

## ANTIBACTERIAL ACTIVITY OF *Arachis hypogaea* L. SEED COAT EXTRACT CULTIVATED IN IRAQ

Ahmed H. AL-Azawi<sup>1\*</sup> and Zainab H. Hassan<sup>2</sup>

<sup>1</sup>Biotechnology Dept., Genetic Engineering and Biotechnology Institute for post graduate studies. Baghdad University, Baghdad, Iraq. <sup>2</sup>Ministry of Agriculture, State Company for Agriculture Supplies, Baghdad, Iraq. E. mail: ahmedharbi\_alazawi@yahoo.com\*

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### ABSTRACT

The present study aimed to assess the antibacterial activity of peanut (*Arachis hypogaea* L.) skin extracts. The phytochemical analysis of the peanut skin extracts was investigated, the result showed a strong presence of flavonoids, phenols, alkaloids and tannins in methanol and ethyl acetate extracts. Antibiotic susceptibility of the bacterial isolates was performed on seven antibiotics represented by Amikacin, Tetracycline, Ciprofloxacin, Chloramphenicol, Ticarcillin, Cefotaxime and Gentamicin by disc diffusion method. The antibiogram for studied isolates revealed high level resistance of *A. baumannii* to all of the antibiotics under test except amikacin, while *Staph. aureus* was resistance to Chloramphenicol and Cefotaxime and sensitive to Amikacin, Tetracycline, Ciprofloxacin, Ticarcillin and Gentamicin. The antibacterial activity of the peanut skin extracts was studied on some pathogenic microorganisms like (*Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Serratia marcescens* and *Escherichia coli*). The results show that the best effect was seen against *Staph. aureus* with inhibition zone ( $10.67 \pm 0.67$ ,  $13.00 \pm 1.00$  and  $14.67 \pm 0.88$ ) in concentration (25, 50 and 100 mg/ml) respectively, with significant difference ( $P < 0.01$ ), while the lowest effect was seen against *A. baumannii* with inhibition zone ( $4.67 \pm 0.33$ ,  $7.33 \pm 0.33$  and  $10.33 \pm 0.33$ ) in concentration (25, 50 and 100 mg/ml) respectively with significant difference ( $P < 0.01$ ) for methanolic extract.

**Keywords:** Peanut, Seed coat Extract, Antibacterial, Antibiotic sensitivity, phenolic compounds

### INTRODUCTION

The powerful tool for the treatment of several diseases and a keystone of modern medical practice is Antimicrobial therapy. However, the increased behavior against of microorganisms to the currently used antimicrobials has created the need to evaluate other agents with potential antimicrobial activity (Abd Alhussain, *et al.*, 2017). In a day a new sources of drugs which have been used effectively in traditional medicine using the natural products in plants of medicinal value. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional system of medicine (Sukanya *et al.*, 2009). Peanut (*Arachis hypogaea* L.) is an important nut, as well as an oilseed crop of the tropical and subtropical world. Peanut skins are low value byproducts of peanut blanching and roasting operations and are currently used as an ingredient of animal ration up to a certain limit (Sobolev and Cole, 2004). Several phytochemicals including resveratrol, flavan-3-ols and proanthocyanidins have been identified in peanuts and evaluated for their potential health benefits (Bolling *et al.*, 2010; Sarnoski *et al.*, 2012 and Hamza and Rashid 2017). Research has shown that peanut consumption provides such health benefits due to high levels of certain phytochemicals (Francisco and Resurreccion, 2008). It is also consist of a suitable amount of phenolics and other health promoting compounds and thus can be

explored for functional food applications (Yu *et al.*, 2005). Peanut stilbenoids show to play roles in plant defense mechanisms, they were evaluated for their effects on economically important plant pathogenic fungi of the genera *Colletotrichum*, *Botrytis*, *Fusarium*, and *Phomopsis* (Sobolev *et al.*, 2011). The aims of this study are detection of active ingredients in peanut skins (*Arachis hypogaea* L.) as well as evaluating the antibacterial activity against pathogenic bacteria.

### MATERIALS AND METHODS

**Plant material:** Raw peanut pods were purchased from the local market in Baghdad city and classified as *Arachis hypogaea* L. by the herbarium of the Biology Department, College of Science, Baghdad University. Pods were manually shelled and the seed coat were collected from the raw peanut kernels. The seed coat were ground using a grinder and stored at  $-20^{\circ}\text{C}$  for future analysis.

**Preparation of Peanut Skin Extracts:** Methanolic, ethyl acetate and aqueous crude extracts were prepared by macerated 100 g of peanut seed coat in 1000 ml of each solvent for 72 hours. After extraction, the mixture was vacuum filtered through Whatman No. 1 paper and the filtrate was dried at  $40^{\circ}\text{C}$  by a rotary evaporator. The resulting extract stored in amber glass vials in a freezer until analyzed. The whole process was completed under dim light to minimize light induced degradation of

phenolics, which are generally light sensitive (N'Guessan *et al.*, 2007).

**General chemical detection methods:** Methanolic, ethyl acetate and aqueous extracts were tested for the presence of the phytoconstituents according to the following standard tests to detected phenols, Flavonoids, Alkaloids and Tannins (Harborne, 1984, Harborne, 1998 and Jaffer *et al.*, 1983).

**Microorganisms tested:** *Acinetobacter baumannii*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Serratia marcescens* and *Escherichia coli*. Can be obtained from Fatima AL- Zahra Hospital in Baghdad, collected from patients with septicemia and diagnosed by using the VITEK-2 System. Bacterial cultures were maintained on nutrient agar (NA) slops. Subcultures were made monthly and stored at 4 °C until required for use.

**Culture preparation:** Three – five colonies from the pure culture were suspended in 5-10 of sterile nutrient broth. The turbidity of the test suspension was compared with 0.5 McFarlandturbidity standards (108 CFU / ml) (Sofia *et al.*, 2007).

**Antibiotic sensitivity test:** Antibiotic sensitivity of the bacterial isolates was determined by the standard disc diffusion method (WHO, 2003). Different antibiotics (Oxoid / England) were used in the present work, Amikacin (Ak), 30 µg; Cefotaxime (CTX), 5 µg; Chloramphenicol (CL) 30 µg; Ciprofloxacin (CIP), 5 µg; Gentamicin (GM), (10 µg); Tetracycline (T), 30 µg; Ticarcillin (TS), 75 µg. The interpretation of antibiotic susceptibility test resistant, (R) intermediate (I), or sensitive (S), according to CSLI, (2011).

**Antibacterial assay:** The diffusion method agar well was employed for detection the antibacterial activity. 0.2 ml volume of the standard inoculums (10<sup>8</sup> CFU/ml) of the test bacterial isolate was spread on Mueller Hinton Agar (MHA) with a sterile glass rod spreader and allowed to dry. Wells (6 mm diameter) were made in each of these plates using sterile cork borer. 100 µl from each concentration (25, 50 and 100 mg/ml) was prepared In dimethyl sulfoxide (DMSO) of the aqueous, methanol and ethyl acetate crude extracts and putting in each hole by using micropipette and allowed to diffuse at room temperature for 30 min. Control (DMSO) experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of any resulting zones of inhibition was measured in millimeters (Valgas *et al.*, 2007).

**Statistical Analysis:** The Statistical Analysis System program was using to study different parameters. LSD test was used to significant compare between means in this study (SAS, 2012)

## RESULTS AND DISCUSSION

**Phytochemical screening of peanut skin extracts:** The preliminary phytochemical screening is a means of evaluating the potential phyto compounds in the skin extract of *Arachis hypogaea*. Phytochemical characterizations of methanol, ethyl acetate and aqueous extracts of *A. hypogaea* are presented in Table 1.

The result showed a strong presence of flavonoids, phenols, alkaloids and tannins in methanolic and ethyl acetate extracts, while alkaloids and tannins were not detected in aqueous extract.

Table 1: Phytochemical screening of peanut seed coat extracts

Phytochemical compound	Methanolic extract	Ethyl acetate extract	Aqueous extract
Flavonoids	++	++	+
Phenols	++	++	+
Alkaloids	+	+	-
Tannins	+	+	-

++ Strongly positive, + Positive, - negative.

The phytochemical finding has been agreed with Chukwumah *et al.*, (2009) which they have reported the presence of tannins, alkaloids and phenols as active compounds in peanut skin. Yu, (2006) has been reported that peanut skins contain phenolic compounds with demonstrated antioxidant properties. Furthermore, earlier study revealed the presence of flavonoids, phenols and coumarins in methanolic extract and the other phytocompounds tannin, saponin, alkaloids were present in trace amounts (Velu *et al.*, 2015).

**Antibiotics susceptibility:** Antibiotic susceptibility of the bacterial isolates was performed on seven antibiotics represented by Amikacin, Tetracycline, Ciprofloxacin, Chloramphenicol, Ticarcillin, Cefotxime and Gentamicin by disc diffusion method Table 2.

The antibiogram for studied isolates revealed high level resistance of *A. baumannii* to all of the antibiotics under test except amikacin. Moreover *K. pneumonia*, *S. marcescens* and *E. coli* were resistant to all of the antibiotics except amikacin and Ciprofloxacin, while *Staph. aureus* was resistance to Chloramphenicol and Cefotxime and sensitive to Amikacin, Tetracycline, Ciprofloxacin, Ticarcillin and Gentamicin.

Table 2: antibiotic Sensitive test

Microorganism	Antibiotic						
	AK	T	CIP	CL	TS	CTX	GM
<i>Staph. aureus</i>	S	S	S	R	S	R	S
<i>E. coli</i>	I	R	S	R	R	R	R

<i>S. marcescens</i>	S	R	I	R	R	R	R
<i>K. pneumonia</i>	S	R	S	R	R	R	R
<i>A. baumannii</i>	I	R	R	R	R	R	R

AK=Amikacin, T= Tetracycline, CIP= Ciprofloxacin, CL= Chloramphenicol, TS= Ticarcillin, CTX= Cefotxime, GM=Gentamicin, S=Sensitive, R=Resistant I = Intermediate.

Drug resistance is one of the natural process whereby organisms develop a tolerance for environmental conditions, these may be due to pre exist factor in the organisms or it may result from acquired some factors, that transfer naturally susceptible strain of bacteria into resistance bacteria. Antibiotic sensitivity *in vitro* is quite different from this obtained *in vivo* because a particular antibiotic is used depending on several factors such as its selective toxicity, drug absorption, metabolism, drug clearance rate, bioavailability and serum attainable level, therefore the risk of increase resistant organi-

sms to antibiotic was developed, on the other hand proper adherence and compliance to drug prescription and dosage on the patients also play a role in the efficacy of the antibiotics in use (Ali, 2011).

**Antibacterial activity of peanuts crude extracts**

Preliminarily, the antibacterial activity of *Arachis hypogaea* skin extracts was qualitatively evaluated by agar well-diffusion method. For each type of *Arachis hypogaea* crude extracts, statistical test were performed between different concentrations, for methanolic extract as seen in Table 3, the best effect was seen against *Staph.aureus* with inhibition zone  $10.67 \pm 0.67$ ,  $13.00 \pm 1.00$  and  $14.67 \pm 0.88$  in concentration 25, 50 and 100mg/ml respectively with significant difference ( $P<0.01$ ), while the lowest effect was seen against *A. baumannii* with inhibition zone  $4.67 \pm 0.33$ ,  $7.33 \pm 0.33$  and  $10.33 \pm 0.33$  in concentration 25, 50 and 100mg/ml respectively with significant difference ( $P<0.01$ ).

**Table 3: Antibacterial activity of methanol peanut seed coat extract**

Isolate	Concentration (mg/ml)			LSD value
	25	50	100	
<i>Staph.aureus</i>	$10.67 \pm 0.67$	$13.00 \pm 1.00$	$14.67 \pm 0.88$	2.577 **
<i>E. coli</i>	$7.33 \pm 0.33$	$9.33 \pm 0.33$	$10.67 \pm 0.33$	1.935 *
<i>S.marcescens</i>	$7.33 \pm 0.67$	$9.67 \pm 0.33$	$12.67 \pm 0.88$	2.704 **
<i>K.pneumonia</i>	$6.67 \pm 0.67$	$9.33 \pm 0.67$	$12.33 \pm 0.33$	2.812 **
<i>A. baumannii</i>	$4.67 \pm 0.33$	$7.33 \pm 0.33$	$10.33 \pm 0.33$	3.066 **
<b>LSD value</b>	1.757 **	1.878 **	1.936 **	---
* ( $P<0.05$ ), ** ( $P<0.01$ ).				

For ethyl acetate extract, Table 4, shows the concentrations 50 and 100 mg/ml were the highest effect on *S.marcescens* with inhibition zone  $21.00 \pm 0.57$  and  $22.67 \pm 0.88$  respectively. While the inhibition zone on gram positive bacteria *Staph. aureus* was  $14.33 \pm 0.67$  in concentration 100mg/ml. More-

over the inhibition zone on gram negative bacteria *A. baumannii*, *E. coli* and *K.pneumonia* were  $11.67 \pm 0.33$ ,  $12.33 \pm 0.33$  and  $13.33 \pm 3.84$  respectively in concentration 100 mg/ml with significant difference ( $P<0.01$ ).

**Table 4: Antibacterial activity of ethyl acetate peanut seed coat extract**

Isolate	Concentration (mg/ml)			LSD value
	25	50	100	
<i>Staph.aureus</i>	$10.33 \pm 0.33$	$11.67 \pm 0.88$	$14.33 \pm 0.67$	2.588 **
<i>E. coli</i>	$8.33 \pm 0.33$	$10.33 \pm 0.33$	$12.33 \pm 0.33$	2.169 **
<i>S.marcescens</i>	$16.33 \pm 0.88$	$21.00 \pm 0.57$	$22.67 \pm 0.88$	3.501 **
<i>K.pneumonia</i>	$6.67 \pm 0.33$	$8.33 \pm 0.33$	$13.33 \pm 3.84$	3.092 **
<i>A. baumannii</i>	$7.00 \pm 1.00$	$8.67 \pm 0.33$	$11.67 \pm 0.33$	2.784 **
<b>LSD value</b>	2.047 **	1.693 **	5.675 **	---
** ( $P<0.01$ ).				

Table 5, illustrates the significant difference ( $P<0.05$ ) and ( $P<0.01$ ) between concentrations and bacterial isolates for aqueous extract, the results show that the highest effect on gram positive bacteria *Staph. aureus* with inhibition zone  $18.67 \pm 0.67$  in concentration 100 mg/ml., likewise, aqueous extract was more active in gram negative bacte-

ria *E. coli* with inhibition zone  $15.67 \pm 0.88$  in the same concentration. While the lowest effect was on gram negative bacteria *K. pneumonia* and *A. baumannii* with inhibition zone  $8.67 \pm 0.33$  and  $9.33 \pm 0.33$  in concentration 100 mg/ml respectively.

**Table 5: Antibacterial activity of aqueous peanut seed coat extract**

Isolate	Concentration (mg/ml)			LSD value
	25	50	100	
<i>Staph.aureus</i>	11.00 ± 0.58	15.33 ± 0.88	18.67 ± 0.67	3.405 **
<i>E. coli</i>	9.33 ± 0.33	12.33 ± 0.88	15.67 ± 0.88	2.956 **
<i>S.marcescens</i>	8.33 ± 0.33	10.67 ± 0.33	13.67 ± 0.88	3.461 **
<i>K.pneumonia</i>	5.67 ± 0.33	6.67 ± 0.67	8.67 ± 0.33	2.706 *
<i>A. baumannii</i>	4.67 ± 0.33	7.33 ± 0.33	9.33 ± 0.33	2.664 *
<b>LSD value</b>	1.242 **	2.101 **	2.101 **	---
* (P<0.05), ** (P<0.01).				

The antibacterial activity in this study has been agreed with Lopes, (2011) who have reported the presence of antibacterial activity in peanut skin. Peanut extracts also exerted antimicrobial effects against *Escherichia coli* and *Listeria monocytogenes* (Quist, 2005). Results show that peanut skin extracted was found to be inhibited all tested bacteria, and show better inhibition for the Gram positive than Gram negative. Generally, plant extracts are usually more active against Gram positive bacteria than Gram negative bacteria. Many researchers suggest that flavonoids possess antibacterial activity (Sato *et al.*, 2000, Simin *et al.*, 2000, Zhao *et al.*, 2001 and Stapleton *et al.*, 2004).

## CONCLUSION

The preliminary phytochemical screening in the seed coat extracts of *Arachis hypogaea* showed a strong presence of flavonoids, phenols, alkaloids and tannins. The methanolic and ethyl acetate extracts of peanut skin possessed the highest phenolic and flavonoid contents than aqueous extract. In the present study, bacterial isolates showed increased resistance to commonly used antibiotics. The peanut skin extracts showed promising antibacterial activity against the resistant bacterial strains. Thus, the study suggests the use of peanut skin extracts in the treatment of various diseases caused by resistant bacteria.

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