

## Evaluation of Inhibitory Activity of the Aqueous & Alcoholic Extract of *Citrullus Colocynthis* towards *Staphylococcus Aureus*

Dr. Aliaa Saadon Abdul Razaq\*<sup>1</sup> Dr. Emad Hamdi Jassim<sup>2</sup> Dr. Jabra Ahmed Ankoosh<sup>3</sup>

1. Market Research & Consumer's Protection Center/ Baghdad University

2. Genetic Engineering & Biotech Institute for Post Graduate Studies/ Baghdad University

3. College of Agriculture/ Baghdad University

\* E-mail of the corresponding author: alia.sadon@yahoo.com

### Abstract

Number of chemical and alcoholic and aqueous extracts have been prepared from four parts of *Citrullus Colocynthis* which is the study focus that includes seeds, core, shell and fruit. The primary chemical diagnosis results show that the plant parts of the *Citrullus Colocynthis* of aqueous and alcoholic extracts contain the active ingredients, such as sponins, Flavonoids, Coumarins, Resins, Alkaloids, Tannins, Phenols, Glycosides and PH ranged between 5.34-5.63 with  $LSD=(P\leq 0.05)$ . All extracts for each part of plant were tested or examined for antimicrobial activity toward *Staph. Aureus* from different cutaneous infections (S1, S2, S3, S4, and S5). The results showed that antimicrobial activity depend on kind of extract and tested microorganism. The inhibition zone of aqueous extracts of dried parts of plants against S1 ranged between 5 mm for core and fruits, 9mm for seeds and 10mm for shell with  $LSD=(P\leq 0.05)$ , while S2 ranged between (3-7) mm for fruits and seeds respectively with  $LSD=(P\leq 0.05)$ , S3 (1-13) mm for shell and seeds respectively with  $LSD=(P\leq 0.05)$ , S4 (1-5) mm for fruits and shell with  $LSD=(P\leq 0.05)$ , and S5 (0-9) mm also for fruits and shell respectively with  $LSD=(P\leq 0.05)$ . While alcoholic extracts for dried parts of plant against S1 ranged between (10-5)mm for all parts of plants, S2(5-15), S3(10-15) mm. S4(5-10)mm and S5(5mm) for all parts with  $LSD=(P\leq 0.05)$ . while fresh alcoholic extracts for all parts of plant against S1(6mm) for core and (4mm) against S5 for core with  $LSD=(P\leq 0.05)$ . The fresh alcoholic extract was not active at different *Staph. Aureus* with  $LSD=0$ .

**Keywords:** *Citrullus Colocynthis*, methanolic extract, aqueous extract, biological activity.

### Introduction

The medical plants play an ultimate key role in human health where 80% of people worldwide rely on using traditional medicine for treatment (Nima *etal*2007). Due to the high use of chemotherapy, many germ and fungi isolates emerged for chemotherapy including the *Staph. Aureus* which is multi resistance against antibiotics as some strains show currently resistance for more than 20 antibiotic compounds including the antiseptics (Shyamala *etal*2009, Nima *etal*2007, Hamooshi and Mahmood 2008) that led to increase in concern in the recent years in the plant kingdom and medical herbs by the researchers; that is endless treasure to be used as major resources for production of medical drugs or as a resource of active materials which are part of medication composition. Also, they are used as raw materials for producing some chemical compounds and for producing some pharmaceutical materials (Nima *etal*2007, Majeed and Al Shati 2002).

*Citrullus Colocynthis* is considered one of the plants which received massive concern in the field of people's medicine such as this medical plant belongs to the Cucurbitaceae and it is called bitter apple and *Citrullus Colocynthis* is annual creeping with heavy branches and ball shaped fruits striped in yellow and when they become ripe, oval seeds form inside and *Citrullus Colocynthis* contains active matters, they are: Aaponins, Alkaloids, Glycosides and Resins and Pictin which is laxative and extreme diuretic and it is used for expelling the parasites from stomach and it is used for treating jaundice, rheumatism, headache, sciatica and back pains. Due to its antiseptic characteristics for treatment of wounds its aqueous extract is used as drink for removing, dyspepsia, diabetic, urinary tract infections, splenomegaly and Leucoderm and the plant is toxic in large quantities and the toxic part is found in the internal shell and seeds (Rodge and Biradar 2013, Prasomth *etal* 2012, Amine *etal* 2013, Mahdi 2011, Majeed and Al Shati 2002, Ali and Al Hasan 2002).

The study is aimed for testing the inhibitory activity of the aqueous and alcoholic extracts of the parts of *Citrullus Colocynthis* fruit on *Staph. Aureus* isolated from different directions.

### The Materials and Research Methods:

#### Plant Sampling:

*Citrullus Colocynthis* fruit has been obtained from the herb sellers in the local markets in the city of Baghdad and fresh *Citrullus Colocynthis* fruit has been obtained from the local markets in Basra during March 2014. The fruit samples have been broken down into seeds and core in the market research and consumer's protection center laboratories- Baghdad University to be prepared for extraction process where dried fruit has been broken down and then electrically crushed and kept in plastic sacks until be used. As for fresh fruit, the fruit has been well washed with water and then the plant parts of the fruit are peeled with sharp and disinfected peeler.

Examining the Groups and Active Compounds Existing in the Plants Parts Subject of the Research:  
The resins, tanins, Glycosides, Alkaloids, Phlyphons, Komarin, saponins and PH have been examined, as shown in (Ajina *etal* 2009).

#### Preparation of Plant Aqueous Extracts:

The extract has been prepared by taking (20) mm of parts of *Citrullus Colocynthis* powder (seeds, shell, core) and each fruit apart (independently per part) and to have them solved in (200) ml of distilled sterilized water in water bath boiled for half an hour and then the mixture is screened through several layers of gauze and then centrifuged at rate of 3000 RPM for quarter an hour and then the extracts have been disinfected when they are passed through 0.2 micrometer diameter membrane screens (Shofalih 2005).

#### Preparation of Alcoholic Plant Extracts:

They have been prepared by taking (20) gm of each part of *Citrullus Colocynthis* as well as the fruit itself apart and extracted in (200) ml of methanol alcohol and then placed in the (500) ml conic flasks in a vibrating incubator under 37° C for 6 hours. The extract then dehydrated in an oven at (5-40)° C for (24) hours, then the dried matter has been collected and kept in disinfected plastic bottles until they are used. (Rani *etal* 2010)

#### Preparation of Bacterial suspension:-

The bacterial suspension has been prepared in concentration of  $10^8 \times 1.5$  bacterial cell/ ml by Mcfoland technique as previously prepared (Jabbar 2007) for five isolations of *Staph. Aureus* shown in table (1) which was obtained from microbiology laboratory of market research and consumer's protection center laboratories-Baghdad University as they have been examined after being biochemically tested and activated on the nutritional broth.

**Table (1): represents the bio- isolations of *Staph. Aureus*:**

Isolation no.	Germ
S1	Skin pus
S2	Gingivitis
S3	Gingivitis
S4	Sputum
S5	Sputum

#### The Evaluation of the Inhibitory Activity of Plant Extracts Against Microorganisms:

The -well diffusion technique has been used including preparation of nutritional agar medium as per the supplier's instructions and poured in Petri dishes and then the medium has been vaccinated with 0.1 ml of the prepared bacterial suspension for each bio isolation of tested *Staph. Aureus* and the suspension is diffused by using a disinfected piece of cotton and the dishes have been left for quarter an hour and then (2-3) wells have been made of 10mm in diameter by using cork driller. The dried alcoholic extract of the plant has been dissolved in dimethyl sulphoxide (DMSO) in concentration of (2) mg/ml and (0.1) ml has been added for each of the prepared raw aqueous and alcoholic extracts by using the mini absorber to the wells on basis of two aureus for each germ type of *Staph. Aureus* isolations for all extracts and then the dishes have been incubated at 37°C for 24 hours and the inhibition diameter has been measured apart from the well diameter (Perez and Bazerque 1990). Then, the concluded findings have been compared with two types of commonly used wide spectrum antibiotics as positive controller Amoxicilin (Amx.25mcg) and Ampicilin (Cloxacillin (Apx.25mcg) in order that the germ isolation sensitivity can be tested of *Staph. Aureus* to the antibiotics.

#### Statistical Analysis System (SAS)

The alcoholic and aqueous extracts of dried and fresh *Citrullus Colocynthis* by using SAS program (2012) for studying the effect of the factors considered in the different characteristics and LSD- Difference Significant among the averages by testing LSD- Less Significant Difference under level of ( $P \leq 0.05$ ) (SAS 2012).

#### Conclusions & Discussion:

##### Testing the groups and compounds existing in parts of *Citrullus Colocynthis* the subject of study

The table (2) and (3) show primary chemical testing on the effective compounds and groups in the aqueous and alcoholic extracts of the plant of *Citrullus Colocynthis* parts the subject of the study. The results showed that all parts used in the study contain resins, tanins, Glycosides, Alkaloids, Phlyphons, Komarin, saponins and PH values of the aqueous extracts were 5.34 as in the core and 5.63 as in the fruit and the alcoholic extract are between 6.25 as in the shell and 6.88 as in the fruit with LSD- Significant Difference of ( $P \leq 0.05$ ). Some findings conformed with the current study results as mentioned (Bander *etal* 2009, Amine *etal* 2013, Prasomth *etal* 2012,

Rodge and Biradar 2013) that the Citrullus Colocynthis fruits contain resins, tanins, Glycosides, Alkaloids, Alkaloids, Phlyphons, Komarin, saponins and this may be a reason in rendering the PH value low due to the existence of the most significant materials in it, they are Colocynthin as they are Glycosides of high bitterness, resins, Pictin and saponin (Majeed and AL Shati 2002).

**Table (2): Results of Primary Specific Tests of dried Citrullus Colocynthis in the aqueous extracts.**

Tests		Plant Aqueous Extracts				LSD List
		Seeds	Core	Shell	Fruit	
1	PH	5,60	5,34	5,50	5,63	NS 0,73
2	Saponins	+	+	+	+	NS 0,00
3	Phlyphon	+	+	+	+	NS 0,00
4	Komarin	+	+	+	+	NS 0,00
5	Resins	+	+	+	+	NS 0,00
6	Alkaloids	+	+	+	+	NS 0,00
7	Tannins	+	+	+	+	NS 0,00
8	Phynols	+	+	+	+	NS 0,00
9	Clycosides	+	+	+	+	NS 0,00

NS is LSD

**Table (3): Results of Primary Specific Testing of Dried Citrullus Colocynthis of the Alcoholic Extract**

Tests		Plant AlcoholicExtracts				LSD List
		Seeds	Core	Shell	Fruit	
1	PH	6,38	6,44	6,25	6,88	0,533*
2	Saponins	+	+	+	+	NS 0,00
3	Phlyphon	+	+	+	+	NS 0,00
4	Komarin	+	+	+	+	NS 0,00
5	Resins	+	+	+	+	NS 0,00
6	Alkaloids	+	+	+	+	NS 0,00
7	Tannins	+	+	+	+	NS 0,00
8	Phynols	+	+	+	+	NS 0,00
9	Clycosides	+	+	+	+	NS 0,00

NS is LSD=( $P \leq 0.05$ )\*

**The Effect of Aqueous and Alcoholic Extracts of Dried and Fresh Citrullus Colocynthis on Inhabitation of the Test Bacteria:-**

Table (4) shows the averages of inhabitation zone formed by the aqueous extract of the fresh Citrullus Colocynthis against antimicrobials of the tested Staph. Aureus where the aqueous extract of Staph. Aureus seeds showed strong inhibitory effect on S3 at inhabitation diameter of 13 mm and the aqueous extract of Citrullus Colocynthis shell has strong effect on S1 and S5 at Citrullus Colocynthis diameter of 10mm and 9mm, respectively with  $LSD = (P \leq 0.05)$ . the aqueous extract of Citrullus Colocynthis core and seeds showed effect of 7mm to S2 and S3 with  $LSD = (P \leq 0.05)$ .

Table (5) shows inhabitation zone diameter in mm for the growth of the antimicrobials when treated with alcoholic extracts of dried Citrullus Colocynthis where it has been found there is sensitivity of S1 towards Citrullus Colocynthis seeds and fruit at inhabitation zone of 25 mm and sensitivity of S1, S2, S3 of Citrullus Colocynthis core and S3 for the Citrullus Colocynthis shell at inhabitation effect of 15mm. the plant parts of Citrullus Colocynthis fruit towards which S1, S2, S3 and S4 showed sensitivity at inhabitation zone of 10mm at  $LSD = (P \leq 0.05)$ .

**Table (4): Inhabitation Zone Diameters (mm) for the Growth of Antimicrobials When Treated with Plant Aqueous Extracts of Dried Citrullus Colocynthis:**

Used Antimicrobials		Plant Aqueous Extracts					LSD
		Seeds	Core	Shell	Fruit	Control (D.W)	
1	S1 Leucoderm	9	5	10	5	0	3,17*
2	S2 gingivitis	7	5	4	3	0	2,33*
3	S3 gingivitis	13	7	1	2	0	3,05*
4	S4	2	2	5	1	0	2,27*
5	S5	5	5	9	0	0	2,84*
LSD		03.82	02.69	03.77*	3.04*	NS 0.00	----

\*NS=(P≤0.05)

**Table (5): Inhabitation Zone Diameters (mm) for the Growth of Antimicrobials When Treated with Plant Alcoholic Extracts of Dried Citrullus Colocynthis:**

Used Antimicrobials		Plant Aqueous Extracts					LSD
		Seeds	Core	Shell	Fruit	Control (D.W)	
1	S1 Leucoderm	25	15	10	25	0	6,26*
2	S2 gingivitis	10	15	10	5	0	4,88*
3	S3 gingivitis	10	15	15	10	0	5,36*
4	S4	10	10	5	10	0	5,29*
5	S5	5	5	5	5	0	2,50*
LSD		4,83*	5,26*	54,77*	4,972*	NS0.00	-----

\*NS=(P≤0.05)

Table (6) shows the pharmaceutical sensitivity of the antimicrobials tested towards some antibiotics where the antibiotics Amoxicillin and Ampicillin gave preference in use as S, S2, S3 showed high sensitivity towards these two antibiotics. S4 and S5 showed high resistance towards them.

**Table (6): Pharmaceutical Sensitivity of the Tested Antimicrobials towards Some Antibiotics**

Used Antimicrobials		Plant Aqueous Extracts		LSD
		Amoxicillin Amx (25 mcg)	Ampicillin/ Cloxacillin Apx (25 mcg)	
1	S1 Leucoderm	25	15	5,20*
2	S2 gingivitis	21	13	4,16*
3	S3 gingivitis	18	10	4,09*
4	S4	0	0	NS0.00
5	S5	0	0	NS 0.00
LSD		5,628	4,79*	-----

\*NS=(P≤0.05)\* not significant

We see from Table (6) that the fresh Citrullus Colocynthis core towards which the sensitivity were shown by S1 and S5 at inhibition diameter of 6mm and 4mm, respectively. With LSD=(P≤0.05). The other antimicrobials had high resistance towards the plant alcoholic extracts of fresh Citrullus Colocynthis parts.

We conclude from the above table (7,5,4) that alcoholic and aqueous extract of dried Citrullus Colocynthis the research subject have better results than and surpass the alcoholic extract of fresh Citrullus Colocynthis. The reason of that is the concentration of the toxic matter of the two Citrullus Colocynthis containing Glycosides, resins and Saponins which resulted in showing high sensitivity by different antimicrobials of Staphylococcus aureus towards the parts of Citrullus Colocynthis and the fruit itself. This means that they have high effect against inflammation.

**Table (7): Inhibition Zones Diameters (mm) of Antimicrobial Growth When Treated with the Plants Extracts of Fresh Citrullus Colocynthis**

Antimicrobials		Plant Alcoholic Extracts				LSD List
		Seeds	Core	Shell	Control (D.W.)	
1	S1 Leucoderm	0	6	0	0	2,42*
2	S2 gingivitis	0	0	0	0	NS 0,00
3	S3 gingivitis	0	0	0	0	NS 0,00
4	S4	0	0	0	0	NS 0,00
5	S5	0	4	0	0	02,05
LSD		NS0,00	2,38	02,38	NS0.00	-----
NS is LSD=( $P < 0.05$ )* not significant						

### Resources

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