

EVALUATION OF ANTIMICROBIAL ACTIVITY OF FLAVONOIDS EXTRACT FROM *CUCURBITA PEPO* LEAVES

Alya'a Sebti Jasim* Nawras Abdulelih Alwan** Wegdan Hussein

Altimimi*** Hhua Kadoum Kareem*

*Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq.

**Department of physiology and chemistry, College of Veterinary Medicine, University of Basrah, Iraq.

***Department of Biology, College of Science, University of Basrah , Iraq.

(Received 18 January 2010 , Accepted 2 March 2010)

Keywords:- *Cucurbita pepo* leaves, Flavonoides compound, antibacterial activity.

ABSTRACT

Investigation of antibacterial activity of flavonoids extract of *Cucurbita pepo* was carried out in this study on Gram positive and Gram negative pathogenic bacteria. The results exhibited variable susceptibilities of microorganism for different concentrations of flavonoids extract. The activity of this extract was associated with high concentration. Using plate methods, flavonoids extract of *Cucurbita pepo* had the highest effect and wide diameter of growth inhibition zones against *serratia* and *Escherichia coli* and it has no effect on growth of *Bulkholderia pseudomallia*. *Staphylococcus aureus* and *Streptococcus sp.*

INTRODUCTION

Bacterial infection contribute largely in general health problems of man and have been reported to be responsible for over 50% of death recorded in developing countries, antimicrobial drugs have failed in their activity against the pathogen due to the development of drug resistance [1]. This increasing resistance to antibiotics has therefore resulted in the search for leads new organic molecules from plants with antimicrobial properties [2]. Plants have been valuable source of natural products for maintaining human health especially in the last decades with more intensive studies for natural therapies and the use has gradually increased according to the world health organization [3].

Cucurbita pepo (pumpkin) is a member of the family curcurbitacea, its an annual plant with yellow flowers it has aclimbing stem up to 12 Cm. long and a fruit with ovoid shape with a curved green shell inside the shell is a flat, round yellow and white seed enclosed in a husk [4].

Cucurbita pepo leaves has been used in traditional medicine as antihelminthic agent and there are for supportive treatment in functional disorders of the bladder and for difficulties in urination [5] as well as childhood enuresis nocturnal and irritable bladder have been treated successfully with pumpkin, it has been also used to eradicate tape worm [6]. Other researchers showed that it is possessed anti-inflammatory antibacterial, antiviral, analgesic effects and cardioprotective activity [7] in addition it has been widely applied in the treatment of benign prostatic hyperplasia in men, obesity, skin problems [8, 9].

Cucurbita pepo have been used in our research because of their antimicrobial material, which are due to compound synthesized in the secondary metabolism of this plant so these products are known by their active substances such as flavonoids compounds [10].

MATERIAL AND METHOD

Plant material and extraction:

Cucurbita pepo leaves were purchase from the local market of Basra. The leaves of *Cucurbita pepo* were sun dried and powdered. Powder (50gm) was extracted by mixing with 250ml ethanol (70%) for 24 hrs. using reflex extraction. The extract was filtered through wattmann No. 31 for removal particles then filtrate was mixed with 1% lead acetate and filtered with wattmann No. 31. residue was dissolved with 25ml acetone and 30ml HCl conc. and left to dry to evaporation the solvent and then obtained black solid residue about 5 gm [6].

Microorganisms Test:

The level of sensitivity of seven types of pathogenic bacteria were previously isolated and identify by other worker these bacteria was determined by using the disk diffusion method (Kirby Bauer) as described by [11]. Circular paper disc measuring 7.0 mm was cut from Whatman No.1 filter paper.

Each group of 2 plates was inoculated with each of the test organism which was fully spread on the Muller-Hinton agar medium. Finally the discs impregnated with extracts (6.25, 12.5, 25, 50, 100 mg/kg.) which contain carefully placed into the culture plates and allowed to stand for a few minutes before being incubated for 24hrs. at 37°C. After this period it was possible to observe inhibition zone overall, cultured bacteria with halos equal to or greater than 7mm. were considered susceptible to the tested extract. They were then examined for growth and signs of inhibition. The zone of inhibition were determined by measuring diameter of clearance a cross the disc with a ruler

RESULTS

Results of disc diffusion test (table 1) which illustrated by photographs which are listed in figure 1 (A-F) and figure 2 (A-H).showed that the flavonids extract of *Cucurbita pepo*, if properly processed, could be used to treat some stubborn *Serratia*, *E.coli* and *Bacillus* infections. This agreement with result of [16] which reported the antimicrobial properties of compounds obtained from *Parthenum argentatum* which inhibited the growth of *Bacillus subtilis*, *E.coli* and *P.aeruginosa*.

It was concluded that the flavonoids extract (ethanolic extract) 70% were toxic to many types of bacteria and showed have antimicrobial activity. The antimicrobial activity from this extract was tested against seven genra of bacteria, which showed it had activity against *Klebselia*, *Bacillus*, *E.coli* and *Serratia* [16].

Table (1): Diameter of inhibition zone with different concentration of extract effect on bacteria used:

Bacteria	Concentration of flavonids extraction (mg/ml)				
	6.25 DIZ(mm)	12.5 DIZ(mm))	25 DIZ(mm))	50 DIZ(mm))	100 DIZ(mm))
<i>Klebsiella pneumoniae</i> 10 ⁻¹	10	8	10	15	0
<i>Klebsiella pneumoniae</i> 10 ⁻²	14	10	8	0	0
<i>Bacillus subtilis</i> 10 ⁻¹	0	35	15	0	20
<i>Bacillus subtilis</i> 10 ⁻²	1	15	17	8	20
<i>Staphylococcus aureus</i> 10 ⁻¹	0	0	0	0	0
<i>Staphylococcus aureus</i> 10 ⁻²	0	0	0	0	0
<i>Bulkholderia pseudomallie</i> 10 ⁻¹	0	0	0	0	0
<i>Bulkholderia pseudomallie</i> 10 ⁻²	0	0	0	0	0
<i>E.coli</i> 10 ⁻¹	15	10	10	15	12
<i>E.coli</i> 10 ⁻²	10	20	10	15	0
<i>Serratia</i> 10 ⁻¹	15	0	10	0	13
<i>Serratia</i> 10 ⁻²	10	10	13	20	10
<i>Streptococcus sp.</i> 10 ⁻¹	0	0	0	0	0
<i>Streptococcus sp.</i> 10 ⁻²	0	0	0	0	0

DIZ= Diameter of inhibition zone measured in millimeter.

*10⁻¹, 10⁻²= dilution of bacterial broth.

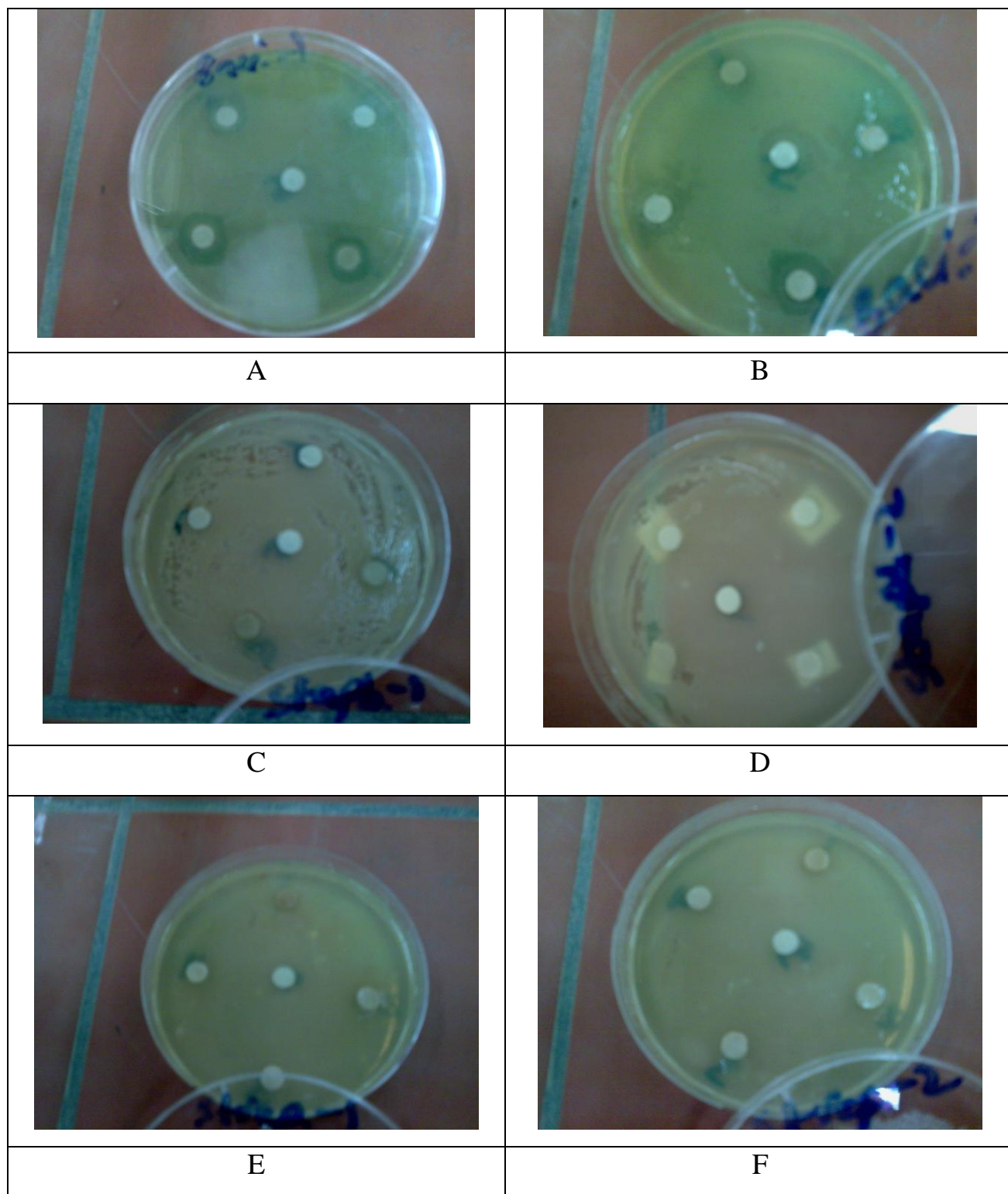


Figure (1): Inhibition zones induced by flavoniods extract on some positive bacteria

A: *Bacillus subtilis* 10^{-1} , B: *Bacillus subtilis* 10^{-2}

C: *Staphylococcus aureus* 10^{-1} , D: *Staphylococcus aureus* 10^{-2}

E: *Streptococcus* sp. 10^{-1} , F: *Streptococcus* sp. 10^{-2}

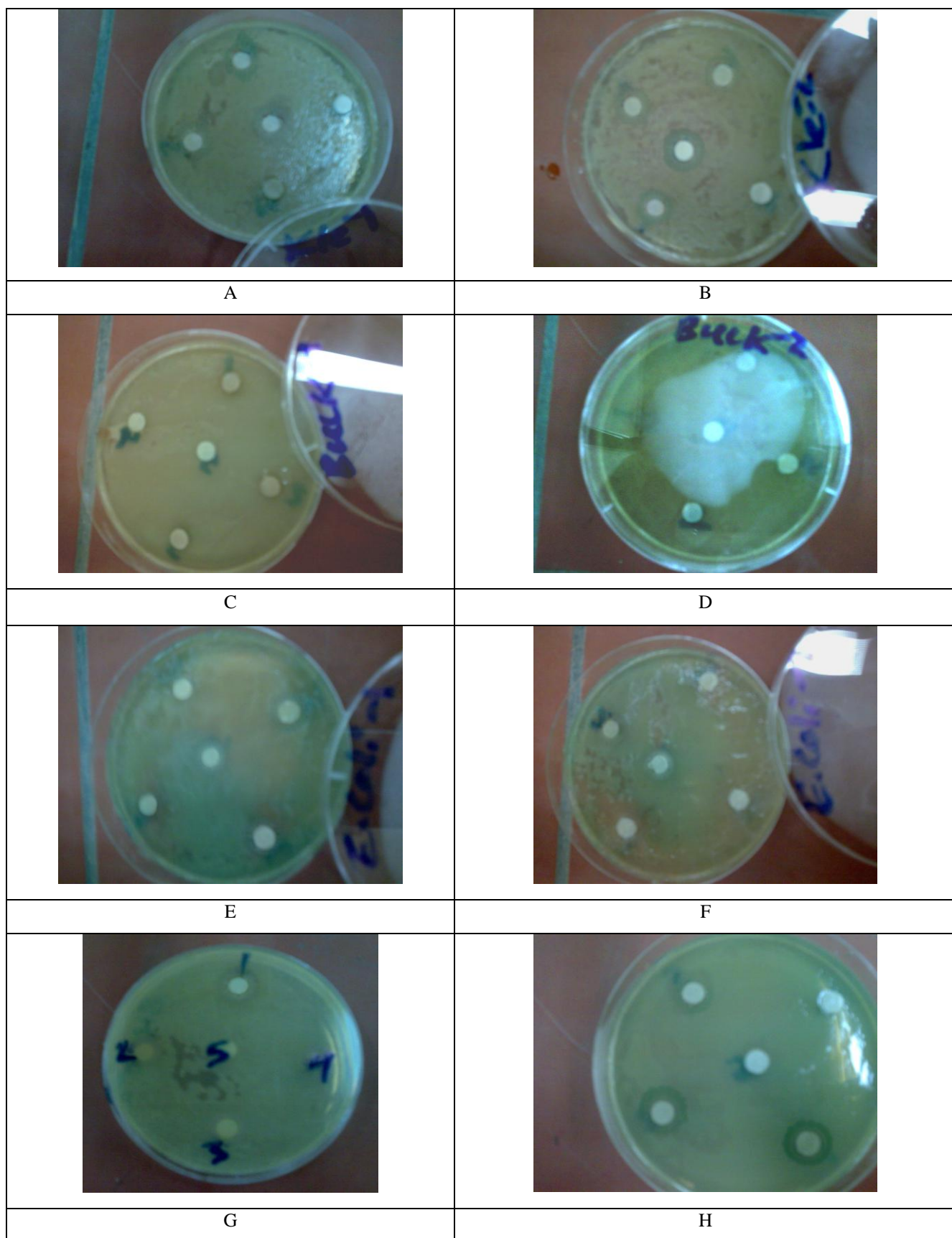


Figure (2): Inhibition zones induced by flavonoids extract on some Negative bacteria

A: *Klebsiella pneumoniae* 10^{-1} , B: *Klebsiella pneumoniae* 10^{-2}
 C: *Bulkholderia pseudomallie* 10^{-1} , D: *Bulkholderia pseudomallie* 10^{-2}
 E: *E. coli* 10^{-1} , F: *E. coli* 10^{-2}
 G: *Serratia* 10^{-1} , H: *Serratia* 10^{-2}

DISCUSSION

The flavonoids extraction of *Cucurbita pepo* in present study revealed the medical importance of this plant through the antimicrobial activity. A variable susceptibility of bacteria against different concentration of the flavonoids extraction of *Cucurbita pepo*.

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the concentration of antibiotic resistant bacteria. The objective of this study was to evaluate the potential of the flavonoids extraction of *Cucurbita pepo* on standard bacteria strains. The flavonoids compounds have effective against bacteria[12,13] because of the site (s) and number of hydroxyl groups on flavonoids group are thought to be related to their relative toxicity to the bacteria that increased hydroxylation results increased toxicity [14 &15], the antimicrobial properties of plant have been investigated by a number of researchers world wide, it was documented that among the compound extracted from these plants, inhibited the growth of *Staph. aureus* , *E.coli* and *Klebsiella pneumoniae* and also the effects of *Cucurbita pepo* may relate to an influence of plant compounds on immunocompetent cells.

Cucurbita pepo possess antibacterial qualities, will surely enhance their application among other uses, as alternative to antibiotics for effective treatment of bacterial infections, *Cucurbita pepo* and also have medicinal uses including antihelminthic as well as natural laxative. In addition it has been widely applied in treatment of benign prostatic hyperplasia in men, obesity, skin problems and irritable bladder (enuresis) in children[16].

تقدير الفعالية المضادة للميكروبات للمستخلص الفلافونيني المعزول من اوراق نبات

اليقطين *Cucurbita pepo*

علياء سبتي جاسم * ، نورس عبدالاله علوان ** ، وجدان حسين التميمي *** ، هدى كاظم كريم *

*فرع الإحياء المجهرية- كلية الطب البيطري – جامعة البصرة – البصرة- العراق

**فرع الفلسفة والكيمياء - كلية الطب البيطري – جامعة البصرة – البصرة- العراق

***كلية العلوم - جامعة البصرة – جامعة البصرة – البصرة- العراق

الخلاصة

لقد تم دراسة الفعالية المضادة للميكروبات للمستخلص الفلافونيني لاوراق نبات اليقطين. وقد اختيرت بعض أنواع البكتيريا الموجبة والسالبة لصبغة كرام. بينت الدراسة أن هذه الميكروبات لها حساسية مختلفة اتجاه المستخلص وحسب نوع الميكروب والتركيز المستخدم وقد لوحظ أن التأثير الأكبر للمستخلص كان على جرثومة *Serratia* وجرثومة *Escherichia coli* أما الجراثيم *Staphylococcus aureus* و *Streptococcus sp.* و *Bulkholderia pseudomallie* فلم تتأثر بالمستخلص

REFERENCES

- 1- Lamikanra , A..K. (1981) African Medicinal plants. /Jnas/ (4) : 29-30.
- 2- Cimanga, K.; Picters, L.; Claeys, M. ; Berghe, D.V. and Vilene, A.J. (1991). Biological activities of cryptolepine, an alkaloid from *Cryptolepis sanguinolenta*. *Planta Medica* 57 (2): 98-99.
- 3- Satish , S. ; Raghavendra , M.P. and Raveesha K.A. (2008) Evaluation of the Antibacterial potential of some plants against Human pathogenic Bacteria. *Adv.Biol.Res.* 2(3-4):44-48
- 4- Winkler ,C. ; Wirleitner ,B. ; Schroecksnadel , K. ; Schennach H. and Fuchs, D. (2005). Extract of pumpkin (*Curcubita Pepo L.*) seeds suppress stimulated peripheral Blood Mononuclear cells *in vitro*. *Am. J. Immunol.* 1 (1): 6-11.
- 5 - Matus, Z.; Molnar , P. and Szabo, L.G. (1993). Main carotenoids in pressed seeds (*Curcubita semen*) of oil pumpkin (*Curcubita Pepo con var. pepo var. styrica*). *Acta pharm. Hung.* ,63:247-56.
- 6- Obi, R.K.; Nwanebu, F.C.; Nadubuis, U.U. and Orji, N.M. (2009). Antibacterial Qualities and Phytochemical Screening of the oils of *Curcubita Pepo* and *Brachening nigra*. *J. Medicinal plant Research* Vol. 3(5) ,p.p. 429-432.
- 7- Obi , R.K. ; Iroagba , I.I. and Ojiako , O.A. (2006). Virucidal potential of some Edible Nigerian vegetables. *Afr. J. Biotechnol.* 5(19): 1785-1788
- 8- Drei Korn, K.R.; Berges, P.L. and Jonas, U. (2002). Phytotherapy of benign prostatic hyperplasia. Current evidence – based evaluation. *Urologe A.* , 41: 447-51.
- 9- Sofowara, E.A. (1982). *Medicinal plants and traditional medicine in Africa*, 3rd ed. Spectrum books limited, Ibadan pp.31-34.
- 10- Tarhan , L. ; Kayali , H.A. and Urek, R.O. (2007). In vitro Antioxidant properties of *Curcubita Pepo L.* Male and Female Flowers Extracts. *Plant Foods for Human Nutrition* 62:49-51.
- 11- Nester, N.E ; Anderson, G.D and Robert, E.C. (2004). *Microbiology , A human perspective* , 4th ed. MC Graw-Hills inc., New York PP.313-315, 319-321.
- 12- Brantner, A. ; Males, Z. ; Papelijnsjak, S. and Antolic, A. (1996). Antimicrobial activity of *Paliurus spina* – Christ Mill. *Ethnopharmacol.* 52: 119-122.

- 13- Thomason, W. A. R. (1978). Medicines from the Earth. MC Graw Hill Book Co. Maiden head , United Kingdom.
- 14- Sealbert, A.(1991) Antimicrobial properties of tannins. Photochemistry 30:3875 – 3883.
- 15- Urs, N.V.R.R and Dunleavy, J.M. (1975). Enhancement of bactericidal activity of a peroxidase system by phenolic compounds (*Xanthomonas phase Var. Sojensis*, soybeans). Phytopathology 65:686-690.
- 16- Nascimento,G.F. ; Locatelli,J. ; Freitas,P.C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on Antibiotic -Resistant bacteria. Braz J. of Microbiology-13:247-256.