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Screening of some Sudanese Plant Extracts Against Common Gram Positive and Gram Negative Bacteria

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Abstract

Acacia nilotica (Garad), *Citrulls colocynthis* (Handal), *Nigella sativa* (Kamoun) and *Trigonella foenum greacum* (Helba) are plants, believed by Sudanese herbalists to have antimicrobial effect. These plants have been tested in the present study to investigate their *in vitro* potential effects against nine Gram positive and Gram negative bacteria. The selected organisms were *Bacillus cereus*, *Corynebacterium ovis*, *Staphylococcus aureus*, *Escherichia coli*, *pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Niesseria genorrhoeae*, *Porteus vulagaris* and *Salmonella typhi*.

The plants were extracted with two solvents, ethanol was used to extract the polar compounds, and the petroleum ether was used to extract the non-polar compounds, the extracts were tested by two methods Minimum Inhibition Concentration method (MIC) and the filter paper disc method.

The MIC of the ethanolic extract of *Acasia nilotica*, inhibited all tested organisms at 8.3 mg/ml. The most sensitive organism was *S. typhi* which was completely inhibited at 8.3 mg/ml (no growth).

S. aureus, *P. vulgaris* and *N .gonorrhoeae* showed no growth at 3.8 mg/ml, 4.2 mg/ml concentration and 2.1 mg/ml concentration they showed variable degrees of inhibition to the tested organisms.

In disc method of ethanolic extract of *A. nilotica*, all tested Gram negative and Gram positive bacteria showed various inhibition zones at the concentration of 500 mg/ ml.

The disc method of petroleum ether extract of the *A. nilotica* was found less effective. The inhibition zones of *S. typhi* were very narrow (9 mm in concentrations 500 mg / ml and 250 ml).

The MIC of the ethanolic extract of *C. colocynthis* was not effective. On the contrary, it enhances the growth of selected organisms.

Keywords: *Acacia nilotica* (Garad), *Citrulls colocynthis* (Handal), *Nigella sativa* (Kamoun) and *Trigonella foenum greacum* (Helba), ethanol extract, petroleum ether extraction. Antibacterial activity antimicrobial effect, nine Gram positive and Gram negative bacteria .

Introduction and Objective

Bark of *A. nilotica* has been used for treating haemorrhages, colds, diarrhoea tuberculosis and leprosy while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions (Shetty, 1977).*A. nilotica* showed antibacterial activities against *Campylobacter* species isolated from sheep in Zaria and Kaduna. In Jordan the antimicrobial activity of ethanol extract of 15 plants used in traditional medicine there and in other countries were tested *in vitro* against 12 pathogenic bacteria. 25 mg/well of 12 plant extracts have antimicrobial activity. Three plants, exhibited broad spectrum antibacterial activity. These plants were *Puncia granatum*, *Quercum infectoria olive*, and *Rhus criarial*. The most susceptible bacteria were *P. aeruginosa*, *B. cereus*, and *S. pyogenes* (ATCC 12351) (Laila *et al*., 1999). The resin of *C. colocynthis* (Cucurbitaceae) is used as a gastro- intestinal stimulant and as a powerful purgative as well as hydrogogue therapeutic and anti-rheumatic cure in traditional medicine in Sudan (Eltohami 2003). Clinical trials have shown that (*Nigella sativa*) Black seed Oil controls Blood Sugar and Cholesterol apart from many diseases and is considered to be one of the greatest healing herbs of all times. Nearly 70% of all traditional ayurvedic formulas contain a special blend of ingredients, which includes Black seeds for this purpose. Black seed is also known as Black Cummin, Black Caraway and many other names (Muhammad *et al.*, 2002).

Tigonalla foenum greacum (Helba) has a wide range of medicinal applications. The seeds are very nourishing and are given to convalescents and to encourage weight gain, especially in anorexia nervosa. The seeds should not be prescribed medicinally for pregnant women since they can induce uterine contractions. Research has shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect (Chevallier, 1996).

Orgamisms may develop resistance to any drug, either rapidly or after long or repeated courses of therapy (Mary *et al*., 2000). *S. aureus* is a common pathogen that infects about 400,000 U.S. hospital patients a year. About one-quarter of them die. For decades, Scientists have been dreading but expecting *S. aureus* strain to emerge that is resistant to Vancomycin (EMILIA, 2002).

Production of extended-spectrum ß-lactamases (ESBLs) by *Klebsiella pneumoniae* is a widespread nosocomial problem. Appropriate infection control and antibiotic management strategies are needed to stem the spread of this emerging form of resistance. Commonly encountered nosocomially acquired gram-negative bacteria, especially *K. pneumoniae*; produce ESBLs as an antibiotic resistance mechanism (David *et al*., 2004). One hundred clinical avian *E. coli* isolates were examined for their general susceptibility to a battery of antibiotics of human and veterinary significance. The prevalence of resistance to aminoglycosides range from 27% for Kanamycin to (97%) for Streptomycin among these isolates. Most *E. coli* isolates (86%) were resistant to the tetracyclin, and oxytetracyclin. Avian *E. coli* isolates were generally resistant to both streptomycin, and sulfonamides (97 of 100%). A high pecentage of the *E. coli* isolates were also found resistant to Ambicillin 30% and Chloramphenicol (10%) (Lydia *et al*., 1999).

Some of the *P. mirabilis* strains were multidrug resistant, which included some aminoglycosides and broad-spectrum cephalosporins.The National Laboratory for Sexually Transmitted Diseases showed the distribution of antibiotic resistance of the strains of *N. gonorrhoeae* which was received and tested in 1998. They found that some strains have chromosomally mediated multiple resistance to the antibiotics tested, e.g. chromosomal resistance toPenicillin/Tetracycline/Erythromycin represents 3.2% (128) of the 4,001 strains tested. Rising ciprofloxacin resistance is associated with importation from Asia.

Emergence of Cephalosporin resistance is likely just around the corner (Canada Communicable Disease Repor, Population and Public Health Branch, 2000).

blood cholesterol levels and also have an antidiabetic effect (Chevallier, 1996).

Material and methods

Experiments of the present study were undertaken in three phases as follows:

1- Collection and cultivation of bacteria.the strain of examined bacteria are brought from collage of laboratory science university of Khartoum

2- Collection and extraction of plants from sudanese market for herbal product,

3- *In vitro* antimicrobial testing by two method Minimum Inhibition Concentration method (MIC) and Disc diffusion Method

The soxhelt extraction method was used in this study (Chairman *et al*., 1965). 200 gram from each ground sample was accurately weight in an empty thimble covered with cotton wool and then placed in soxhel- extraction apparatus. Ethanol was used as solvent, 350-400 ml were added, pre-weighed round bottomed flask full of 350-400 ml of solvent was fitted to the extractor. The apparatus was assembled, the extraction process was allowed to continue for 12 hours. The apparatus was then carefully dismounted, and the solvent was evaporated first at room temperature, then dried in an oven at 105 ˚C of the polar content:

The polar extract was calculater as follows:

$$\text{Polar content \%} = \frac{\text{The weight of the polar content extract (gm).}}{\text{Weight of sample (gm)}} \times 100$$

(Chairman *et al*., 1965).

Petroleum ether extraction

Finely ground sample, from each plant was accurately weight, and put in an empty thimble. The thimble covered with cotton wool, then it was placed in soxhlet extraction apparatus. In this study petroleum ether was used as solvent. Pre- weighed round bottomed flask full of 350-400 ml of petroleum ether was fitted to the extractor, the apparatus was assembled, the extraction continued for 12 hours. The apparatus was evaporated first at room temperature. Then dried in an oven at 105 ˚C. The oil constant weight was expressed as percentage of the oil content.

The non-polar extract was calculater as follows:

$$\text{Oil \%} = \frac{\text{The weight of oil extracted (gm)}}{} \times 100$$

Weight of sample (gm)

(Chairman *et al*., 1965).

In-vitro antimicrobial testing

The two most commonly used methods to determine antimicrobial susceptibility are the minimum inhibition concentration method (Balair *etal*., 1970), and the disc or agar well diffusion assay (Navarro *et al*., 1996). Both method were used in this study for both ethanolic and petroleum ether extracts of each of the four plants.

Minimum Inhibition Concentration method (MIC)

Four gram of ethanolic extracts of each plant was added to 20 ml sterile deionized water, which was putt in sterile test tube. The tube was shaken until the contents were homogenized, the concentration at this stage was 200 mg/ ml. Then 10 ml of that homogenized solvent were taken, and added to another test tube, which containeds 10 ml of sterile demonized water, the concentration was 100 mg/ ml. then 10 ml. was taken and added to a third tube which contained 10 ml sterile deionized water; the concentration was then 50 mg/ ml. Then 10 ml from the third tube which contain 10 ml sterile deionized water, the concentration was 25 mg/ ml. The serial dilution was repeated until the sixth dilution.

The content of the six tubes were added to six plates each one contained 20 ml sterile nutrient agar. The plates were left to solidify. The concentrations used were as follows:

66.7 mg/ ml, 33.3 mg/ ml, 16.7 mg/ ml, 8.3 mg/ ml, 4.2 mg/ ml, and 2.1 mg/ ml medium Plates contained *C. colocyrthis* extrat, and *T. foenum greacum* extract were solidified in all above six concentrations. Plates contained *A. nilotica* at 66.7 mg/ml medium, 33.3 mg/ml medium, and 16.7 mg /ml medium, did not solidified, so antimicrobial testing in that connection was not examined. Plates contained *N. satva* extract at 8.3 mg/ ml medium, did not solidified, so antimicrobial effects in that concentration was not examined.

Antimicrobial testing

The negative control plates which contained nutrient agar, and the possitive control were plates contained nutrient agar mixed with chlorotetracyclin at a concentration of 26.7 mg/ml medium. Tested plates contained extracts of plants under study, mixed with nutrient agar. They were each inoculated by tested organisms, and results were compared with negative and positive controls. The petroleum ether extracts for all tested plants, could not be examined by M I C method, because petroleum ether extract is oily, so that extracts of examined plants could not be homogenized. therefor, results had not been taken.

Disc Method

The discs was prepared from small filter paper of 5 mm diameter (Whatman Co.). Before used discs for antimicrobial testing, they were sterilized by oven, then saturated by each of the six examined plants extract (Brander and Bugh, 1977). Extraction concentration were as follows:

One gram from plant extract was dissolved in 2 ml solvents (ethanol or petroleum ether). One ml from that 2 ml was then taken and, added to another tube contained one ml solvent. This sterile dilution continued, for six concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml.) Discs were saturated in all above concentrations, then left to dry at room temperature before antimicrobial testing was done. The antimicrobial test started by culturing tested organisms on nutrient agar surface. Prepared discs were then placed on surface of media to examine antimicrobial effects. The result were taken by measuring the diametre of inhibition zones around saturated discs after incubation period of 18 hours at 37 ˚C.

Ethanolic extract

Ethanolic extract of *A. nilotica, C. colocyntlis N. sativa*, and *T. foenum greacum.* were diluted to series of concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml.). Filter paper discs were soaked into the extracts with the above concentrations, and then left to dry out. The selective Gram negative, and Gram positive bacteria were cultured on nutrient agar media free of extract. The

discs were then placed on the surface of culture in a clockwise direction starting from low to high concentration.

2-4-3-2 Petroleum ether extract

Petroleum ether extract of *A. nilotica. C. colocyntlis, N. sativa,* and *T. foenum greacum* were diluted to a series of concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, and 15.625 mg/ml.). Dry filter paper which were saturated firstly by the suitable concentrations, placed on the surface of nutrient agar, which had already been cultured by the selected Gram positive and Gram negative organisms. The cultures were incubated for 18 hours

Result

The results of ethanolic extract of *A. nilotica* against test strains using disc method are shown in Table 4. Figures 1, 2 and 3. At the concentration of 500 mg/ml all tested Gram negative, and Gram positive bacteria were inhibited with various inhibition zones. But there was good result with *C. ovis* theinhibition zone at 500 mg/ml was 21 mm. and *S. typhi* which inhbition zone was 26 mm. at 500 mg/ ml. *N. gonorrhoea* was inhibited after 6 days post incubation at 38 ˚C and the inhibition zone at the concentration 500 mg/ml was 6 mm; 250 mg/ml was 8 mm and at 125 mg/ml was 11 mm. In case of *N. gonorrhoea* the inhibition zones were observed at 500 mg/ml, 250 mg/ml and 125 mg/ml figure 2 the inhibition zones increased when the concentration was decreased.

Minimum Inhibition Concentration method (M I C)

The effects of *A. nilotica* extract against tested strains using MIC method are shown in Table 5, Figure 4, 5, 6 and 7. *S. aureus, N. gonorrhoeae*. *P. aeruginosa*, and *B. cereus* were completely inhibited at the concentration 8.3 mg/ ml. The growth of *P. vulgaris* in this concentration was similar to that in the positive control. The growth of *E. coli* and *S. typhi* was inhibited at 8.3 mg/ ml, but the degree of inhibition of these organisms using *A. nilotica* extract was found less than that obtained by chlortetracycline, the negative growth control.

Table 4: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extract of *Acacia nilotica*

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ ml
Bacillus cereus	15	11	8	8	0	0
Corynebacteria ovis	21	17	17	16	14	12
Staphylococcus aureus	12	11	8	6	6	0
Escherichia coli	18	20	13	10	0	0
Klebsiella pneumoniae	11	9	7	0	0	0
Neisseria gonorrhoeae	6	8	11	0	0	0
Proteus vulgaris	8	0	0	0	0	0
Pseudomonas aeruginosa	10	6	6	6	6	6
Salmonella typhi	26	16	16	16	15	13

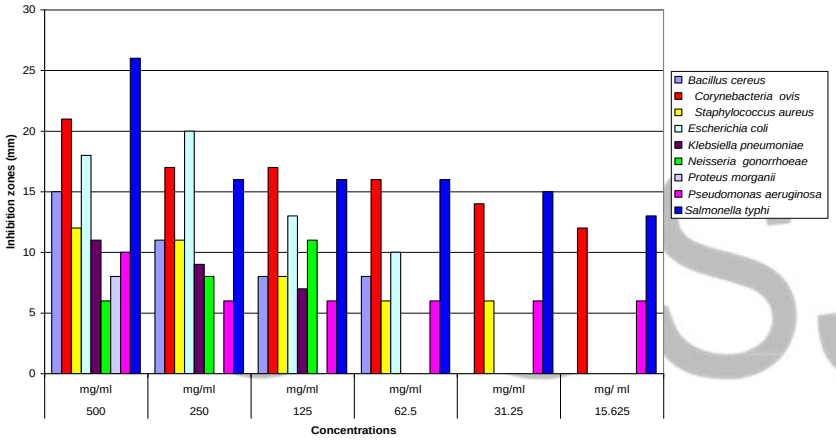


Figure 1: Inhibition zone (mm) of some gram positive and gram negative bacteria using ethanolic extract of *Acacia nilotica*

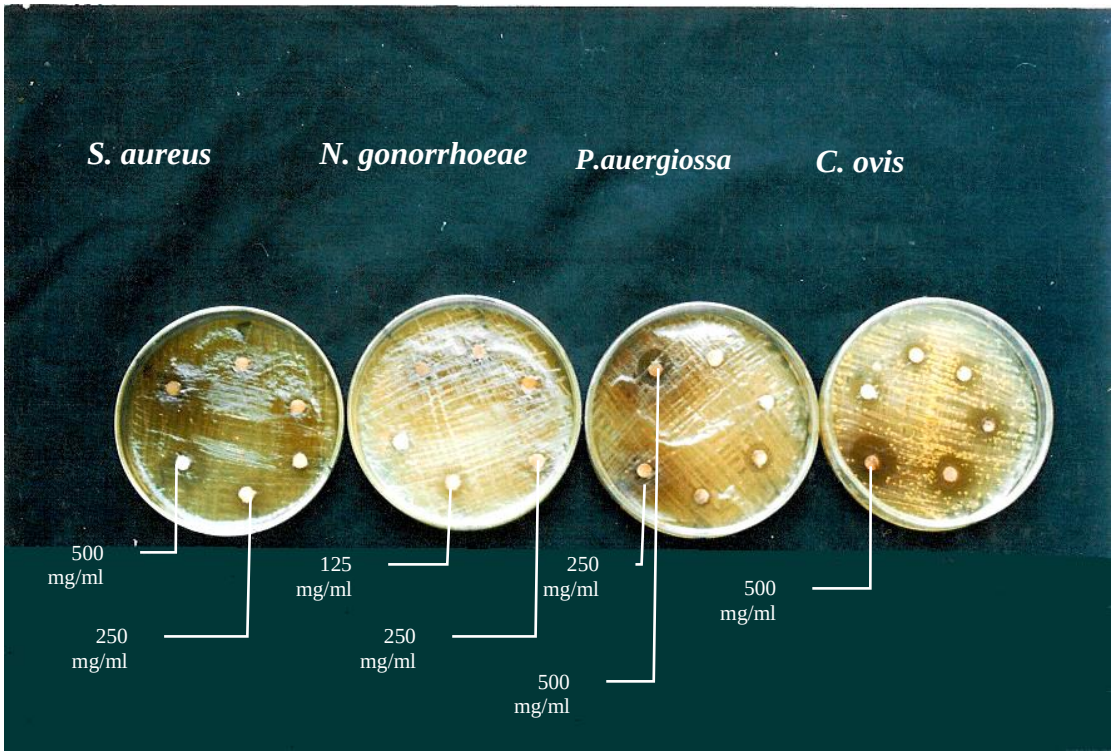


Figure 2: Sensitivity of some gram positive and gram negative bacteria to ethanolic extract of *Acacia nilotica*.

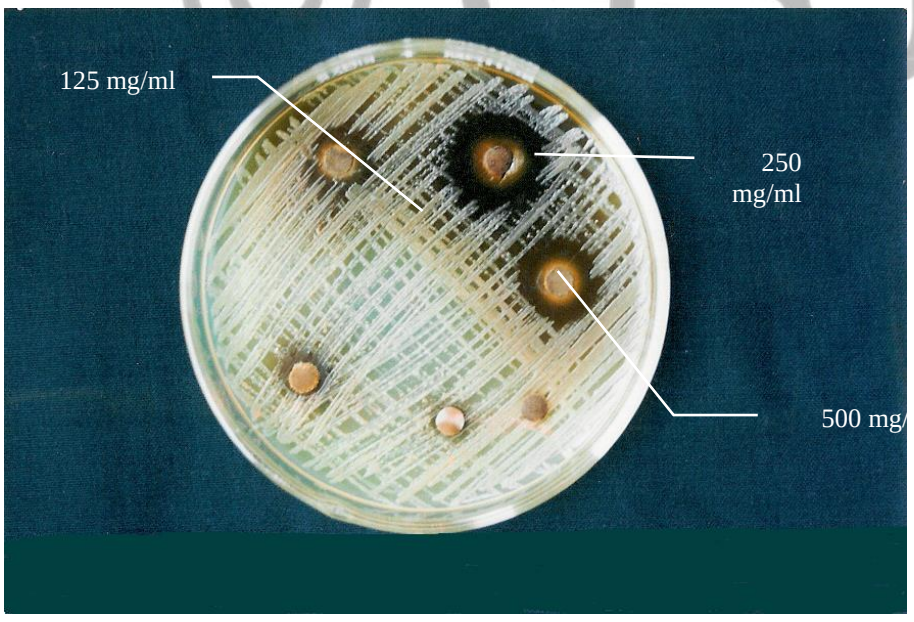


Figure 3: Sensitivity of *E. coli* to ethanolic extract of *Acacia nilotica*.

Table 5: Inhibition of growth of test organisms with ethanolic extract of *Acacia nilotica* using MIC method.

	A. nilotica (8.3 mg/ml)	*A. nilotica* (4.3 mg/ml)	*A. nilotica* (2.1 mg/ml)	Positive Control Chlortetracycline (26.7 mg/ml)	Negative control
Bacillus cereus	++++	+++	+++	++++	_
Corynebacteria ovis	++++	++++	+++	++	_
Staphylococcus aureus	++++	++	++	+++	_
Escherichia coli	++++	+++	++	++++	_
Klebsiella pneumoniae	++	++	++	++++	_
Neisseria gonorrhoeae	++++	++	++	+	_
Proteus vulgaris	++++	++	_ _	++	_
Pseudomonas aeruginosa	+++	+ + +	++	++	_
Salmonella typhi	++++	+++	+++	+++	_

++++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than

negative control; + Growth equal with negative control - Good growth; - - Growth more than negative control

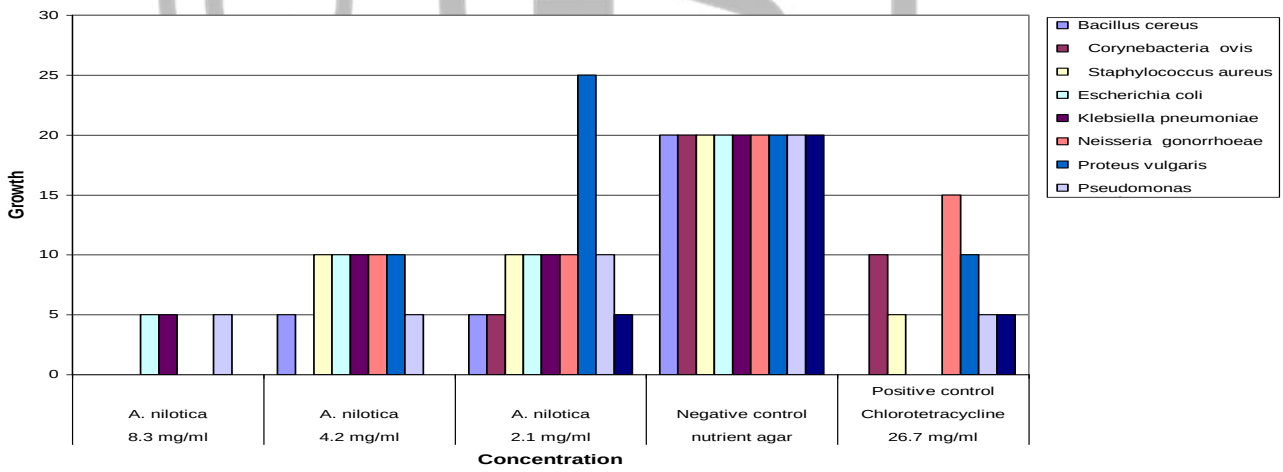
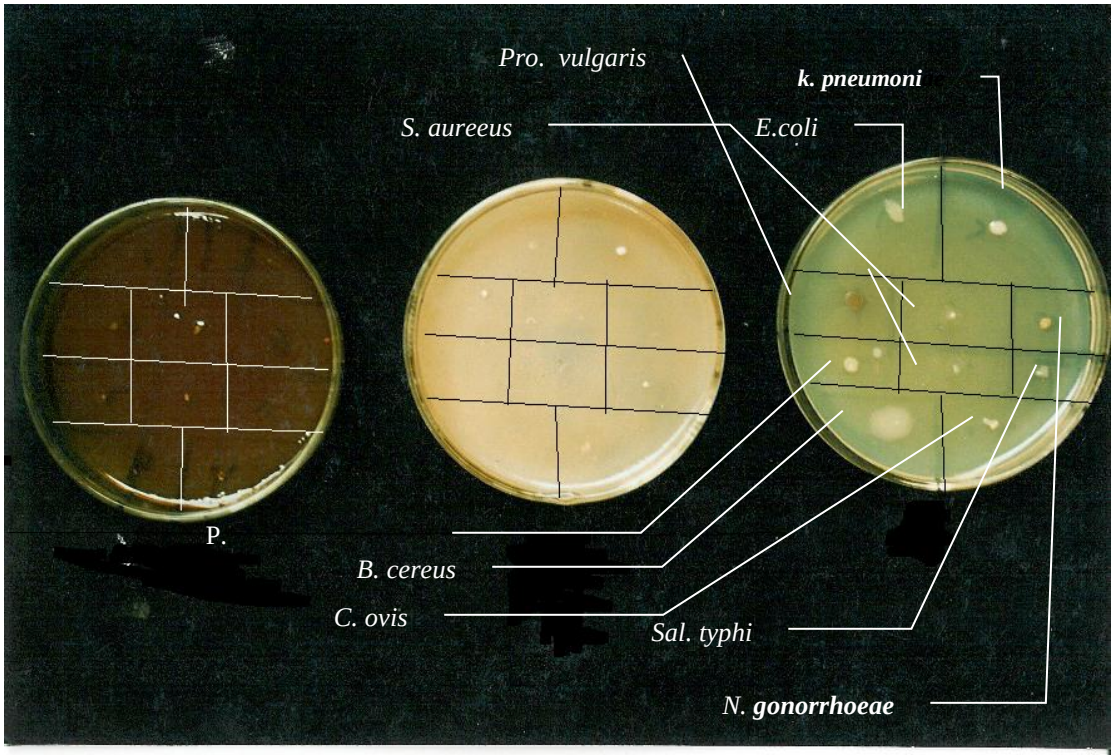


Figure 4: Inhibition of growth of some gram possitive and gram negative bacteria with ethanolic extract of *Acacia nilotia* using MIC method



Possative control *Acacia nilotica* Negative control

Figure 5: Inhibition of growth of test organisms at concentrations 8.3 mg/ml of the ethanolic extract of *Acacia nilotica* using MIC method.

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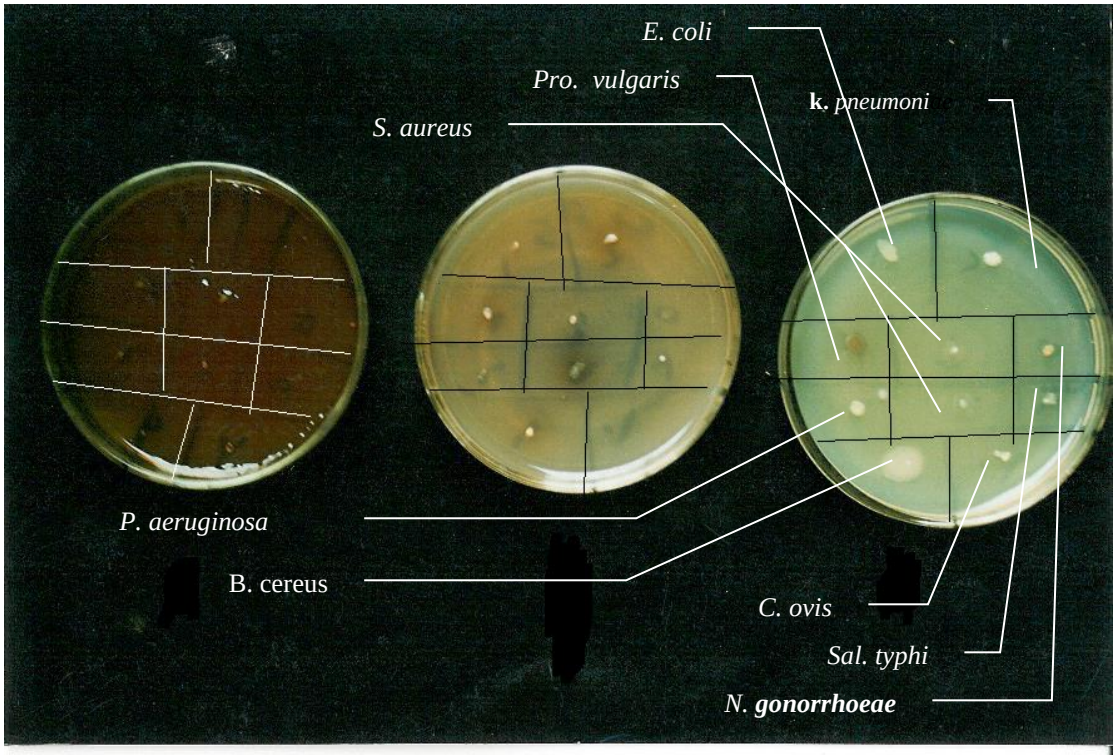


Figure 6: Inhibition of growth of test organisms at concentrations 4.3 mg/ml of the ethanolic extract of *A. nilotica* using MIC method

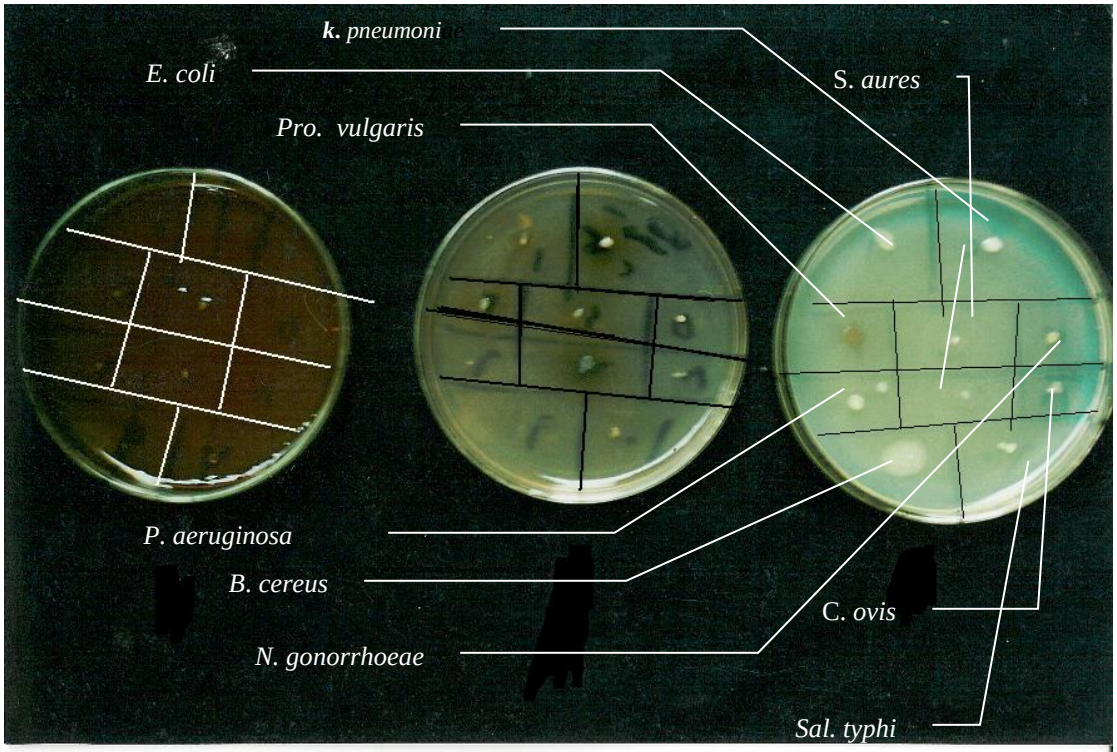


Figure 7: Inhibtion of growth of test organisms in concentrations 2.1 mg/ml of the ethanolic extract of *Acacia nilotica* using MIC method.

The result of ethanolic extract of *C. colocynthis* against tested organisms using MIC method are shown in Table 6 and Figure 8. *N. gonorrhoeae* and *S. aureus*, showed poor growth (+++) at concentration 66.7 mg /ml, while others test organisms were found resistant.

The results of ethanolic extract of *C. colocynthis* against test strain using disc method are shown in Table 7 and Figure 9. At the concentration 15.624 mg/ml, and 31.25 mg/ml *K. pneumoneia* was inhibited, the inhibition zones were 9 mm and 8 mm, respectively.

At 62.5 mg/ml, and 31.25 mg/ml *P. vulgaris* was inhibited. The inhibition zones were 10 mm and 7 mm, respectively. At 62.5 mg/ml, and 31.25 mg/ml *S. aureus* was inhibited, the inhibition zones were 9 mm and 8 mm, respectively. The inhibition zones were *N. gonorrhoeae* was inhibited the inhibition zones were 10 mm and 9 mm, respectively.

At the concentration 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml *P. aeruginosa* was inhibited the inhibition zones were 6 mm, 6 mm, 7 mm, and 9 mm, respectively. At the concentration 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 g/ml *C. ovis* was inhibited The inhibition zones were 11 mm, 8 mm, 8 mm, and 6 mm, respectively.

The results of ethanolic extract of *N. sativa* against tested strain using MIC method are shown in Table 8, Figure 10 and 11. At 66.7 mg/ml *N. gonorrhoeae* was inhibited (++++). *E. coli* was inhibited (++++) at concentration 66.7 mg/ml. All other test organisms were resistant.

The results of ethanolic extract of *N. sativa* against test organisms using disc method are shown in Table 9, Figures 12 and 13. At 500 mg/ml, *S. aureus* was inhibited the inhibition zone was 16 mm, at 250 mg/ml inhibition zone was 10 mm; at 125 mg/ml the inhibition zone was 9 mm, and at 31250 µg/ml the inhibition zone was 8 mm. *N. gonorrhoeae* was inhibited at 250 mg/ml and 125 mg/ml, inhibition zone was 8 mm at the two concentrations. At all other concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 g/ml, 31.25 mg/ml, and 15.624 mg/ml) other test organisms were resistant.

The results of ethanolic extract of *T. foenum greacum* against test organisms using MIC method are shown in Table 10 figure 14 and 15. *S. aureus* and *N. gonorrhoeae* had low growth when they were compared with possitive and negative control.

The results of ethanol extract for *T. foenum greacum* against tested strain using disc method are shown in Table 12. Figure 16. All test organisms were resistant, except *C. ovis* which was inhibited at 500 mg/ml and the inhibition zone was 10 mm. The inhibition zones which are shown at 62.5 mg/ml and 31.25 mg/ml were very narrw. So it was not considered as a positive result.

Table 6: Inhibition of growths of test organisms with ethanolic extract of *Citrullus colocynthis* using MIC method.

	C. colocynthis 66.7 mg/ml	*C. colocynthis* 33.3 mg/ml	Positive Control Chlortetracycline (26.7 mg/ml)	Negative control
Bacillus cereus	++	_	++++	_
Corynebacteria ovis	_ _	_ _	++	_
Staphylococcus aureus	+++	++	+++	_
Escherichia coli	_ _	_ _	++++	_
Klebsiella pneumoniae	_ _	_ _	++++	_
Neisseria gonorrhoeae	+++	++	+	_
Proteus vulgaris	_	_ _	++	_
Pseudomonas aeruginosa	++	++	+++	_
Salmonella typhi	++	++	+++	_

++++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than negative control; + Growth equal with negative control

- Good growth; - - Growth more than negative control

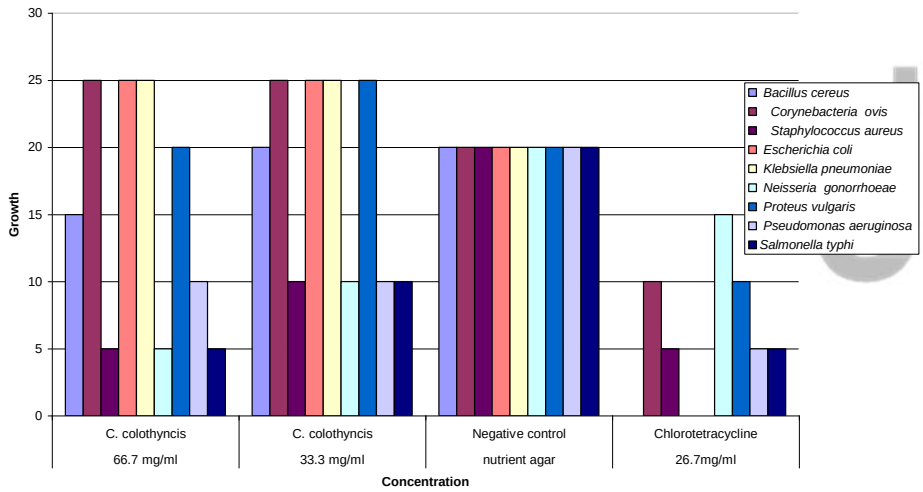
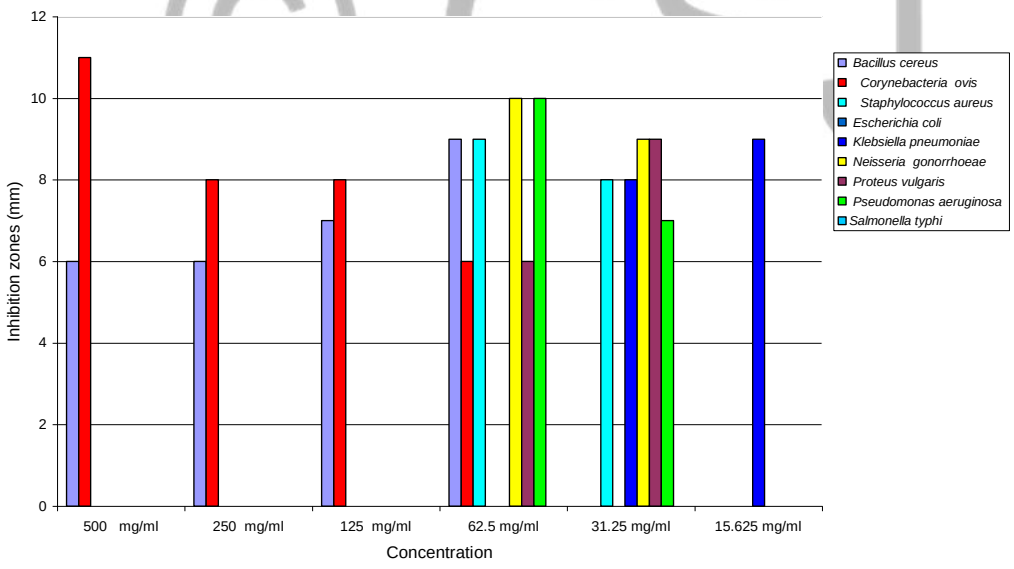


Figure 8: Inhibition of growth of test organisms with ethanolic extract of *Citrullus colothyncis* using MIC method

Tabel 7: Inhibition Zones (mm) of some Gram spositive and Gram negative bacteria produced by ethanolic extract *Citrullus colocynthis*

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
Bacillus cereus	6	6	7	9	0	0
Ccorynebacteria ovis	11	8	8	6	0	0
Staphylococcus aureus	0	0	0	9	8	0
Escherichia coli	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	0	8	9
Neisseria gonorrhoeae	0	0	0	10	9	0
Proteus vulgaris	0	0	0	6	9	0
Pseudomonas aeruginosa	0	0	0	10	7	0
Salmonella typhi	0	0	0	0	0	0



Figuer 9: Inhibition zones (mm) of some gram positive and gram negative bacteria produced by ethanolic extract of Citrullus colothyncis

Table 8: Growths of test organisms at three different concentrations of of *Nigella sativa* extracts and MIC method.

	N. sativa 10.00µg/ml	*N. sativa* 100µg/ml	*N. sativa* 10µg/ml	Positive Control Chlortetracycline (26.7 mg/ml)	Negative control

Bacillus cereus	+	+	_	++++	_
Corynebacteria ovis	_ _	_ _	_ _	++	_
Staphylococcus aureus	+	_	_ _	+++	_
Escherichia coli	++++	+ +	_	++++	_
Klebsiella pneumoniae	_ _	_ _	_ _	++++	_
Neisseria gonorrhoeae	++++	++	++	+	_
Proteus vulgaris	_	_	_ _	++	_
Pseudomonas aeruginosa	_ _	_ _	_ _	+++	_
Salmonella typhi	_ _	_	_ _	+++	_

++++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than

negative control; + Growth equal with negative control

- Good growth; - - Growth more than negative control

Figure 11: Growth of tested organisms in concentration 10.00µg/ml of the mixture of *Nigella sativa* extracts and nutrient agar MIC method.

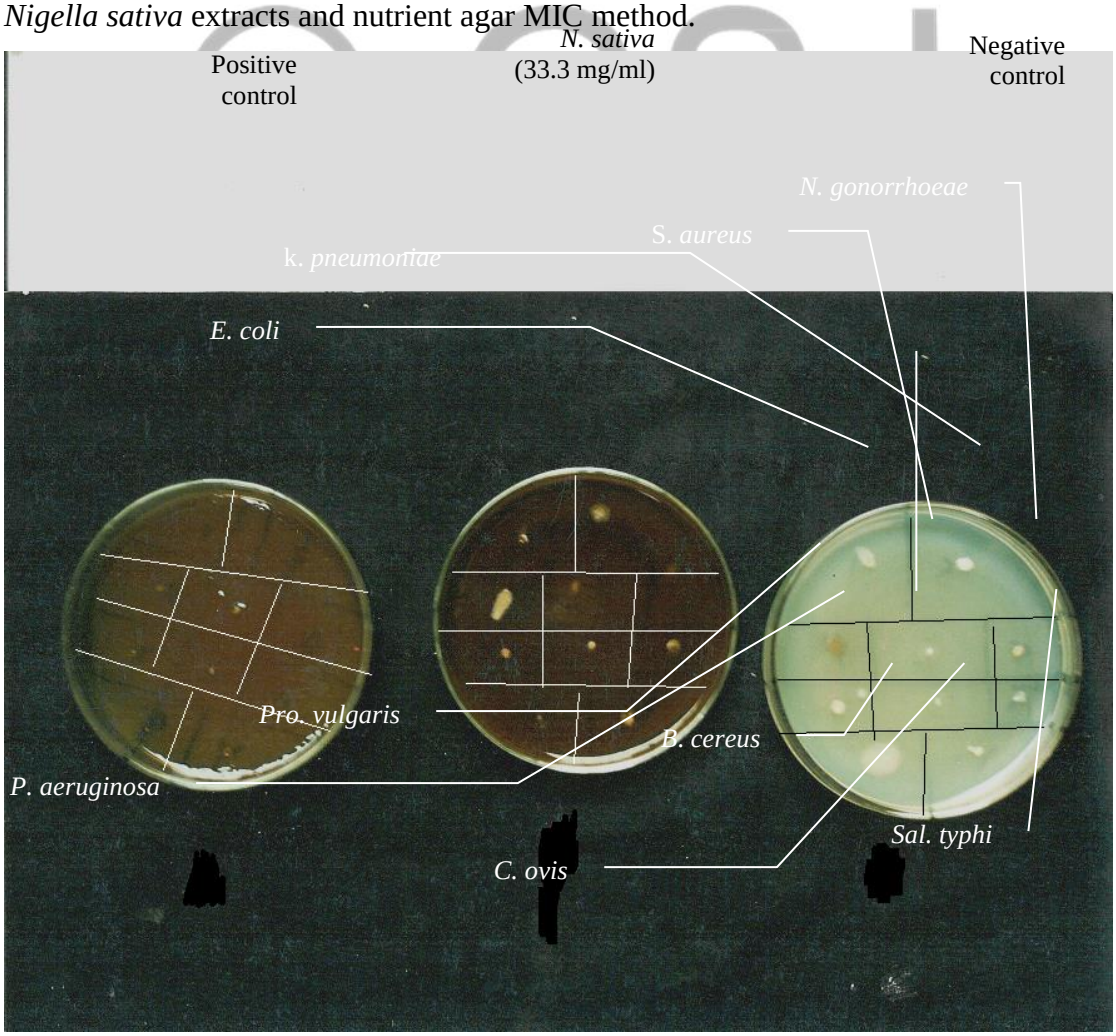


Table 9: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extracts of *Nigella sativa*.

	500 mg/ ml	250 mg/ ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg /ml
Bacillus cereus	0	0	0	0	0	0
Corynebacteria ovis	0	0	0	0	0	0
Staphylococcus aureus	16	10	9	8	8	0
Escherichia coli	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	0	0	0
Neisseria gonorrhoeae	10	8	8	0	0	0
Proteus vulgaris	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0
Salmonella typhi	0	0	0	0	0	0

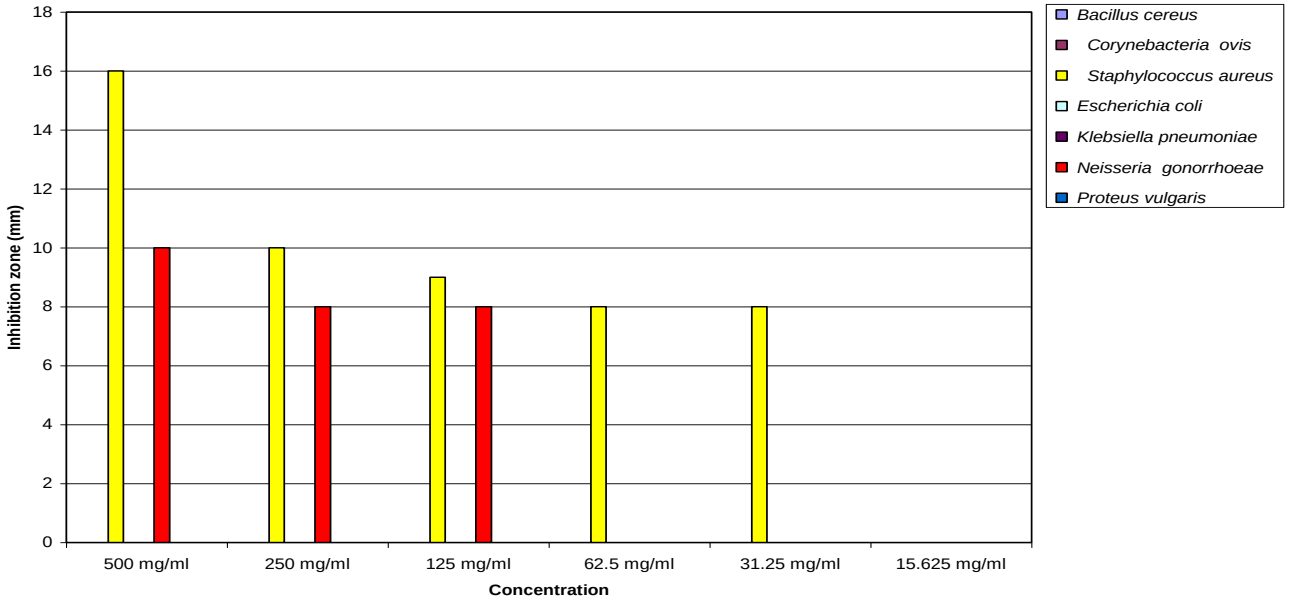


Figure 12: Inhibition zones (mm) of some gram positive and gram negative bacteriaproduced by ethanolic extract of *Nigella sativa*.

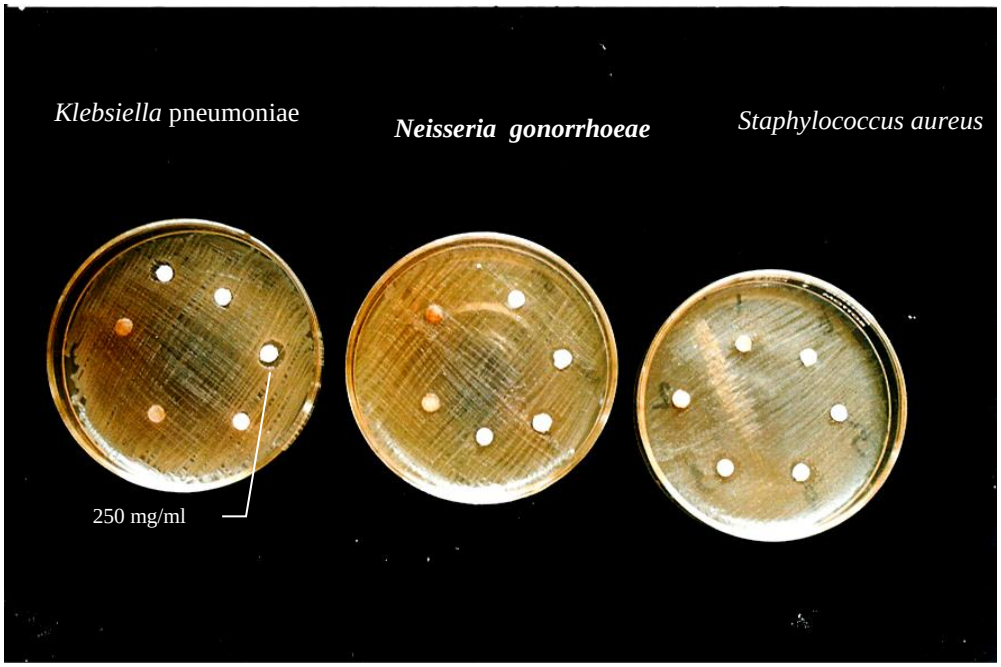


Figure 13: Inhibition zone (mm) at different concentrations of ethanolic extract of *Nigella sativa.*

Table 10: Growth of test organisms at four different concentrations of *Trigonella foenum greacum* extracts MIC method.

	T. foenum (66.7) mg/ml	*T.foenum* (33.3) mg/ml	*T.foenum* (16.7) mg/ml	*T .foenum* (8.3) mg/ml	Positive Control Chlortetracycline (26.7) mg/ml	Negative control
Bacillus cereus	++	++	+	– –	++++	–
Corynebacteria ovis	++	– –	– –	– –	++	–
Staphylococcus aureus	+++	++	– –	– –	+++	–
Escherichia coli	++	– –	– –	– –	++++	–
Klebsiella pneumoniae	– –	– –	– –	– –	++++	–
Neisseria gonorrhoeae	++	++	– –	– –	+	–
Proteus vulgaris	– –	–	– –	– –	++	–
Pseudomonas aeruginosa	+++	++	– –	– –	+++	–
Salmonella typhi	++	+++	– –	– –	+++	–

++++ No growth; +++ Poor growth; ++ Growth was more than positive control and little than negative control; + Growth equal with negative control

- Good growth; - - Growth more than negative control

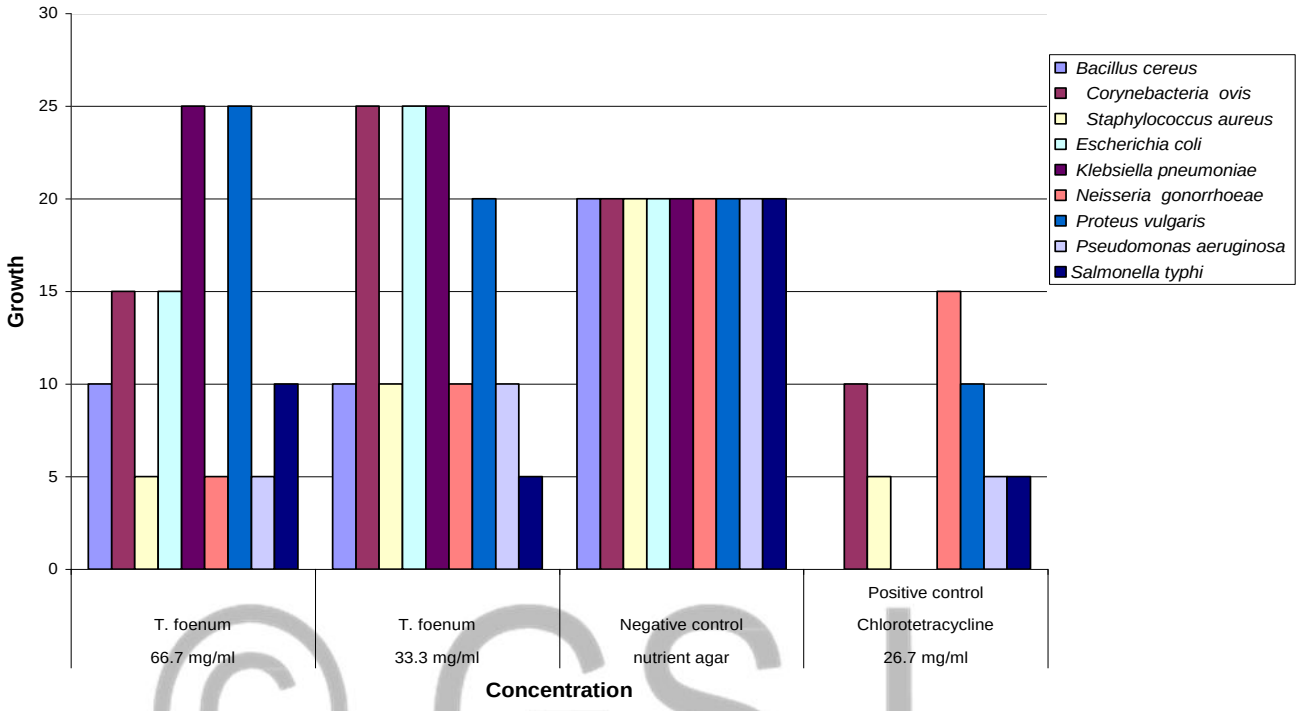


Figure 14: Inhibition of growth of test organisms with ethanolic extract of Trigonella foenum using MIC method

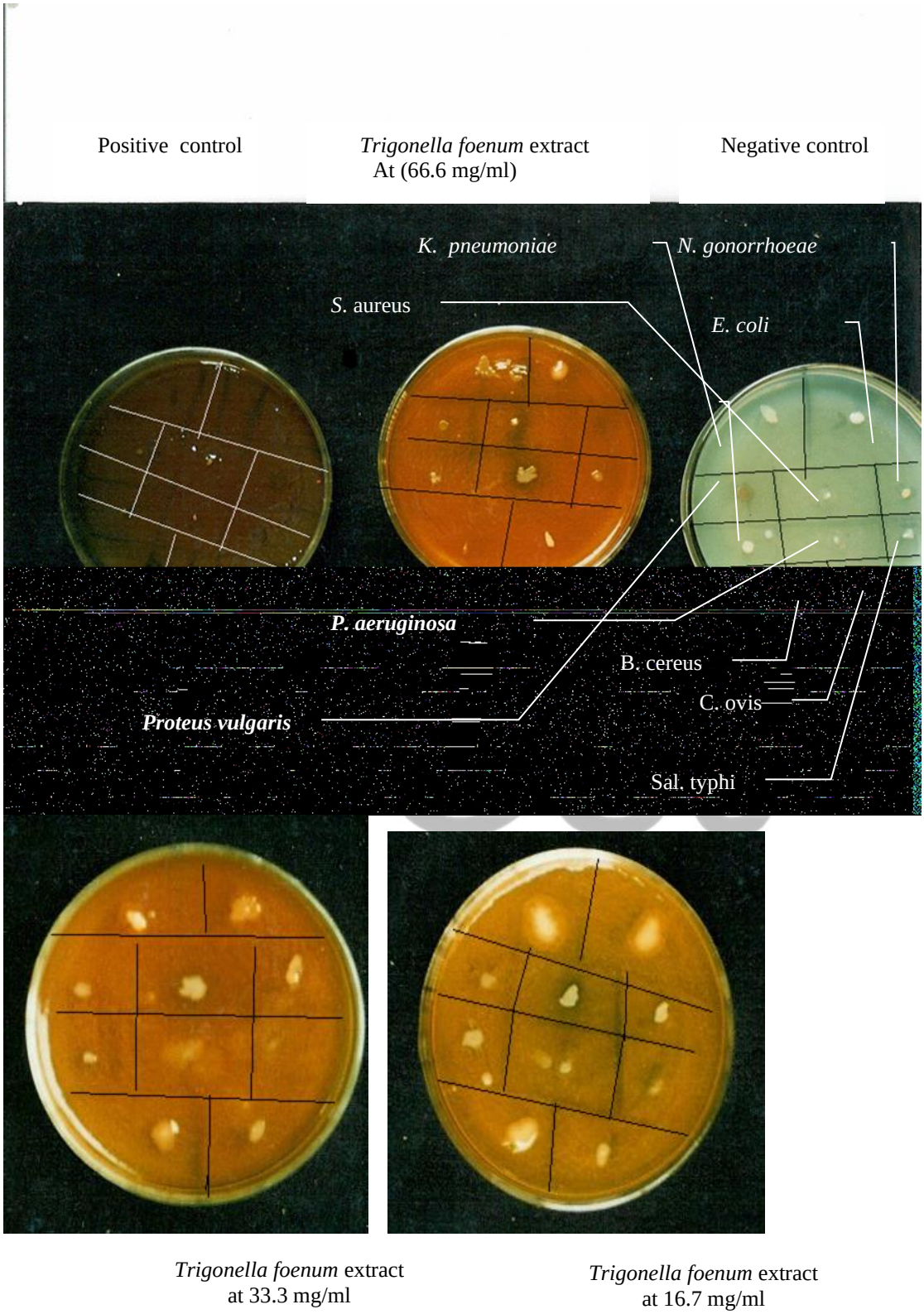


Figure 15: Growth of test organisms at four different concentrations of *Trigonella foenum greacum* extracts MIC method.

Table 11: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by ethanolic extracts of *Trigonella foenum greacum*.

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg/ml
Bacillus cereus	0	0	0	0	0	0
Corynebacteria ovis	10	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	7	9	0	0
Neisseria gonorrhoeae	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0
Salmonella typhi	0	0	0	0	0	0

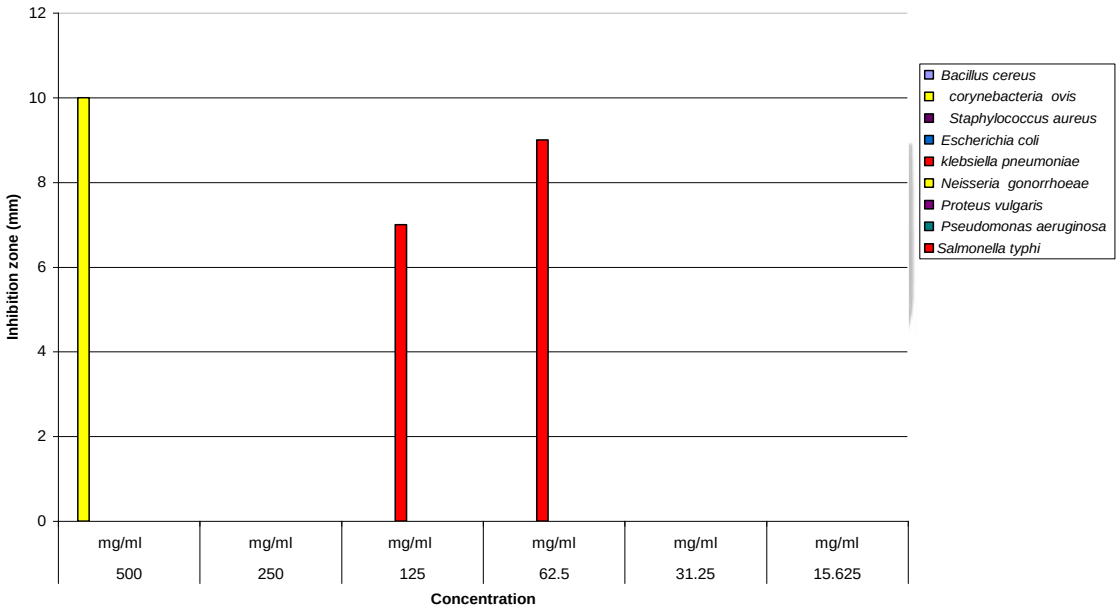


Figure 16: Inhibition zones (mm) of test organisms produced ethanolic extract of *Trigonella foenum*

3-2 Petroleum ether extracts.

MIC method could not be applied with petroleum ether extracts, because these extracts are oily, and in MIC method water was used as a solvent of plants extract, so the resulted mixture of extracts, with media was non homogeneous.

3-2-1 Disc method

3-2-1-1 *Acacia nilotica*

The results of petroleum ether extract of *A. nilotica* against test organisms using the disc method are shown in Table 12 and Figure 17.The results using filter paper discs saturated with petroleum ether extract against all selected organisms showed resistant patterns (*E. coli, K. pneumonia, N. gonorrhoeae*, *P. vulgars*, *P. areuginossa, B. cerus* and *C. ovis,*). Except *S. tiphy* and *S. aureus* which were found sensative. *S. tiphy* was inhibited at 500 mg/ml, 250 mg/ml, inhibtion zone were 9 mm. and 10 mm, respectively. At other concentrations *S. tiphy* was inhibited with equal inhibition zones. at 250 mg/ ml *S. aureus* was inhibited, the inhibition zone was 11 mm.

3-2-1-2 *Citrullus colocynthis*

All tested organisms *(E. coli, K. pneumonia, N. gonorrhoeae*, *P. vulgaris*, *P. aeruginosa S. tiphy*, *B. cerus, C. ovis,* and *S. aureus*) were resistant at all concentration petrolium ether extract of *C. colocynthis* (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml, and 15.624 mg/ml). Table 13.

3-2-1-3 *Nigella sativa*

N. gonorrhoeae was the most affected organisms it was inhibited at 500 mg/ml, 250 mg/ml, 125 mg/ml, and 625 mg/ml. The inhibition zones were 13 mm, 10 mm, 10 mm, and 8 mm. *K. pneumonia* was found sensitive and showed inhibition zones at 62.5 mg/ml, 31250 µg/ml. The inhibition zones were 10 mm and 7 mm. respectively. *P. areuginossa* was inhibited at 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml. The inhibition zones were 11 mm, 10 mm, and 9 mm, respectively. *S. aureus* was inhibited at 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml. the inhibition zones were 11 mm, 10 mm, and 8 mm, respectively. *E. coli*, *P. vulgaris*, *S. typhi*, *B. cerus,* and *C. ovis,* were resistant at all concentration of petrolium ether extract of *N. sativa* (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, 31.25 mg/ml, and 15.624 mg/ml). Table 14. Figure 19.

3-2-1-4 *Trigonella foenum greacum*

E. coli, K. pneumonia, N. gonorrhoeae, *P. vulgaris*, *P. areuginossa S. typhi*, *C. ovis,* and *S. aureus* were resistant at all concentration of petrolium ether extract of *T. foenum greacum* (500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, 31.25

mg/ml,and 15.624 mg/ml). Excep *B. cerus* was inhibited 500 mg/ml the inhibition zone was 10 mm. Table 15

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg ml
Bacillus cereus	0	0	0	0	0	0
Corynebacteria ovis	0	0	0	0	0	0
Staphylococcus aureus	0	11	0	0	0	0
Escherichia coli	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	0	0	0
Neisseria gonorrhoeae	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0
Salmonella typhi	9	10	7	7	7	7

Table 12: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Acacia nilotica*

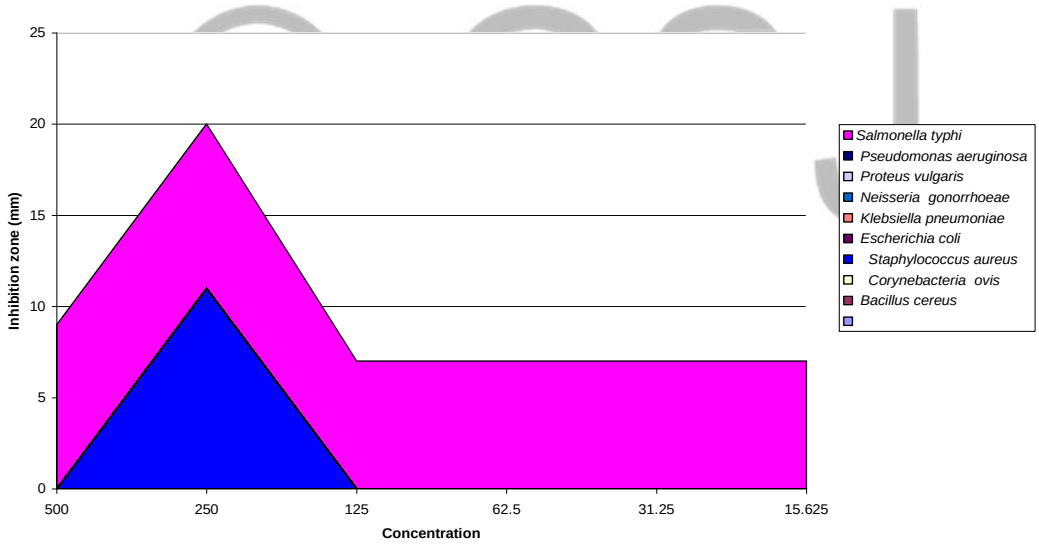


Figure 17: Inhibition zone (mm) of test organisms produced by petrolium ether extract of *Acacia nilotica*

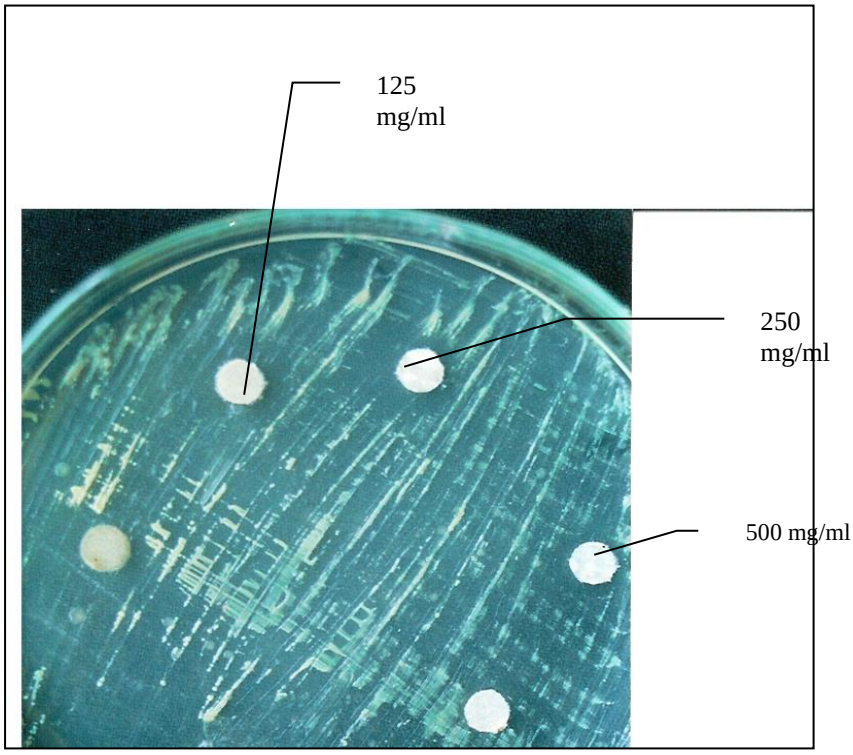


Figure 18: Inhibition zones (mm) of *Salmonella typhi* produced by petroleum ether extracts of *Acacia nilotica*.

Table 13: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Citrullus colocynthis*

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg ml
Bacillus cereus	0	0	0	0	0	0
Corynebacteria ovis	0	0	0	0	0	0
Staphylococcus aureus	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	0	0	0
Neisseria gonorrhoeae	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0
P. aeruginosa	0	0	0	0	0	0
Salmonella typhi	0	0	0	0	0	0

Table 14: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Nigella sativa*.

	500	250	125	62.5	31.25	15.625

	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg ml
Bacillus cereus	0	0	0	0	0	0
Corynebacteria ovis	0	0	0	0	0	0
Staphylococcus aureus	0	0	11	10	8	0
Escherichia coli	0	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	10	7	0
Neisseria gonorrhoeae	13	10	10	8	0	0
Proteus vulgaris	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	11	10	9	0
Salmonella typhi	0	0	0	0	0	0

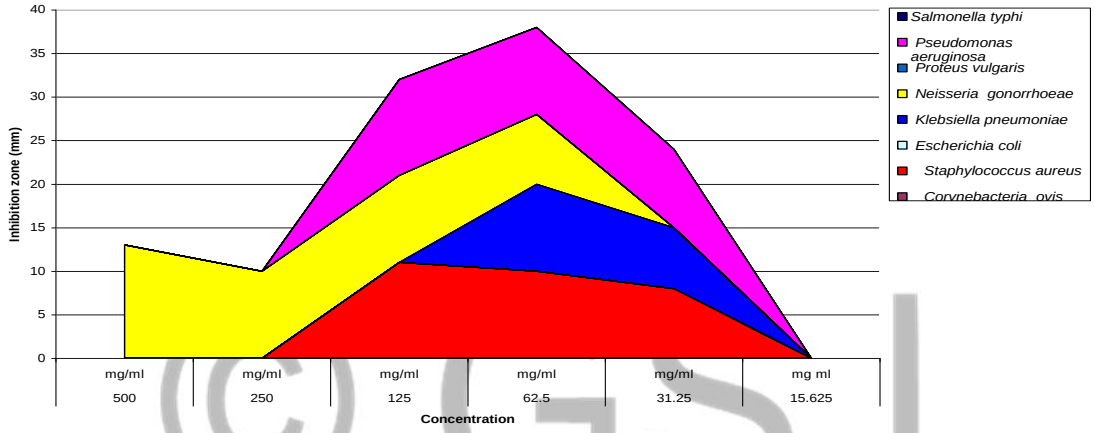


Figure 19: Inhibition zones (mm) of some gram positive and gram negative bacteria produced by petrolum ether extract of *Nigella sativa*

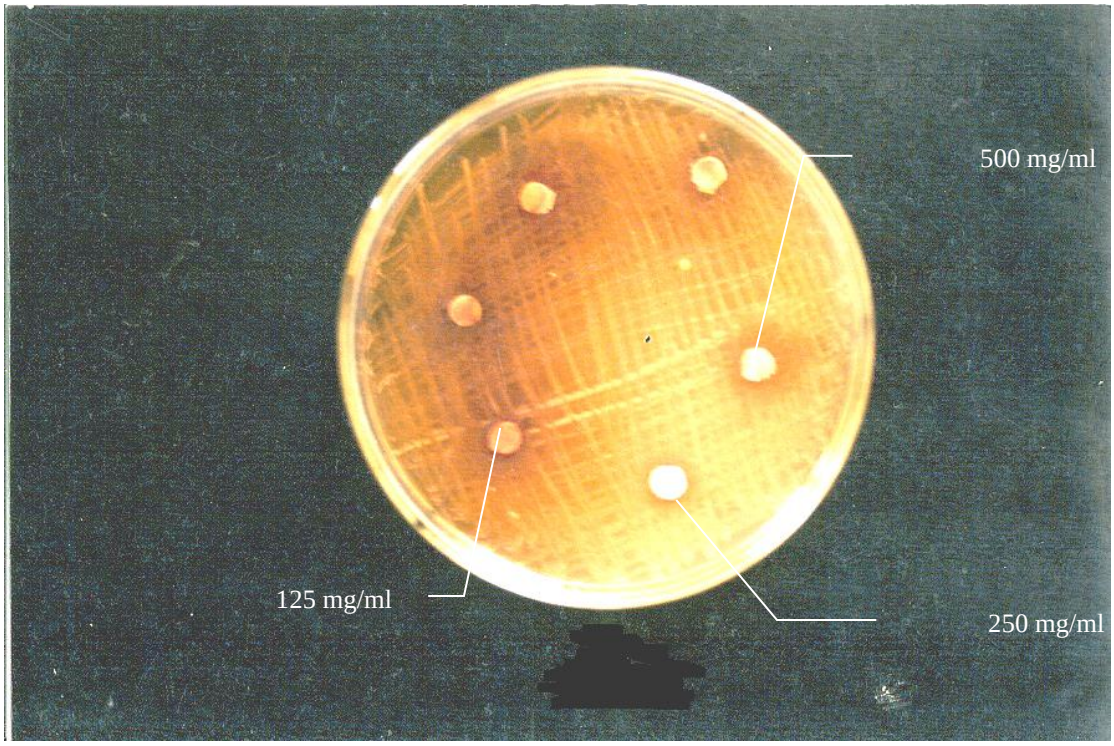


Figure 20: Inhibition zones of *Neisseria gonorrhoeae* produced by petrolum ether extract of *Nigella sativa*.

Table 15: Inhibition Zones (mm) of some Gram positive and Gram negative bacteria produced by petroleum ether extracts of *Trigonella foenum greacum.*

	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	15.625 mg ml
Bacillus cereus	10	0	0	0	0	0
Corynebacteria ovis	0	0	0	0	0	0
S.aureus	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0
K. pneumoniae	0	0	0	0	0	0
N.gonorrhoeae	0	0	0	0	0	0
Proteus vulgaris	0	0	0	0	0	0
P. aeruginosa	0	0	0	0	0	0
Salmonella typhi	0	0	0	0	0	0

Discussion

There has been a lot of talk about plants having medicinal value. Some plants have been used for centuries as a treatment of infections and other illness. Some Sudanese plants are commonly used for treatment of bacterial diseases, this is why it was important to investigate their antimicrobial activities or refute these claims. In the present study four plants namely *Acacia nilotica* fruit (Garad) *Citrullus colocynthis* seeds (Hanal) *Nigella sativa* seeds (Kamoun) *Trigonella foenum greacum* seeds (Helba) which are believed amongst Sudanese herbal therapists as antimicrobial agents, were examined. Tests were made to find their possible *in vitro* effects by observing the inhibition of growth of nine selected gram positive and gram negative bacteria.

Two solvents were used to extract the four plants, ethanol used to extract polar compounds, and petroleum ether (40- 60%) used to extract the fatty compounds of these plants. The plant extracts were tested using two methods, the Minimum Inhibition Concentration (MIC) method, and filter paper discs method. This latter method was found to be more reliable than the former. Because the results of the extract efficacy is very easy to read, the result had been taken by measuring the inhibition zone by transparent ruler in millimeter.

The presnt study revealed some successful result. Which was ethanolic extracts of *A. nilotica* against *S. typhi, C. ovis, E. coli*, and *B. cereus*. Although, experimentss in the present study have shown that the disc method of the ethanolic extract of *A. nilotica* inhibited all tested organisms with various inhibition zones. So the ethanolic extract of *A. nilotica* was the most effective as antimicrobial agent among the four plants tested.

But ethanolic extract of *A. nilotica* has poor effect against *P. aeruginosa* and *N. gonorrhoea* which was inhibited after 6 days post incubation at 38 ˚C at 125 mg/ml and 250 mg/ml. The petroleum ether extract of *A. nilotica,* also has antimicrobial effect but it was less than that shown in ethanolic extract of the same plant. *S. aureus*, and *S. typhi* were mostly affected by petrolium ether extract of *A. nilotica.* The minimum inhibition concentration of them was 250 mg/ml, and 15.625 mg/ml, respectively. this means that the ethanolic extract of *A. nilotica* was more effective than petroleum ether extract of the same plant as antimicrobial fraction. Raji (2002) found that the ethanolic extract of *A. nilotica* had minimal inhibition concentration

(MIC) of 80 mg/ml, while water extract of the same plant gave MIC of 250mg/ml Ethanol extract of *A. nilotica* at concentration of 200 mg/ml and 20 mg/ml. had inhibitory diameters zone of 6 mm and 4 mm, respectively when were used against *E. coli* and *C. laridis* (Raji *et al*., 2002).

Plants commonly used in Sudanese traditional medicine had been screened for their inhibitory effects on hepatitis C virus (HIV) and protease (PR) using *inviter* as methods. Of these, methanol extracts of *A. nilotica* was one of the most active extracts (Hussein, *et al*., 2000). In China they found that aqueous extract of *A. nilotica*, had an inhibitory effect on carrageen an induced paw edema and yeast- induced pyrexia in rats (Dafallah and Al-Mustafa, 1996). The potential toxicity of *Acacia nilotica* was investigated in rats maintained 2 % and 8 % *Acacia nilotica* diet for 2 and 4 weeks. It is concluded that *Acacia nilotica*, at 2 % and 8 % levels, has low toxicity potential (Al-Mustafa and Dafallah, 2000).

The ethanolic extract of *C. colocynthis* was very mild as antimicrobial agent, the affected organisms were *B. cereus*, which was inhibited at 500 mg/ml and 250 mg/ml. The inhibition zones in the two concentrations were 6 mm, and at 125 mg/ml and at 62.5 mg/ml the inhibition zones were 7 mm, and 9 mm, respectively. That means the inhibition of ethanolic extract of *C. colocynthis* against *B. cereus* was increased when the concentration of the extract was decreased.

The petroleum ether extract of *C. colocynthis* did not show any inhibition effect. So, from this study I found that ethanolic extract of *C. colocynthis* have a mild effect as antimicrobial agent. Because inhibition zones which was shown in case of *B. cereus* were very narrw to consider ethanolic extract of *C. colocynthis* as antimicrobial agent. This result is there for considered not good result and not encouraging for further future investigation.

Petroleum ether extract and the ethanolic extract of *N. sativa* were effective as antimicrobial agent. *S. aureus* and *N. gonorrhoea* were inhibited. *S. aureus* was inhibited, the minimmum inhibition concentration was 31.25 mg/ml. *N. gonorrhoea* inhibted the minimmum inhibition concentration was 62.5 mg/ml. Ethanolic extract of *N. sativa* was inhibited *N. gonorrhoea* and *S. aureus.* In case of *S. aureus* the

inhibition zone was 16 mm. at 500 mg/ ml, the minimmum inhibition concentrationwas 31.25 mg/ml.

In case of *N. gonorrhoea* the Inhibition zones was 8 mm. at two concentrations 250 mg/ml and 125 mg/ml and 10 mm . at 500 mg/ ml. In this study the ethanolic extract of *N. sativa* was found less effective as antimicrobial agent when it compared with petroleum ether extract against this organisms.

Beside *N. gonorrhoea* and *S. aureus* the petroleum ether extract of *N. sativa* inhibited *K. pneumonia* and *P. aeruginosa* the minimmum inhibition concentration of them was 31.25 mg/ml (Table 14).

All above results show that *N. sativa* has good antbacterial effect, and *N. sativa* has antibacterial effect in two polar compound (Table 9) and the fatty one (Table 14). This result is in agreement with Fadadalla (2002). who found that, *N. sativa* is strong antimicrobial agent, and he also found methanolic extrac of *N. sativa* was less effective than the petrolum ether extract. Alcoholic seeds extract of *N. sativa* showed antibacterial activity against *Micrococcus pyogenes* var. *aureus* and *E. coli* (Muhammad *et al*., 2002). *N. sativa* have regulation effects on blood pressure and on blood sugar levels (Gamal *et al*., 1998).

Topical application of *N. sativa* and *Corocus sativa* extracts, inhibited skin carcinogenesis in mice (Salomi *et al*., 1991). The plant also has immunomodulatory and interferon like active (Medicina *et al*., 1997).

The ethanolic extract and the petroleum ether extracts of *T. foenum greacum* were found very poor antimicrobial agent. they inhibited only one organism in the ethanolic extract of the plant. This organism was *K. pneumonia* it was inhibited at 125 mg/ml, and 62.5 mg/ml and the inhibition zones were 7 mm and 9 mm, respectively. These were relatively very narrow inhibition zones.

The petroleum ether extract inhibited *B. cereus* at 500 mg/ml, the inhibition zone was 10 mm. This result was considered not good and is not encouraging for further investigation. has shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect (Chevallier, 1996). The seed and leaves are anticholesterolemic, anti-inflammatory, antitumor, carminative, demulcent, deobstruent, emollient, expectorant, febrifuge, galactogogue, hypoglycaemic, laxative, parasiticide, restorative and uterine tonic(Bown, 1995). The seed yields a strong mucilage and is therefore useful in the treatment of inflammation

and ulcers of the stomach and intestines (Chevallier, 1996). Taken internally, a decoction of the ground seeds serves to drain off the sweat ducts (Chiej 1984). The seed is very nourishing and body-building and is one of the most efficacious tonics in cases of physical debility caused by anaemia or by infectious diseases, especially where a nervous factor is involved (Phillips and Foy, 1990). It is also used in the treatment of late-onset diabetes, poor digestion (especially in convalescence), insufficient lactation, painful menstruation, labour pains etc (Bown, 1995). The seeds freshen bad breath and restore a dulled sense of taste(Chevallier, 1996). Externally, the seeds can be ground into a powder and used as a poultice for abscesses, boils, ulcers, burns etc, or they can be used as a douche for excessive vaginal discharge (Phillips and Foy, 1990). The leaves are harvested in the growing season and can be used fresh or dried. The seeds are harvested when fully ripe and dried for later use (Bown, 1995). Compounds extracted from the plant have shown cardiotonic, hypoglycaemic, diuretic, antiphlogistic and hypotensive activity (Duke 1985). One of its constituent alkaloids, called 'trigonelline', has shown potential for use in cancer therapy. The seed contains the saponin diosgenin, an important substance in the synthesis of oral contraceptives and sex hormones (Phillips and Foy, 1990).

In this study the minimum inhibition concentration method (MIC) was found to be complicated, and the results were not consistent. Results in this method had been taken by comparison between growth of tested organisms in media containing plant extracts, with growth of the positive control, and negative control on the other side. In MIC method, the petroleum ether extract could not be applied, because the fatty compounds can not be homogenized in distil water.

In the (MIC) method *A. nilotica* fruit extract at the concentrations 66.7 mg/ml, 33.3 mg/ml; and 16.7 mg/ml; did not allow the media medum to be solidify, so antimicrobial activity of *A. nilotica* extract in these concentrations were not taken. Finnally, it is consider that *A. nilotica* fruit, and *N. sativa* seeds are strong antimicrobial agents, and the believe of the Sudanese herbalist on them are true. The disc method is a good method to screening antimicrobial activities of plant extracts.

References:

Al-Mustafa Z. H. and Dafallah A.A., 2000. A study in the toxicology of *Acacia nilotica*. *Am J. Chin Med*. 28: 123-129.

Balair, J. E., Lenett, E. H., Truant, J. P., eds. 1970. Manual of Clinical Microbiology. American Society for Microbiology, William and Wilkins Company.

Barrow, G. I., Feltham, R. K. A., eds. 1993. Cowan and Steelś Manual for the Identification of Medical Bacteria. 3rd edition, Cambridge University Press Cambridg.

Bebawi, F. F., Neugebohrn, L., eds. 1991 In: A Review of Plants of Northern Sudan with special Reference to their uses. Technical Cooperation-Federal Republic of Germany. Eschborn

Bown D., Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London. 1995 ISBN 0-7513-020-31

Brander, G. C., and Bugh, D. M., eds. 1977. Veterinary Applied Pharmacology and Therapetics, 3rd edition, Lea and Febiger, Philadelphia

Canada Communicable Disease Report. *Population and Public Health Branch (PPHB)*. Volume: 26S6 - October 2000. 1998/1999 Canadian Sexually Transmitted Diseases (STD) Surveillance Report

Chevallier A. The Encyclopegia of Medicinal Plants Dorling Kindersley. 1996 ISBN 9-780-3-3148 An excllent guide to over 500 of the more well known medicinal herbsfrom around the world

Centers for Disease Control and Prevention (CDC) National Center for Infectious Diseases Division of Bacterial and Mycotic Diseases, What are the most common foodborne diseases?, 2003

Christian, Janet and Greger, Janet, *Nutrition for Living*. Benjamin/Cummings Publishing Company, Fourth Edition, 1994., U.S. Food and Drug Administration,

Center for Food Safety and Applied Nutrition, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, *The Bad Bug Book*. *Clint Infect Dis 2002, 34:634-640. [PubMed Abstract] [Publisher Full Text*]

Clark, A. M. 1996 Natural products as a resource for new drugs. *Pharm. Res. 13: 1996.*

Cruickshank, J. P., Duguid, B. P., Marmion, R. H. A., Swain, eds. 1973 Medicinal Microbiology Volume one. Microbial infection 12th edition. Churchill livingstone, Edinburgh and London.

Dafallah, A. A., al-Mostafa, Z. 1996. Investigation of the anti-inflamotary activity of *Acacia nilotica* and *Hibiscus Sabdariffa.* In *Am J Chin Med* 24 pp.: 263-239.

David L. Paterson, MBBS, FRACP; Wen-Chien Ko, MD; Anne Von Gottberg, MBChB; Sunita Mohapatra, MD; Jose Maria Casellas, MBBS; Herman Goossens, MD; Lutfiye Mulazimoglu, MD; Gordon Trenholme, MD; Keith P. Klugman, MBChB; Robert A. Bonomo, MD; Louis B. Rice, MD; Marilyn M. Wagener, MPH; Joseph G. McCormack, MD, FRACP; and Victor L. Yu, MD; *Annals of Internal Medicine*; volume 140 Issue 1; Pages 26-32; 6 January 2004.

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Duke J. A. and Ayensu. E. S. Medicinal Plants of China Reference Publications, Inc. 1985 ISBN 0-917256-20-4

Eltohami, M. S., 2003 Medicinal and Aromatic Plants Research institute, htt: www. Fao. Orag/ docrep/X5402e/x5402e16.htm.

EMILIA ASKARI. 2002. Drug-resistant bacteria discovered in Detroit. Germs develop a deadly defense. FREE PRESS STAFF WRITER. http://www.freep.com/news/health/nstaph12_20021112.htm

Gamal, E. B., El-Gazali, Hassan, E. khalid, Mahagoob, S., El-Tohami, Wail, E. Abdalla and Salma, M. A. Yagi. 1998. Medicinal Plant in khartoum state. Medicinal a

Hussei G., Miyashiro H., Nakamura N., Hattori M., Kukiuchi N., Shimotohno K., Inhibitory effects of Sudanese medicinal plant extracts on hipatitis C. virus (HCV) protease. Phytother Res. 2000 Non; 14(7): 510-6.

Laila F. nimri, M. M. Meqdam and A. Alkofahi, 1999. *Pharmaceutical Biology.* vol. 37, No. 3, pp. 196-201. Antibacterial Activity for Jordanian Medicinal plants. (http: www.szp. swets.nl/szp/journals/pb373196.htm).

Lydia Bass, Cynthia A. Leebert, Margie D. Lee, Anne O. Summers, David G. White, StephanG. Thayer, and John J. Maurer. 1999. Incidence and Characterization of Intergrons, Genetic Elements Mediating Multiple- Drug Resistance, in Avian *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, Vol. 43, No. 12 p. 2925-2929.

Medicina R., Janssen, J., Tarasenco, A., Lazovi, G., Corbitt, W., Powell, D., Jocic, D., and Majovic, V. 1997. Anti-angiogenic activity of *Nigella sativa* plant extract in Cancer therapy. Proc Annu Meet Am Assoc Cancer Res;38: A 1377, 1997

Muhammad A. Randhawa, Mastoor S. Al-Ghamdi Department of Pharmacology, College of Medicine, King Faisal University, Dammam, Saudi Arabia A review of the pharmaco-therapeutic effects of *Nigella sativa* Pakistan J. Med. Res. Vol.41, No.2, 2002

Phillips R. & Foy N. Herbs Pan Books Ltd. London. 1990 ISBN 0330-30725-8

Raji, M. A., Adekeye, J. O., Kwaga, J. K. P., and Bale, J. O. O., Bello, A. *African Journal of Science & Technology* Volume 3 Number 2 December 2002.

Shetty, K. A. B. 1977. Social forestry in Tamil Nadu. Indian Farming 26-82.