



# Antibacterial Activity of Ethanol Extract of *Phoenix dactylifera* Leaves against some Gram Negative Bacterial Isolates

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## ABSTRACT

This research was conducted at Gombe State University, Gombe State, Nigeria from January to July, 2013. The research aimed at determining the antimicrobial activity of ethanol extract of *Phoenix dactylifera* leaves against four clinical isolates of Gram negative bacteria using Agar Disc diffusion technique. The Gram negative bacteria screened included; *Escherichia coli*, *Morganella morganii*, *Proteus mirabilis* and *Yersinia enterocolitica*. Four different concentrations tested included 20µg/disc, 40µg/disc, 80µg/disc and 160µg/disc which revealed a strong in vitro antibacterial activity against all the test organisms. Of these organisms tested, *E. coli* was found to be the most sensitive, followed by *P. mirabilis* and *Y. enterocolitica* producing the same zones of growth inhibition and the least sensitive organism being *M. morganii*. Minimum Inhibitory Concentration (MIC) was determined at 25µg/ml for all the test organisms while the Minimum Bactericidal Concentration (MBC) was probably above 100µg/ml at the highest concentration used because the organisms still managed to grow when sub-cultured on the solid media. This indicates that *Phoenix dactylifera* leaf extract is bacteriostatic against the test isolates used in this study.

### Keywords:

Antibacterial activity, *Phoenix dactylifera* leaf, Extracts, Clinical isolates

## INTRODUCTION

Date palm (*Phoenix dactylifera*) is a major fruit tree in most of Arabian Peninsula and it is considered one of the most important commercial crops. The fruits of date palm (Dates) are a vital element of the daily diet in the Arabian world. According to the international Bibliography, three (3) principal cultivar groups of *P. dactylifera* have been identified mainly according to sugar content of their fruit namely; soft type (e.g. "Barhee"), semi-dry type (e.g. "Dayri") and dry type (e.g. "thoory"). The beneficial health and nutrition values of date palm for human and animal consumption have been claimed for centuries (Duke, 1992; Vayali, 2002; Tahraoui et al., 2007).

Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the fact that these medicinal herbs are safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. Antimicrobial agents can also be derived from herbs, and over 1000 plants exhibit antimicrobial effects (Nychas, 1995).

According to World Health Organization, medicinal plants can be a good source of variety of drugs. Various societies across the world have shown great interest in curing diseases using plant/plant based drugs. Microorganisms are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As a preventive and curative measure, plants and their products have been used in the treatment of infections for centuries. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare (Alagesabooopathi, 2011).

As the global interest towards traditional medicines over the conventional treatment is increasing due to their safer action (in terms of tolerance and side effects) for acute and chronic diseases, this research aimed at determining the antibacterial activity of ethanol extract of *P. dactylifera* leaves against some clinical isolates of Gram negative bacteria obtained from Gombe State Specialist Hospital, Gombe State, Nigeria.

## MATERIALS AND METHODS

### Collection, identification and preparation of date palm leaves

Fresh samples of date palm leaves used in this research were collected at Gombe State University date farm and identified at the department of Biological Sciences, faculty of Science of the same University. The leaves were washed individually under running tap water to remove all the possible soil particles and dirt. The leaves were air-dried under shade for two weeks (14 days). The

dried leaves were ground into powder using clean laboratory Motor and pestle (Muktar and Tukur, 1999). The powder was kept in air tight plastic container at room temperature before extraction process.

### Extraction

The leaves extract of date palm was prepared using the standard percolation method of Alade and Irobi, (1993). Twenty grams (20g) of the powdered plant material was weighed and percolated with 200ml of 95% ethanol in Conical flask and allowed to stand for two weeks with regular shaking after which it was filtered using Whatman No. 1 filter paper and the filtrate obtained was reduced to dryness by removing solvent in air-dried oven at 40°C for further analysis.

### Disc preparation

Sensitivity discs of the extract were prepared by serial doubling dilution of the extract in Dimethyl sulfoxide (DMSO) followed by placing sterile Whatman No. 1 filter paper discs of 6mm diameter that take up 0.01ml to make the required disc potency. Disc potencies of 20, 40, 80 and 160µg/disc were prepared.

### Biochemical identification of the test bacterial isolates

The bacterial isolates used in this research were clinical isolates obtained from Gombe State Specialist Hospital, Gombe State. They were subjected to standard identification procedures described by Cheesebrough, (2000) and confirmed to be Gram negative isolates of *Escherichia coli*, *Morganella morganii*, *Proteus mirabilis* and *Yersinia enterocolitica* as follows:

### Triple Sugar Iron (TSI)

This test was conducted based on the ability of the Gram negative isolates to ferment lactose and glucose and the production of hydrogen sulphide and gas in the media (Cheesebrough, 2000).

### Citrate utilization test

This test was carried out by inoculating the Gram negative isolates into bijou bottles containing Simon's citrate agar. Colour change in the medium was also noted after incubation for 24hrs (Cheesebrough, 2000).

### Urase test

This test was performed by inoculating the test organism into a bijou bottle containing Urea agar. This was followed by incubation at 37°C for 24hrs and the colour change was noted (Cheesebrough, 2000).

### Indole test

The test for indole production was carried out by inoculating the Gram negative isolates into bijou bottles containing Peptone water. This test was achieved by the addition of 0.5ml of Kovac's reagent, shaken gently and noted the colour change within 10 minutes after 24hrs incubation at 37°C (Cheesebrough, 2000).

### Motility test

A small suspension of the clinical isolate grown in peptone water was placed on clean, grease free glass slide and then covered with a cover glass. The preparation was examined microscopically first with 10x and then 40x objectives (Cheesebrough, 2000). Motile isolates appeared in the form of motile rod shape.

### Standardization of the test bacterial isolate

The inocula of the test organisms were standardized by emulsifying a few colonies of the isolates in sterile distilled water to match 0.5 Mcfarland standard described by Cheesebrough, (2000). The Mcfarland standard was prepared by mixing 0.6ml of 1% (w/v) dehydrate barium chloride solution with 99.4ml of 1% (v/v) sulphuric acid solution.

### Determination of antibacterial activity of the plant extract

The crude extract was screened for antimicrobial activity against the test organisms using Disc diffusion method according to standard procedure of Kirby-Bauer described by Cheesebrough, 2000. In this method, sterile swab sticks were used to inoculate the standardized inocula of the test organisms onto the surface of prepared Mueller-Hinton agar plates. Incorporated discs with the plant extract were placed on the inoculated plates with Augmentin disc ( $\mu\text{g}$ ) at the centre serving as a control after which the inoculated plates were incubated at 37°C for an overnight. Zones of growth inhibition were measured following 24hours of incubation.

### Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration was determined by serial doubling dilution of the plant extract in sterile distilled water and achieved four different concentrations of 100 $\mu\text{g/ml}$ , 50 $\mu\text{g/ml}$ , 25 $\mu\text{g/ml}$  and 12.5 $\mu\text{g/ml}$ . 2ml of each concentration was pipette into test-tubes containing 2ml of nutrient broth. 0.1ml of standardized suspension of the test organisms was then introduced into these test-tubes. Two test-tubes labeled as positive and negative controls containing plant extract plus nutrient broth and nutrient broth plus test organism respectively were also set up along side. All the test-tubes were incubated at 37°C for 24hours after which the MIC was determined. MBC was determined by the sub culture of all test-tubes without an evidence of growth during MIC and incubated at 37°C for 24 hours.

## RESULTS

The physical properties of the extract revealed that 3.3g of it was recovered which was dark green in colour and gummy in texture (Table 1).

Table 2 presents the zones of growth inhibition of the different concentrations of plant extract against the bacterial isolates tested. All the concentrations inhibited the organisms tested with varying zones of growth inhibition (Figure 1) recorded in mm which increased with the increase in concentration from the lowest (20 $\mu\text{g/disc}$ ) to the highest (160 $\mu\text{g/disc}$ ).

Minimum inhibitory concentration of the leaves extract was determined at 25 $\mu\text{g/ml}$  for the entire test organisms while the minimum bactericidal concentration appeared to be above 100 $\mu\text{g/ml}$  which was the highest concentration used. This is because the test organisms were able to grow when all the test-tubes which showed no evidence of growth were sub-cultured on solid media (Table 3).

**Table 1: A physical property of *Phoenix dactylifera* leaves extract**

S/N	Properties	Extract
1	Weight of plant material	25g
2	Weight of extract recovered	3.3g
3	Colour	Dark green
4	Texture	Gummy

**Table 2: Antibacterial activity of ethanol extract of *P. dactylifera* leaves against the test Gram negative bacteria using disc diffusion method**

S/N	Bacteria	Concentrations/inhibition zones (MM)			
		20µg/disc	40µg/disc	80µg/disc	160µg/disc
1	<i>Escherichia coli</i>	9	12	14	15
2	<i>Morganella morganii</i>	8	11	12	13
3	<i>Proteus mirabilis</i>	9	12	13	14
4	<i>Yersinia enterocolitica</i>	9	12	13	14

**Table 3: Antibacterial activity of the ethanol extract of *P. dactylifera* leaves using broth dilution method**

S/N	Bacteria	Concentrations (µg/ml)						
		MIC				MBC		
		100	50	25	12.5	100	50	25
1	<i>Escherichia coli</i>	-	-	-	+	***	**	**
2	<i>Morganella morganii</i>	-	-	-	+	***	**	**
3	<i>Proteus mirabilis</i>	-	-	-	+	***	**	**
4	<i>Yersinia enterocolitica</i>	-	-	-	+	***	**	**

Key: MIC= Minimum Inhibitory Concentration

MBC= Minimum Bactericidal Concentration

\*\*= Growth observed (Turbid)

\*\*\*= MBC above 100µg/ml

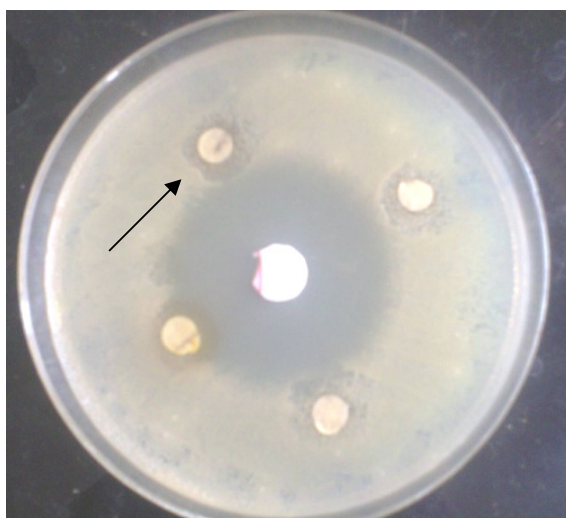


Figure F

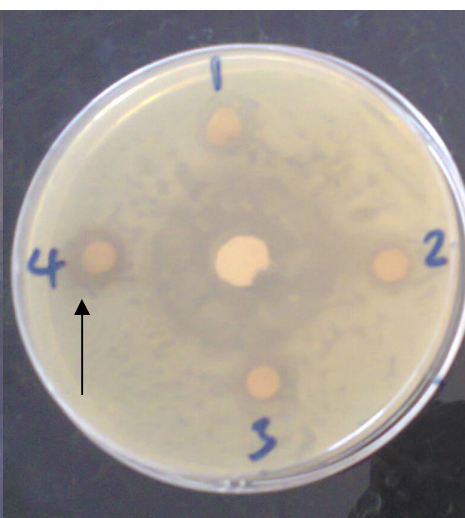
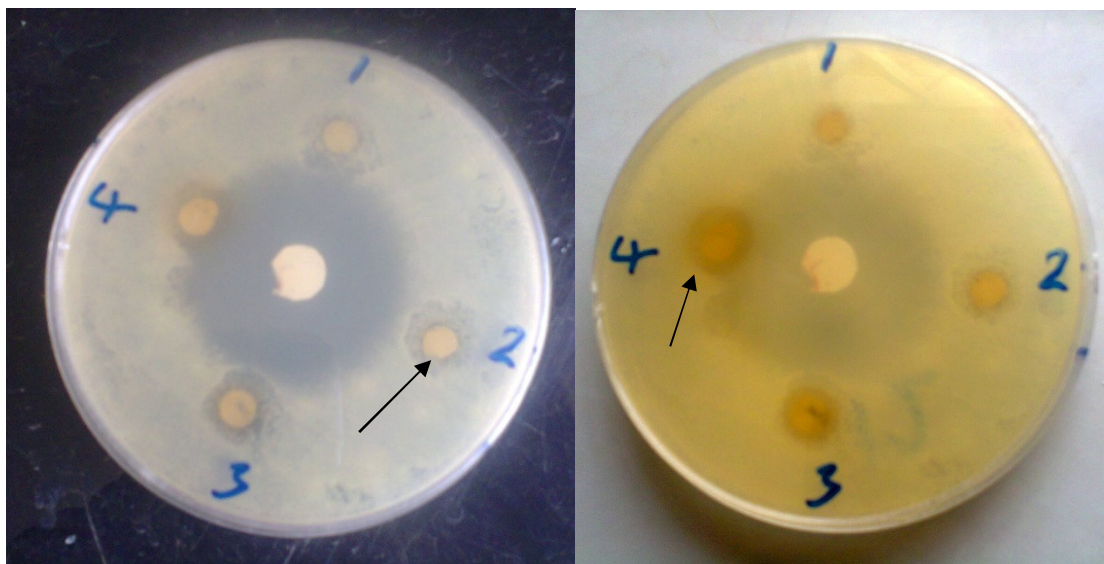


Figure G



**Figure 1: Zones of growth inhibition produced by the extract of *P. dactylifera* against the test Organisms**

Plates showing the zones of growth inhibition of ethanol extract of *P. dactylifera* leaves indicated by arrows against the test organisms (F= *E. coli*, G= *M. morganii* and the rest represent *P. mirabilis* and *Y. enterocolitica*) with Augmentin disc (10µg) at the centre serving as a control.

## DISCUSSION

The ethanol extract of *P. dactylifera* leaves screened for the antibacterial activity in this research revealed a very strong activity inhibiting the growth of all the test organisms at all concentrations used. Of the four bacterial isolates tested, *Escherichia coli* was found to be the most sensitive organism producing zones of growth inhibition of 15mm, 14mm, 12mm and 9mm at 160µg/disc, 80µg/disc, 40µg/disc and 20µg/disc respectively. This was followed by *Proteus mirabilis* and *Yersinia enterocolitica* with 14mm (160µg/disc), 13mm (80µg/disc), 12mm (40µg/disc) and 9mm (20µg/disc) for each (Figure 1). The least sensitive organism based on the research was *Morganella morganii* with 13mm (160µg/disc), 12mm (80µg/disc), 11mm (40µg/disc) and 9mm (20µg/disc) (Table 2).

Minimum Inhibitory Concentration for all the test organisms was observed at 25µg/ml while the Minimum Bactericidal Concentration was probably above 100µg/ml which was the highest concentration used. This is because the growth of the entire test organism was observed even at this concentration following the sub culture on the solid media of all the test-tubes which showed no evidence of growth during MIC (Table 3).

Kahkashan (2012) reported the antibacterial activity of *Phoenix dactylifera* leaf and pit extract against some selected Gram negative and Gram positive pathogenic bacteria at King Saud University in Saudi Arabia. Their research revealed that the acetone and methanol extracts showed good antibacterial activity

against *Bacillus subtilis*, *Escherichia coli*, *pseudomonas aeruginosa*, *Shigella flexineri*, *Staphylococcus aureus* and *Streptococcus pyogenes* while the water extract showed very little effect on all the test bacterial species. Antifungal activity of *P. dactylifera* leaves and pits using different solvents have also been reported. According to their research, water extracts, acetone and methanol extracts showed varying degree of growth inhibition of *Fusarium oxysporum*, *Fusarium sp.*, *Fusarium soloni*, *Aspergillus flavus*, *alternaria altanta*, *Alternaria sp.* and *Trichoderma sp* (Najat and Kahkashan, 2012).

## CONCLUSION

In conclusion, the ethanol extract of *Phoenix dactylifera* leaves demonstrated a strong *in vitro* antibacterial activity against all the Gram negative organisms tested.

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