez J., Navasa M., Gomez J. (2002). Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology*, 35, pp.140-148.

Foreman MG., Mannino DM., Moss M. (2003). Cirrhosis as a risk factor for sepsis and death: analysis of the National Hospital Discharge Survey. *Chest*, 124, pp. 1016–1020.

Garrity G.M. (editor in chief). Bergey's Manual of Systematic Bacteriology. 2nd ed. Vol. 1, Taxonomic Outline of the Archaea and Bacteria. New York, Berlin ,Heidelberg: Springer; 2001.

Geeta Singh and Padma Kumar (2013). Extraction, gas chromatography–mass spectrometry analysis and screening of fruits of *Terminalia chebula* Retz. for its antimicrobial potential. *Pharmacognosy Res*., 5(3), pp. 162–168.

Gislene, G. F. N., Juliana, L., Paulo, C. F., and Giuliana, L. S. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant Bacteria. Brazilian J. Microbiol. 31, pp. 247-256.

Gurdip Singh, Shashi Kiran,Palanisamy Marimuthu, Valery Isidorov , Vera Vinogorova (2008). Antioxidant and antimicrobial activities of essential oil and various oleoresins of *Elettaria cardamomum* (seeds and pods) *Journal of the Science of Food and Agriculture*, 88(2), pp. 280–289.

Gustot T., Durand F., Lebrec D., Vincent JL., Moreau R. (2009). Severe sepsis in cirrhosis. *Hepatology*, 50, pp. 2022–2033.

Islambulchilar M., Sattari M.R., Sardashti M., Lotfipour F., (2011). Effect of Taurine on the antimicrobial efficiency of gentamicin, *Adv Pharm Bull*. 1(2), pp. 69–74.

Javier Fernandez and Thierry Gustot (2012). Management of bacterial infections in cirrhosis. *Hepatology*, pp.1-12. S1–S12.

Kannan P., Ramadevi S.R., Waheeta Hopper, (2009). Antibacterial activity of *Terminalia chebula* fruit extract *African Journal of Microbiology Research*, 3(4), pp. 180-184.

Kim, Jeong-Keun, Narae Kim, and Young-Hee Lim (2009). Evaluation of the antibacterial activity of rhapontigenin produced from rhapontin by biotransformation against *Propionibacterium acnes*. *J. Microbiol. Biotechnol*., 20(1), pp. 82–87.

Libster, M. (2002). Delmar's integrative herb guide for nurses. 1st Ed., Albany, USA, *Delmar Thamson Learning*. PP: 360-370.

Mahboubi M., Kazempour N., Khamechian T., Fallah M.H.& Kermani M. M., (2011).Chemical composition and antimicrobial activity of *Rosa damascena* Mill Essential Oil, *Iran J Microbiol*, 3(4), pp. 194-200.

Mohammad Abu Bin Nyeem, Mohammad Nazrul Islam, Md Khairul Alam, Md. Masum Billah, Bijoya Islam and Mahbub Mostofa, (2016). Antidiarrhoeal and antimicrobial activities of the methanolic extract of the flowering tops of *Rosa damascena*. *International Journal of Current Research*, 5(8), pp. 30547-30551.

Moreillion P., Que Y.A., and Glauser M.P., (2005). *Staphylococcus aureus* (Including Staphyloccal Toxic shock). In 'Principles and Practice of Infectious diseases.' (Ed.) Mandell G.L., Bennett J.E., Dolin R., Churchill livingstone Pennyslyvania 6th ed. 2, pp. 2333-2339.

Ozkan, G., Sagdic, O., Baydar, N.G. and Baydar, H. (2004). Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol. Int*., 10, pp. 277-281.

Perez, C. Pauli, M. and Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. *Acta Biologiae et Medecine Experimentaalis*, 15, pp. 113-115.

Plessier, A., Denninger, M.H., Consigny, Y., Pessione, F., Francoz, C., Durand, F. (2003). Coagulation disorders in patients with cirrhosis and severe sepsis. *Liver Int*., 23, pp. 440–448.

Raid Al Akeela, Yazeed Al-Sheikha, Ayesha Mateenb, Rabbani Syeda, K. Janardhanc, V.C. Guptab (2014). Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains. *Saudi Journal of Biological Sciences*, 21, pp. 147–151.

Sana Eltayeb Mahjoob Hamed, Hatil Hashim EL-Kamali. (2016). Antibacterial properties of methanolic and aqueous extracts of some plants against some *Enterobacteriaceae* species. *Open Access Library Journal*, 3, e2488, pp. 1-10.

Shahidi Bonjar, G. H. (2004). Evaluation of antibacterial properties of Iranian medicinal plants against *Micrococcus aureus, Serratia marcescens, Klebsiella pneunomiae* and *Bordella bronchoseptica*. *Asian Journal of Sciences*, 3(1), pp. 82-86.

Shohayeb M., El-Sayed S. Abdel-Hameed, Salih A. Bazaid, Ibrahim Maghrabi, (2014). Antibacterial and antifungal activity of *Rosa damascena* MILL. Essential Oil, different Extracts of Rose Petals, *Global Journal of Pharmacology* 8(1), pp. 01-07.

Shokouhinejad, N., Emaneini, M., Aligholi, M. Jabalameli, F. (2010). Antimicrobial effect of *Rosa damascena* extract on selected endodontic pathogens. *J. Calif. Dent. Assoc.*, 38, pp. 123-126.

Stewart, P. and Costerton, W. (2001). Antibiotic Resistance of Bacteria in Biofilms. *The Lancet*, 358 (9276), pp.135-138.

Thierry Gustot and Richard Moreau (2015). Spontaneous bacterial peritonitis and other infections, Cirrhosis: A practical guide to management, First Edition: pp.164-174.

Ulusoy, S., Boşgelmez Tinaz, G., Seçilmiş Canbay, H. (2009). Tocopherol, carotene, phenolic contents and antibacterial properties of rose essential oil, hydrosol and absolute. *Curr. Microbiol*.,59, pp. 554-558.

Velanganni J., Kadamban D., Ramamoorthy D. (2011) Phytochemical screening and antimicrobial activity of the stem of *Mallotus philippensis* (Lam.) Muell. Arg. Var.*Philippensis*. *Int J Pharmacol Pharmaceut Sci*., 3(2), pp.160–163.

Velioglu, Y.S. and Mazza, G. (1991). Characterization of flavonoids in petals of *Rosa damascena* by HPLC and spectral analysis. *J. Agric. Food Chem*., 39, pp. 463-467.

Verma, RS., Padalia, RC., Chauhan, A., Singh., A. and Yadav, AK. (2011). Volatile constituents of essential oil and rose water of Damask rose (*Rosa damascena* Mill). cultivars from north Indian hills. *Nat. Prod. Res*., 25, pp. 1577- 1584.

Vonshak A., Barazani O., Sathiyomoorthy P., Shalev R., Vardy D., Golan-Goldhirsh A. (2003). Screening South Indian medicinal plants for antifungal activity against cutaneous pathogens. *Phytother Res*.,17, pp. 1123–5.