

Evaluation of Antibacterial Effect of *Cucurbita pepo* (Yakten) Extracts on Multi-antibiotic Resistance Bacterial Strains Isolated From Human Urinary Tract Infections

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ABSTRACT

The aqueous and methanolic extracts of *Cucurbita pepo*, were used as antimicrobial activity against multi-antibiotic resistance strains (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Proteus mirabilis*) which were isolated biochemically from human urinary tract infections. The *in vitro* antimicrobial activity was performed by using Kirby-Bauer diffusion. The methanolic extracts of *Cucurbita pepo* fruit-cortex showed for the first time a high to moderate antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* respectively whereas the aqueous extract showed a moderate effect against *Staphylococcus aureus* only. Weak antimicrobial activity was seen on *Klebsiella pneumonia*, and *Proteus mirabilis* using both extract. Using core fruit methanolic and aqueous extraction showed no effect on any pathogenic bacteria. Whole fruit methanolic extract gave moderate and low antimicrobial effect on *Staphylococcus aureus* and *Escherichia coli* respectively. The results concluded that the application of *Cucurbita pepo* cortex extract against human multiresistant urinary tract pathogens may represents a new source as adjuvant to antibiotics for effective treatment of bacterial urinary tract infections.

Keywords: Antimicrobial activity, methanolic extract, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Cucurbita pepo*.

Cucurbita pepo

Cucurbita pepo

Escherichia coli, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Proteus*)

.Kirby-Bauer diffusion

.(*mirabilis*

Escherichia coli

Staphylococcus aureus

Proteus mirabilis Klebsiella pneumonia

Staphylococcus aureus

Escherichia coli

INTRODUCTION

The numbers of pathogenic multiresistant bacterial strains that carry resistance to many antibiotics were increased gradually. This increasing creates an emergent need to produce new antimicrobial agents. The most important antibiotic sources is the plants which provide us with different antimicrobial agents. Secondary metabolites of plants provide efficient pharmaceutical agents such as terpenoides, xanthones, benzophenones, coumarins, alkaloids, saponins, tannins, flavonoids, glycolipid, and galactolipids (Belguith *et al.*, 2010). Many parts of the plant (leaves, roots, flowers, whole plants, seeds, fruits and stems) were examined as antimicrobial agents in many researches against different pathogenic bacteria (Maeda *et al.*, 2009).

Researchs carried out in last few decades have validated several claims of the uses of traditional medicine plants. Popularity of *Cucurbita pepo* in various systems of traditional medicine for several ailments (antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, antihypercholesterolemia, intestinal antiparasitic, antiinflammation, and antalgic) focused the investigators' attention on this plant (Fu *et al.*, 2006).

Cucurbita pepo gives scavenging capacities due to the presence of flavonoid and phenylpropanoid glycoside-compounds known as potential hydrogen donors and radical scavengers. The scavenging activities for OH radical may be due to phenolic substances which stimulate hydrogen donating ability of hydroxyl substitutions (Dorman *et al.*, 2003). Leman *et al.*, showed in 2007 that *Cucurbita pepo* also showed antioxidant capacities. The carotenoids of *Cucurbita pepo* have been investigated for some time, but the studies were mostly limited to provitamin A. Carotenoids in terms of human health, reduce the risk of degenerative diseases such as cancer, cardiovascular diseases, cataracts, and macular degeneration, thus the importance of determining the vitamin A-inactive carotenoids became evident (Godoy and Rodriguez-Amaya, 1998). Other important contents in *Cucurbita pepo* are Oleic acid which is significantly the most concentrated followed by linoleic and palmitic acid. With the exception of magnesium and manganese, all other endogenous mineral contents varied significantly among seed samples. Potassium,

magnesium, and calcium were the most prevalent minerals, respectively (Ahmed *et al.*, 1996).

Other study revealed that cucurbitaceae plants are a great source of folic acid and are often prescribed for those who are lethargic, anemic or pregnant (Landrum and Bone 2001). In addition to that, it was investigated that *Cucurbita pepo* has phenolic contents with antioxidant capacity (Leman *et al.*, 2007). This plant also contains carotenoids which used as provitamin A carotenoids, for human health, that is, reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataracts (Godoy and Rodriguez, 1998).

Chromatographic purification of *Cucurbita pepo* fruit extracts showed that there is many important components such as: triglyceride fatty acid mixture, tetrahydro-thiophene, linoleic acid, calotropoleanly ester, cholesterol oleanen-3-ol. All components revealed antimicrobial, antiviral and antitumour activities (Badr *et al.*, 2010). No reports refer to plant fruit cortex as a source for pharmaceuticals.

The current study investigated that the antimicrobial components of three parts of *Cucurbita pepo* are cortex, core and whole fruit against multi-antibiotic resistance strains (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Proteus mirabilis*) which were isolated biochemically from human urinary tract infections.

MATERIALS AND METHOD

Samples

The Fresh fruits of *Cucurbita pepo* used in this work are taken from a local farm in Mosul city and divided into three parts; cortex, core and whole fruit. For effective extraction of the plant material, a freeze-drying method was performed. Freeze-drying is known to have a high extraction efficiency because ice crystals formed within the plant matrix can rupture cell structure, which allows exit of cellular component and access of solvent, and consequently better extraction (Chan *et al.*, 2009).

Preparation of extract

Each part of fruit was extracted with 70% alcoholic and distilled water in a specific glass compressor (Germany) for 10 minutes using 5gm of fruit/5ml of extract solution. The whole extract was then filtered with the help of whatman no. 4 filter paper. The collected solvent was then evaporated to dryness with rotary vaccum evaporator at 40 °C to afford a thick residue. The thick residue was then converted in dried powder in desiccators. This dried mass was dissolved in alcohol and in aqueous to get 20 mg/ml concentration of extract (Obi and Nwanebu, 2009).

Test bacteria

Four multiresistant bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia*) were isolated biochemically from urine specimens of in-patients and out-patients of General Hospital of AL-Baida city/ Libya and used for antimicrobial activity (Cheesbrough, 2002; Powers, 1991; Cappuccion *et al.*, 1992). Cultures identification was carried out by using standard techniques (Udo *et al.*, 2008). The test bacterial multiresistant strains include *Staphylococcus aureus* as two strains resistant to 12 and 13 antibiotics, two multiresistant strains of *Escherichia coli* resistant to 13 antibiotics for each, one multiresistant strain of *Proteus mirabilis* resistant to 12 antibiotics

and two strains of *Klebsiella pneumonia* resistant to 12 and 13 antibiotics were used as tested bacteria (Table 1).

Culture media and Antibiotics

Muller Hinton Agar and Nutrient agar were used for agar diffusion assay. Amoxicillin (Amo) (10mcg, for gram +ve), Gentamycin (C) (10mcg, for gram-ve) were used as control.

Antimicrobial Activity Test

The Antimicrobial susceptibility testing was done using Kirby-Bauer diffusion technique. The tests were realized by diffusion method using Muller Hilton agar while the antibacterial effect was determined depending on the inhibition area diameter (Nicodim *et al.*, 2009). Two standardized overnight cultures of each strain (containing 10^8 colony-forming units, CFU/ml) were used to flood surface of Muller Hilton agar. The antibiotic discs impregnated in 20mg/ml of three extract; cortex, core and whole fruit and the controls (Amoxicillin 10mcg, Gentamycin 10mcg) were then aseptically placed at reasonable equidistance on the inoculated plates and allowed to stand for 1h. The plates were then incubated at 37C° for 18h (Ehinmidu, 2003).

RESULTS AND DISCUSSION

The results revealed that alcoholic and aqueous cortex extract (cxt) of *Cucurbita pepo* gave high effect on *Staphylococcus aureus*. Alcoholic extract (Aex) showed a higher effect with a high inhibition zone on bacteria (16mm), whereas aqueous extract (aqex) showed a moderate effect (10mm) compared with control (18mm). For *Escherichia coli* cxt gave moderate effect on this pathogen (Table 2). Higher effect was noticed using Aex (16mm), whereas Aqex showed lower effect (4mm). Aqex or Aex of the cortex showed weak effect on both *Proteus mirabilis* and *Klebsiella pneumonia*. Using core fruit extraction (Aqex or Aex) the results showed no effect on any pathogenic bacteria. Whole fruit extract gave moderate antimicrobial effect on *Staphylococcus aureus* ranged between 6mm using Aqex and 8mm using Aex. With *Escherichia coli* Aqex and Aex gave low antimicrobial effect with 2mm of inhibition zone for each extract (Wex and Aex) compared with the control (Figure 1). The same treatment showed no effect on *Proteus mirabilis* and *Klebsiella pneumonia* compared with the control.

Form these results we conclude that cortex extract (cxt) of *Cucurbita pepo* may contains the main specific components that responsible for antimicrobial effect on gram positive *Staphylococcus aureus*. This means that these components may react specifically with murine layer in gram positive bacteria since moderate and low effect were seen on gram negative bacteria. The fruit core not contains any antimicrobial component therefore not showed any antimicrobial effect on the four tested pathogenic bacteria. When the whole fruit extract used, only moderate and low effect was seen on *Staphylococcus aureus* and *Escherichia coli*. This low effect (compared with ctx) may be due to the interaction between the components of cortex and core thereby leads to reduce the effect of cortex components.

Table 1: Multi-resistance pattern strains and their frequencies used as tested bacteria.

Bacteria	Number of resistant antibiotics	Frequency and frequency (%)	Patterns of Multi-resistance strains													
			Amp	Aml	Amc	Car	Te	Ot	Do	Sxt	Cn	Na	F	E	Ob	Cr
<i>P. mirabilis</i>	12	2(13.33)	Amp	Aml	Amc	Car	Te	Ot	Do	Sxt	--	--	F	E	Ob	Cr
<i>K.pneumonia</i>	13	3(20)	Amp	Aml	Amc	Car	Te	Ot	Do	Sxt	--	Na	F	E	Ob	Cr
	12	2(13.33)	Amp	Aml	Amc	Car	Te	Ot	Do	--	--	Na	F	E	Ob	Cr
<i>S. aureus</i>	13	4(2.1)	Amp	Aml	Amc	Car	Te	Ot	Do	Sxt	Cn	Na	-	E	Ob	Cr
	12	2(1.02)	Amp	Aml	----	Car	Te	Ot	Do	Sxt	Cn	Na	-	E	Ob	Cr
<i>E. coli</i>	13	7(3.58)	Amp	Aml	Amc	Car	Te	Ot	Do	Sxt	Cn	Na	-	E	Ob	Cr
		6(3.07)	Amp	Aml	Amc	Car	Te	Ot	Do	Sxt	----	Na	F	E	Ob	Cr

Ampicillin (Amp), Amoxicillin (Aml), Augmenten (Amc), Carpenicillin (Car), Tetracyclin (Te), Oxytetracyclin (Ot), Deoxytetracyclin (Do), Suphamethoxazol (Sxt), Gentamycin (Cn), Naldixic acid (Na), Nitrofurantoin (F), Erythromycin (E), Cloxacillin (Ob), Cephaloridin (Cr).

Table 2: Inhibition zone (mm) of cortex, core and whole fruit extracts.

Bacteria	Cortex		Core		Whole fruit		Control	
	Aqex	Aex	Aqex	Aex	Aqex	Aex	Amo	Cn
<i>Staph. aureus</i>	10	16	----	----	6	8	18	
<i>E. coli</i>	4	6	----	----	2	2		20
<i>Proteus mirabilis</i>	2	2	----	----	----	----		18
<i>Klebsiella pneumonia</i>	2	2	----	----	-----	-----		18

Aqex=aqueous extract, Aex= Alcoholic extract
Amoxicillin (Amo), Gentamycin (Cn).

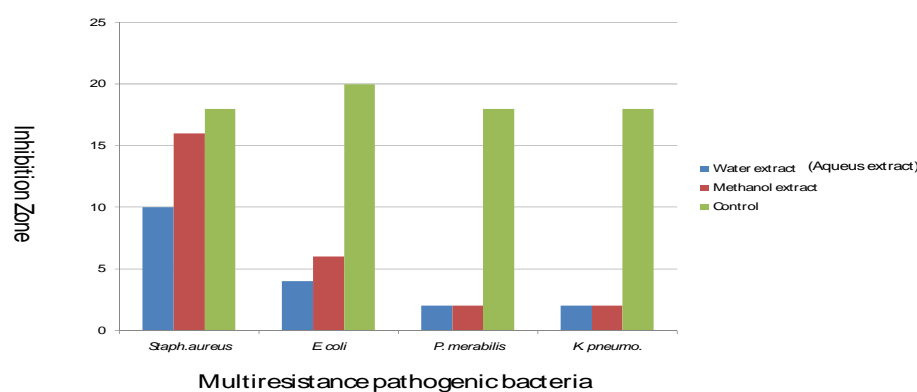


Fig. 1: Antimicrobial activity of Aqueous and alcoholic *Cucurbita pepo* cortex extract (Inhibition zone mm) on pathogenic multi-resistant antibiotic strains.

The results obtained from previous study showed that two oil extraction of *Cucurbita pepo* produced a higher zone of inhibition of 60% on *Staphylococcus aureus* and

Escherichia coli. Phytochemical analyses of *Cucurbita pepo* extracts reveal the presence of tannins, flavonoids, saponins, cyanogenic glycosides and cardiac glycosides, These extracts, apart from their roles as food additives and supplements, may also be utilized as effective and cheap sources of antibacterial agents for the treatment of bacterial infections (Obi *et al.*, 2009).

CONCLUSIONS AND RECOMMENDATIONS

The alcoholic and aqueous cortex of *Cucurbita pepo* possess antibacterial activity against multiresistant pathogenic bacteria that infect the human urinary tract. *Cucurbita pepo* is available locally and at cheap affordable prices, which will surely enhance their application among other uses, as adjuvant to antibiotics for effective treatment of bacterial infections. These results may suggest that total antimicrobial activities of cortex extract are the come from the individual activities of many components present in the cortex with different effects. It is important to investigate the efficiency of these components in the cortex of *Cucurbita pepo* with advance methodology to assess the real effect of each component on multiresistant microbes.

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