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Effect of Arak (*Salvadora persica*) and Cinnamon (*Cinnamomum spp.*) Extracts on Foulbrood Bacteria *In vitro*

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ABSTRACT

The antibacterial activity of the aqueous and ethanolic extracts of both Arak (*Salvadora persica*) and Cinnamon (*Cinnamomum spp.*) plants was evaluated on the foulbrood bacteria under the laboratory conditions instead of the synthetic antibiotics [it is surmised that is first time to evaluate Arak plant against the honeybee foulbrood pathogens]. The major antimicrobial constituents (total phenolics, flavonoids and tannins) were determined quantitatively in the extracts using the spectrophotometer technique. The results showed these extracts had significant antibacterial effects against the selected pathogens, *Paenibacillus larvae* and *Melissococcus pluton*.

Keywords: Siwak extracts, Cinnamon extracts, American & European Foulbrood.

INTRODUCTION

Honeybee colonies exposed to numerous pathogens caused severe damage to beekeeping industry worldwide, and the economically most important diseases of honeybees are the bacterial brood diseases as European Foulbrood (EFB) and American Foulbrood (AFB) (Shimanuki, 1997 and Genersch, 2010).

AFB was caused by only *Paenibacillus larvae* subsp. *larvae* (*P. l. l.*) spore_bacterium by coordination of Shimanuki & Knox (1991), Alippi (1997), Hansen & BrØdsgaard (1999) and Scuch *et al*. (2001). While EFB caused by another non spore_bacterium, *Melissococcus pluton* (*M. pluton*) according to Shimanuki & Knox (1991).

Since year of 1996 to now the known symptoms of these diseases had appearing in several Egyptian apiaries according to observations of several researchers such as Khattab (1997), Mattar (2001), Khattaby (2004 & 2006), Zakaria (2007), Owayss (2007), Mostafa *et al*. (2008), Hashish *et al*. (2008), Mabrouk (2008), Ibrahim (2009), Gomaa (2009), Sanad & Al-Barrak (2010), Goda (2011), Fathy *et al*. (2012), Kamel (2012), Abou_Lila (2012), Omar *et al.* (2013), Masry *et al.* (2014) and Ma'moun (2014).

To avoid problems of the synthetic antibiotics, there are many plant extracts and essential oils were laboratory examined and used previously for controlling these pathogens in the honeybee colonies like as *Cinnamomum spp*., *Sysygium aromaticum*, *Mentha piperita*, Clove, eucalyptus, thyme and lemon grass (Calderon *et al*., 1994; Alippi *et al*., 1996; Al_Hojaymi, 2005 and Goda, 2011).

So, the thought in this presented study was a using of extracts of two natural materials which were sticks of Arak plant (Siwak) (*Salvadora persica*) and bark of Cinnamon plant (*Cinnamomum spp.*) harmonization with the later researchers in the same trend aimed to avoidance off the chemical antibiotics with their negatives because they always cause in weakening the honeybee and in contamination of the bee's products by their disintegrated residues which were more seriousness acceptance to results of Albo *et al*. (2003), Reybroeck (2010) and Kamel *et al*. (2013), furthermore allowing with creation of new *P. l. l.* strains or varieties are resistances to the cures such as what happened by using of OTC antibiotic acceptance to results of Alippi *et al.* (1996), Miyagi *et al*. (2000) and Evans (2003).

MATERIALS AND METHODS

The presented work was carried out in year 2011 under the suitable sterilized conditions, and the study was mainly divided into compartments, the first one was Microbial Tests at the specific microbial laboratory for examination of honeybee diseases, Fac. Agric., Moshtohor, Benha Univ., and the other was determination of certain active compounds in the extracts at laboratory of honeybee products analysis, Honeybee Res. Dep., Agric. Res. Cent., Egypt.

1. Microbial tests

a. The isolated bacteria which used

Two different bacteria were used for this assay, which were *Paenibacillus larvae* subsp. *larvae* (*P. l. l.*), the caused spore-bacterium of American foulbrood (AFB) disease, and *Melissococcus pluton* (*M. pluton*), the caused non spore-bacterium of European foulbrood (EFB) disease, whereas they were isolated according to Anderson (1990), Shimanuki & Knox (1991), Hornitzky & Clark (1991), Hornitzky & Nicholls (1993), Nordstrom & Fries (1995), Djordjevic *et al*. (1998) and Hornitzky (1998a, b) on Sheep Blood Agar (SBA) medium and on Bailey medium respectively from samples appeared the known diseased symptoms whether respecting to AFB according to Shimanuki & Knox (1991), Alippi (1997), Hansen & BrØdsgaard (1999) and Scuch *et al*. (2001) «Figs. 1 and 2», or respecting to EFB according to Shimanuki & Knox (1991), «Figs. 3 and 4». Also, for confirming of the isolates, some tests were performed such as description of the bacterial colonies characters according to Djordjevic *et al*. (1998) and Hoyo *et al*. (2001), catalase production, Gram stain test and the negative stain were examined according to Shimanuki & Knox (1991).

b. Preparation of aqueous and ethanolic extracts of both Arak sticks (Siwak) and Cinnamon bark

Deduction from Almas (2001), Al-Bayati & Sulaiman (2008), Mahfuzul *et al*. (2008), Muthiah

(2008) and Shkr *et al*. (2011), the extracts were prepared as following:

1kg dried sticks of Arak stems named Siwak (*Salvadora persica*) and 1kg of powdered Cinnamon barks (*Cinnamomum spp.*) were purchased from a local retail market, Arak sticks were cut into small pieces and ground by a grinder (SONAI, Model MAR 3000), and then powders of both Arak sticks and Cinnamon bark were separately sieved in a screen cloth to obtainment on a very fine powder. For preparation of the aqueous extracts, 200gm fine powder of each plant were separately good mixed with 800ml of deionized sterile distilled water and soaked in which in a 1000ml sterilized beaker and were kept at 40°C for 48hrs, then filtered by using of Whatman No. 1 filter paper (the soaking process was repeated several times until the upper liquid of the mixture became like colorless). The filtrates were evaporated in a vacuum and dried by heating at 40-50°C by using an oven or incubator until thick pastes were formed which were considered 100% concentration of extracts. The extraction was repeated by following the same procedure exception of the solvent which was replaced with ethyl alcohol 95% for preparation of the ethanolic extracts. Thus, plant extracts already became presence as 100% in concentration, and they were Aqueous Siwak Extract (ASE), Ethanolic Siwak Extract (ESE), Aqueous Cinnamon Extract (ACE) and Ethanolic Cinnamon Extract (ECE), and these pastes or final dried materials were stored in labeled sterile bottles and kept in freezer of the refrigerator until the using time.

c. Assay the antibacterial activity of the different extracts on the foulbrood bacteria (*in vitro*)

Firstly, three serial concentrations 10, 30 and 50% (*w/v*) were prepared from each extracted paste (100% conc) of the four extracts separately by mixing with appropriate volumes of the similar solvent (deionized sterile distilled water or ethyl alcohol 95%) according to Pirson square of the dilution.

For subculture and treating of *P. l. larvae* bacterium, MYPGP medium was used, whereas it was assembled and prepared according to Nordstrom & Fries (1995), but Bailey medium which previously mentioned was used for subculture and treating of *M. pluton* bacterium. The plant extracts were tested on the desired bacteria by the filled wells method (ditch plate method), whereas by using of aseptic tube was 4mm diameter, three circular hollows were made in 5 dishes of each medium, then hollows of 1 dish of each medium were pipetted and filled with 50µl from only one extract of the plant four extracts (ASE, ESE, ACE or ECE) in concentrations 50, 30 and 10% separately (the result was 4 dishes of each medium were treated by the extracts). But hollows of the 5th dish of each medium differed in the treatment, whereas in them, 1st and 2nd hollows were pipetted and filled with 50µl just from whether of the two solvents, deionized sterile distilled water or ethyl alcohol 95% as a negative control, while for comparison or as a positive control, the 3rd hollow was pipetted and filled with 50µl from the veterinary antibiotic which named Tylosin diluted to concentration 100ppm used by Mostafa (2009) «Antibiotic powder manufactured by ADWiA company & each 1gm contained on 228mg of tylosin tartrate and equaled to 200mg of tylosin base, and then a sterilized cotton swab was used to spread portions of a spore suspension from *P. l. larvae* isolated bacterium streaky on surface of whole MYPGP dishes, finally the plates were left for 1hr at room temperature and then incubated aerobically at 37 ± 1˚C 72hrs. While another sterilized cotton swab was used to spread portions of a cell suspension from *M. pluton* isolated bacterium streaky on surface of whole Bailey dishes, finally the plates were left for 1hr at room temperature and then anaerobically incubated at 34 ± 1˚C 4 days. The whole plates (Petri dishes) examined for inhibition zones of the bacterial growth around the extract or the treated wells, the values of these zones were recorded in millimeters by using of a ruler. The tests in the Petri dishes were performed triplicate then whole values were subjugated to the statistical analysis of variances in a randomized complete block design (ANOVA) by MSTAT-C version 1.41 pursuant to Sendecor & Cochran (1980), then all means were compared by Duncan's multiple range test at level 0.05 pursuant to Steel & Torri (1980).

2. Determination of certain active compounds in the extracts

Firstly, only 1gm of each extract pastes 100% concentration that resulted from the aqueous or ethanolic extraction of both the two plants was separately dissolved and completed to 100ml of suitable solvent (deionized sterile distilled water or ethyl alcohol 95%), thus four extracts 1% concentration were obtained and they were Aqueous Siwak Extract (ASE), Ethanolic Siwak Extract (ESE), Aqueous Cinnamon Extract (ACE), and Ethanolic Cinnamon Extract (ECE).

a. Total Phenolics (TP) Content

The samples were prepared according to Shahidi & Naczk (1995) and Al_Waterhouse (2002) and the absorbance was determined using biochrom Libra S12 UV-Vis spectrophotometer at 765nm by colorimetric assay, whereas gallic acid standard curve was first prepared from 0-320mg/ l and total phenolics were expressed in mg gallic acid equivalent (GAE) / gm dry matter and calculated from the prepared standard curve with 0 to 100mg/ gallic acid (GA).

b. Total Flavonoids (TF) Content

The samples were prepared according to Proestos *et al*. (2006) and the absorbance was read by using biochrom Libra S12 UV-Vis spectrophotometer at 510nm by colorimetric assay, whereas a standard curve of quercetin dissolved in 80% ethanol was initially prepared from 0-1000µg/ ml. and total flavonoids were expressed in mg Quercetin equivalent (QE)/ gm dry matter.

c. Total Tannins (TT) Content

The samples were prepared according to Tamilselvi *et al*. (2012) and absorbance was read by using biochrom Libra S12 UV-Vis spectrophotometer at 725nm, whereas gallic acid standard solution were first prepared from 0-320mg/ l and the total tannins were expressed as GAE/ gm dry matter and calculated from the prepared standard curve with 0-100 mg/ GA.

Respecting to all assays, the analysis of both sample and standard were made in triplicates, and percentages of the value means were calculated according to the equation:

$$\% \text{ Content} = \frac{\text{Sample Abs}}{\text{Standard Abs}} \times \frac{\text{Sample Volume}}{\text{Standard Volume}} \times \frac{\text{Standard Weight}}{\text{Sample Weight}} \times 100$$

Then whole values were statistical analyzed, finally the % mean of content ± standard error were presented.

"Laboratory experiments"

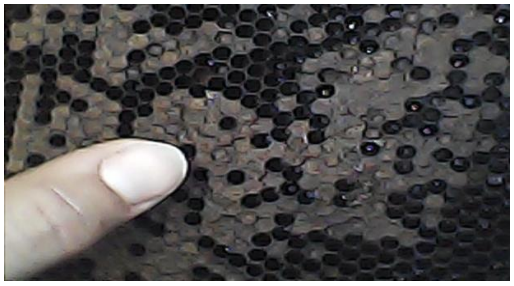


Fig. 1; AFB Symptoms.

Fig. 2; A ropy threadlike material AFB only.

Fig. 3; EFB Symptoms.

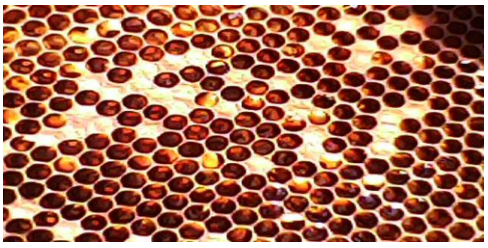


Fig. 4; Conglobated and dried larvae

RESULTS AND DISCUSSION

These results comprised the bioassay of Arak and Cinnamon extracts against the microbial causative agents of American and European foulbrood diseases, whereas the bacteria were isolated, purified and accurately identified before the bioassay.

1. Confirmation of the isolated bacteria

a. Morphology describe of the bacterial colonies

The bacterial colonies which were cultured from AFB sample agreed with *P. l. l.* bacterium as description of Drobnikova *et al*. (1994), Nordstrom & Fries (1995), Hornitzky (1998 b), Hoyo *et al*. (2001) and Chantawannakul & Dancer (2001), whereas the colonies appeared slowly and singly on sheep blood agar (SBA) medium, measured about 4mm diameter, had irregular edges, flat, light grey, non-pigmented «Fig. 5». While the bacterial colonies which cultured from EFB sample, appeared very slowly on Bailey medium, were single colonies, very small measured 1-2mm diameter, biconvex, shape circular, had regular edges, white of color and sometimes grow deeply inner the medium «Fig. 6», and these growths belonged to *M. pluton* bacterium agreed with Anderson (1990) and Djordjevic *et al*. (1998)

b. Catalase production test

The bacterial colonies isolated on SBA medium gave a negative reaction with catalase test per reason was non-ability of them on producing to catalase enzyme which analyses hydrogen peroxide (H_2O_2) and this result agreed with Shimanuki & Knox (1991).

c. Gram stain test

By the microscopic examination to the stained films which were prepared from SBA culture, it was seen that flooded rod forms with a blue or a violet color, measured as 1.5-6.0µm long and as 0.5-0.6µm wide «Fig. 7», blue rods during transformation to endo-spores «Fig. 8», and very small ellipsoidal bright spores measured as 1.2µm length and 0.6µm in diameter «Fig. 9», and Piccini & Zunino (2001) had steered similar descriptions for the *P. l. l.* bacterium.

d. The negative staining

The prepared films of culture on SBA medium appeared rods flooded with light violet color and the spores remained bright on dark blue background «Fig. 10», while the prepared films of culture on Bailey medium appeared big spherical or lanceolate forms in pairs or chains and bright on black ground «Fig. 11», whereas similar descriptions were stated by Alippi (1991) and Djordjevic *et al*. (1998) for *P. l. l.* bacterium and *M. pluton* bacterium respectively.

2. Assay the antibacterial activity of the different extracts on the foulbrood bacteria (*in vitro*)

It came to light from Table (1) that all used extracts (aqueous and ethanolic extracts of Arak (*Salvadora persica*) and Cinnamon (*Cinnamomum spp.*) plants had an antibacterial activity at their cleared concentrations against both of the two foulbrood bacterial growth on the culturing media «Figs. 12 - 19» but with varying degrees and without inducing of a complete inhibition, whereas;

Table (1): Mean of inhibition zone's diameters (IZD) of bacterial growth in mm by Siwak extracts, Cinnamon extracts and Tylosin

Extract	Concentration	IZD in mm	
		$LSD_{0.05}$ = 0.340	$LSD_{0.05}$ = 0.404
		P. l. l.	*M. pluton*
ASE	50%	$9.7^{j} \pm 0.667$	$12.0^{j} \pm 1.001$
ASE	30%	$6.2^{k} \pm 0.602$	$7.7^{k} \pm 0.883$
ASE	10%	$0.0^{m} \pm 0.000$	$2.5^{m} \pm 0.765$
ESE	50%	$24.0^{e} \pm 1.001$	$25.7^{e} \pm 0.883$
ESE	30%	$13.7^{i} \pm 0.667$	$14.5^{i} \pm 0.867$
ESE	10%	$4.5^{l} \pm 0.867$	$5.3^{l} \pm 1.203$
ACE	50%	$27.0^{c} \pm 1.156$	$29.5^{c} \pm 1.042$
ACE	30%	$21.3^{f} \pm 0.883$	$24.2^{f} \pm 0.441$
ACE	10%	$16.7^{h} \pm 0.667$	$20.0^{h} \pm 1.156$
ECE	50%	$33.3^{a} \pm 0.882$	$35.8^{a} \pm 0.883$
ECE	30%	$24.7^{d} \pm 0.334$	$28.0^{d} \pm 1.001$
ECE	10%	$19.3^{g} \pm 0.883$	$21.8^{g} \pm 0.883$
Tylosin	100ppm (0.01%)	$28.8^{b} \pm 1.203$	$35.3^{b} \pm 0.667$

IZD; Inhibition zone's diameter
$LSD_{0.05}$; low significant difference at alpha 0.05
P. l. l; Paenibacillus larvae subsp. Larvae bacterium causative of American foulbrood disease
M. p; Melissococcus pluton bacterium causative of European foulbrood disease
ASE; Aqueous Siwak Extract
ESE; Ethanolic Siwak Extract
ACE; Aqueous Cinnamon Extract
ECE; Ethanolic Cinnamon Extract
(A, b, c …); Marks of the ranks

In case of Arak (Siwak) plant

By scrutiny of the inhibition areas in Table (1) and through the different concentrations, we found that Ethanolic Siwak Extract (ESE) had preceded by its highest concentration which 50% on Aqueous Cinnamon Extract (ACE) in its two lower concentrations, while in the same concentration 50%, ESE had surpassed on Aqueous Siwak Extract (ASE) in relation to its ability on inhibition of *P. l. l.*, which the latest extract gave inhibition zones were 9.7, 6.2 and 0.0 mm at concentrations 50, 30 and 10% respectively, while ESE gave IZDs were 24.0, 13.7 and 4.5mm at same concentrations respectively. Also, we found for inhibition of *M. pluton* that same trend had happened with those, whereas at concentrations 50, 30 and 10%, ESE induced IZDs 25.7, 14.5 and 5.3mm respectively, but ASE gave IZDs 12.0, 7.7 and 2.5mm respectively.

Al-Bayati *et al*. (2010) in the dental medicine field, examined the ethanolic extract of Arak plant by certain concentrations as antibacterial for protective the tooth and curative of plaque comparison with 0.2% chlorhexidine digluconate as chlorhexidine mouth wash also with placebo, and they assessed the acute toxicity of the extract on group of 32 male albino mice then on group of Male dental student volunteers according to specific and restricted conditions, and lastly, they concluded from their results that *Salvadora persica* alcohol extract produced remarkable antibacterial activity *in vitro* at concentration 10mg/ ml extract, was well tolerated and safe. As a mouth rinse was less effective than chlorhexidine in preventing plaque accumulation and more effective than placebo on dental plaque accumulation.

Successively Shkr *et al*. (2011) had also examined alcohol extracts of *Salvadora persica* L. (fresh and dry plant) which were screened *in vitro* for activity against *B. subtilis, E. coli, Lactobacillus brevis, Proteus vulgaris, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* using standard Agar Disc-Diffusion assay. Chloramphenicol and Miconazole were used as referee standards, and authors demonstrated that antimicrobial activity of alcohol extracts of herbaceous parts of *S. persica* L. revealed a higher inhibition zone to *Proteus vulgaris* followed by other tested organisms which exhibited less zone of inhibition such as *B. subtilis, Lactobacillus brevis, Staphylococcus aureus, E. coli, Candida albicans* and *Aspergillus niger*. And this result confirmed that *S. persica* L. contained substances with antimicrobial properties.

In case of Cinnamon plant

Respecting to spore_bacterium *P. l. l.*, we found within each concentration that Ethanolic Cinnamon Extract (ECE) had surpassed in its antibacterial power on Aqueous Cinnamon Extract (ACE) besides the other extracts, which the latest gave inhibition areas were 27.0, 21.3 and 16.7mm at concentrations 50, 30 and 10% respectively, while ECE gave IZDs (inhibition zone diameters) were 33.3, 24.7 and 19.3mm at same concentrations respectively. Also, ECE had surpassed within each concentration on all other extracts in its ability for inhibition of *M. pluton*, whereas the IZDs of

ECE at the mentioned concentrations respectively were 35.8, 28.0 and 21.8mm, while ACE gave inhibition zones 29.5, 24.2 and 20.0mm respectively.

Mukhtar & Ghori (2012) demonstrated that ethanolic extract of the Cinnamon plant was more effective than the aqueous extract, whereas the first exhibited inhibition zones as 16mm against *B. subtilis* DSM 3256 spore_bacterium (this bacterium interlaces in several characters and in the culturing conditions with *P. l. l.* bacterium) and as 17mm against non spore_bacterium *E. coli*, subsequently the Cinnamon ethanolic extract was equally effective against both Gram negative and Gram positive bacteria.

In contrast with our results, Goda (2011) examined the ethanolic extract of Cinnamon, but this extract didn't success for inhibiting whether of *P. l. l.* or *M. pluton*, while Al_Hojaymi (2005) stated that both of ethanol and hexane alcohol extracts of *Cinnamomum celanicum* plant were examined for inhibition *B. larvae* in the laboratory by loading method with disc diffusion assay, and the results appeared that Hexane alcohol extract of Cinnamon was the most effective on *B. larvae* at 2000µg concentration whereas it completely inhibited the bacterial growth to area 50mm, but the used concentrations of the ethanol alcohol extract weren't stated furthermore the inhibition areas which were induced by them.

Noteworthy in our work that solvents which were used for extraction both of the two plants didn't inhibit the bacterial growth whether *P. l. l.* or *M. pluton* bacterium on the culturing media, and by comparison, the veterinary antibiotic Tylosin tartrate had inhibited the bacterial growth around the injection an area 28.8mm in case *P. l. l.* bacterium and an area 35.3mm in case *M. pluton* bacterium «Figs. 20- 21». On basis of our results and the previous presentation there were five facts;

1st fact The concentrations which higher than or upper 50% of whether Siwak or Cinnamon extracts exactly have good antibacterial activities and will definitely give better results against the foulbrood bacteria.

2nd fact Overall, Cinnamon was more effective than Siwak and the ethanolic extracts were superior on the aqueous extracts for inhibition of the foulbrood bacterial growths in Petri dishes.

3rd fact *M. pluton* bacterium was more sensitive than *P. l. l.* bacterium for the treating by extracts of Siwak and Cinnamon.

4th fact Antibiotic Tylosin stilled have a high effect against the foulbrood bacteria with full reservation and caution from using it because of its contribution in generation and in development of foulbrood bacterial races are most resistances when controlling in the bee hive as well as Kamel *et al*. (2013) referred.

5th fact Power of the two plants' extracts for affecting on growths of the foulbrood bacteria exactly was due to these extracts contained on contents or ingredients which had a high antibacterial act, and this thinking or reasoning we tried to its evidence by the chemical analysis which their results came next.

3. Determination of certain active compounds in the extracts

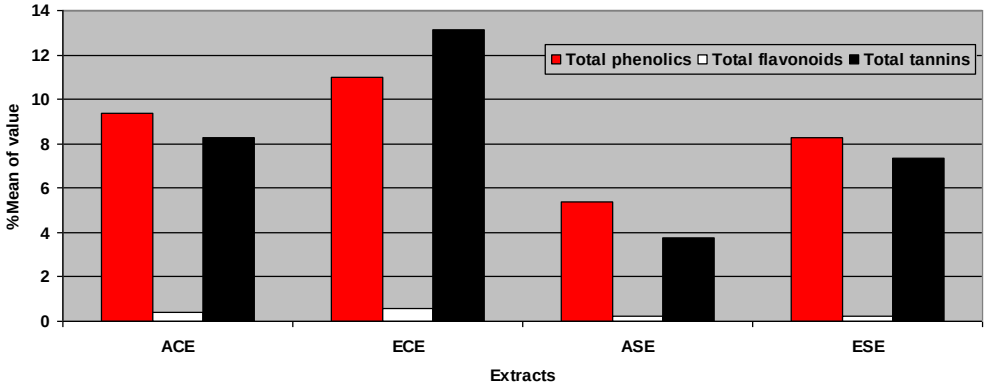
The results which tabulated in Table (2) and represented in Figure (4) were percentages of mean values of triplicate assay of some active ingredients; total phenolics, flavonoids and tannins compounds in the two plant extracts (Arak and Cinnamon) which extracted by ethanol 95% and deionized distilled water as extraction solvents, and it was obvious that ethanolic extraction recorded the highest content of total phenolics, flavonoids and tannins in compared with water extraction, and those were supported by what said by many researchers, whereas Khuwijitjaru *et al*. (2012) decided that extracts from Cinnamon bark using subcritical water treatment at 200°C contained higher amounts of the total phenolics content than those extracted at 150°C for every extraction time, also the total phenolics content and DPPH (1,1- Diphenyl - 2-picryl hydrazyl), radical scavenging activity from the organic solvent extraction (50% methanol) were 139±6mg GAE/ g dry sample. On other hand, Kamil *et al*. (1999), Garboui *et al*. (2009) and Abdillahi *et al*. (2010) stated that *Salvadora persica* was known to containing on several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates

Table (2): Differences between Siwak and Cinnamon extracts in mean percentage of total phenolics, flavonoids and tannins contents

Plant extract	Siwak		Cinnamon		
Active ingredient	Aseptic Water (ASE)	Ethanol 95% (ESE)	Aseptic Water (ACE)	Ethanol 95% (ECE)	$LSD_{0.05}$
Total phenolics	$5.362^{d} \pm 0.043$	$8.273^{c} \pm 0.216$	$9.345^{b} \pm 0.129$	$11.015^{a} \pm 0.015$	0.438
Total flavonoids	$0.203^{d} \pm 0.006$	$0.260^{c} \pm 0.017$	$0.399^{b} \pm 0.012$	$0.550^{a} \pm 0.016$	0.044
Total tannins	$3.761^{d} \pm 0.105$	$7.358^{c} \pm 0.355$	$8.250^{b} \pm 0.021$	$13.130^{a} \pm 0.039$	0.602

ASE; Aqueous Siwak Extract
ESE; Ethanolic Siwak Extract
ACE; Aqueous Cinnamon Extract
ECE; Ethanolic Cinnamon Siwak Extract
$LSD_{0.05}$; Low significant difference at alpha 0.05
(A, b, c …); Marks of the ranks

Fig.(22): Differences between Siwak and Cinnamon in percentage mean of total phenolics, flavonoids and tannins contents



ASE; Aqueous Siwak Extract **ESE; Ethanolic Siwak Extract**
ACE; Aqueous Cinnamon Extract **ECE; Ethanolic Cinnamon Siwak Extract**

The highest value in total phenolics was in ethanolic extract of Cinnamon (ECE) (11.015±0.015%) and the lowest value was recorded in water extract of Siwak (ASE) (5.362±0.043%), and data of the total phenolics showed a significant difference between the two plants (cinnamon and Arak), also between the two extract solvents in the one plant.

The data in same table (2) showed a high significant difference in total flavonoids, and the extract ASE showed the lowest value (0.203±0.006%), on other hand ECE extract showed the highest value (0.550±0.016%).

Generally, experimental data of total tannins statistically revealed differences between Arak and Cinnamon plants, the extract ESE recorded (7.358±0.355%), ASE extract recorded (3.761±0.105%), ECE extract recorded (13.130±0.039%) and ACE extract recorded (8.250±0.021%).

All previous measures were nearby to certain other results, whereas respecting to Cinnamon, there were Muchuweti *et al*. (2007) who said that concentration of the phenolics compounds in the following spices ranged from 6.90 to 15.83mg $GAEg^{-1}$ and they were in the order oregano > cinnamon > sweetbasil > bay leaves > mint > sage > rosemary > parsley > marjoram, in addition to the concentration of tannins after treating the sample with PvPp (polyvinyl-polypyrolidone) was found to range from 0.31 to 13.66 mg $GAEg^{-1}$, also the order of the concentration of tannins in the studied spices was as follows: cinnamon > oregano > sweetbasil > mint > bay leaves > parsley > sage > rosemary > marjoram.

Also, Vallverdu *et al*. (2014) stated that total phenolics content was ranged from 1.12mg GAE/ g DW in bay leaves to 5.82mg GAE/ g DW in cinnamon, and Deepshikha (2013) decided that total phenolics content in cinnamon was 279.64mM GAE/ g.

Sree *et al*. (2012) used different organic solvents such as methanol, ethanol, ethyl acetate and water to extract the optimum yield of cinnamaldehyde, total phenolics content and eugenol from cinnamon species, whereas for cinnamaldehyde and total phenolics content, methanol showed best results and the concentrations were 23.625mg/L and 9mg/L respectively, then ethanol showed best results in extraction of eugenol and its concentration was 12.4mg/L. While respecting to Arak, then Ibrahim *et al*. (2015) said that total phenolics content for *S. persica* collected from the southern region was 794.6mg as D-catechin equiv/ 100g Miswak, and the total flavonoids for the same Miswak samples was 503.8 as quercetin equiv/ 100g Miswak, on other hand, the ratio of total flavonoids/ total phenolics was 0.63 to 0.68 in the present samples indicated high proportions of flavonoids.

Also, Alali *et al*. (2007) recorded that total phenolics in aqueous and in methanol extracts of *Salvadora persica* were 12.8 and 10.1mg GE/ g dry weight respectively.

In generally, this study draw a conclusion that ethanolic extracts of Arak (Siwak) and Cinnamon plants exhibited high phenolics, flavonoids and tannins contents as compared to water extraction method of the same plants which can be reflected to a biological activity of these plants because of the phenolic compounds, flavonoids and tannins played an important role for the main part of antimicrobial activity of these plants (Arak and Cinnamon).

Mukhtar & Ghori (2012) explained the reason was that antimicrobial component of the cinnamon bark was more soluble in ethanol as compared to water, but its activity was reported less as compared to the garlic plant, also the ethanolic extract of cinnamon showed better zones at all concentrations against *E. coli* as compared to the aqueous extracts which were effective at only higher concentration and when compared with impenem, it produced a smaller zone. Both the aqueous and ethanolic extracts were effective against *E. coli*, but ethanol extract showed comparatively better results

Odhav *et al*. (2002) suggested that mechanism of antibacterial action of spices involved the hydrophobic and hydrogen bonding of phenolics compounds to

membrane proteins, this mechanism named membrane destruction and cell wall disruption of electron transport systems. Darout *et al*. (2000) added that antimicrobial activity of aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulphates, in addition to many other compounds naturally presented in the plants. Cowan (1999) said the ethanolic extracts showed better results as compared to aqueous as being organic dissolved more organic compounds resulting in the release of greater amount of active antimicrobial components. In addition, Wendakoon & Sakaguchi (1995) suggested the antibacterial activity of cinnamon might be due to the presence of cinnamaldehyde compound which inhibited the amino acid decarboxylation activity in the cell which leads to energy deprivation and microbial cell death. Finally, Saleh & Khan (2013) denoted there were several studies had focused on chewing stick miswak, *Salvadora persica* L. and on the chemical components which had antimicrobial activity.

"Laboratory experiments"

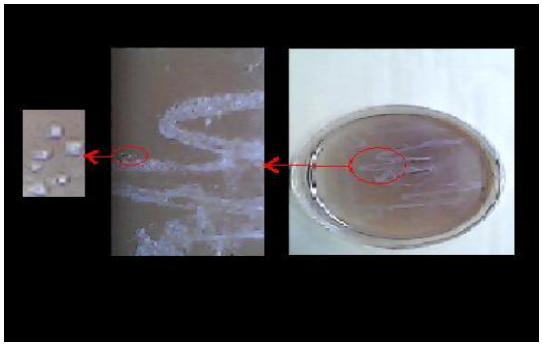


Fig. 5; *P. l.* subsp. *larvae* colonies on SBA medium.

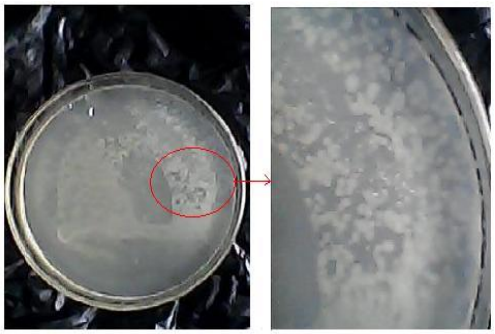


Fig. 6; *M. pluton* colonies on Bailey's medium.

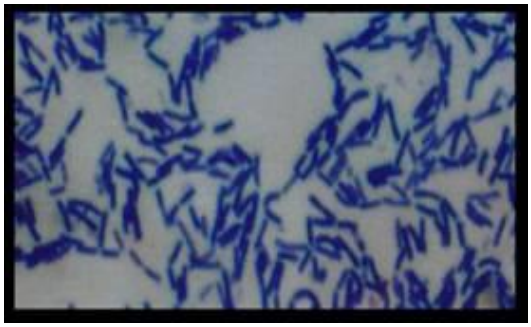


Fig. 7; Gram +ve Rods of *P. l.* subsp *larvae* stained with violet crystal dye, Magnify 1000 once.

Fig. 8; Rods of *P. l.* subsp. *larvae* turn to spores, Magnify 1000 once.

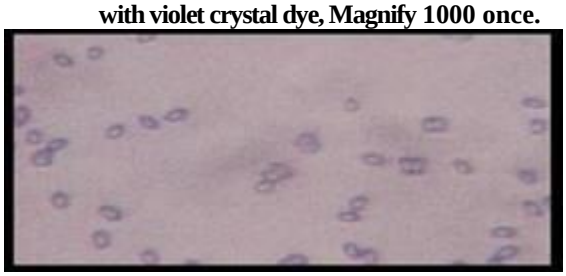


Fig. 9; *P. l. larvae* spores Magnify 1000 once.

Fig. 10; *P. l. larvae* spores stained by negative staining method, Magnify 1000 once.

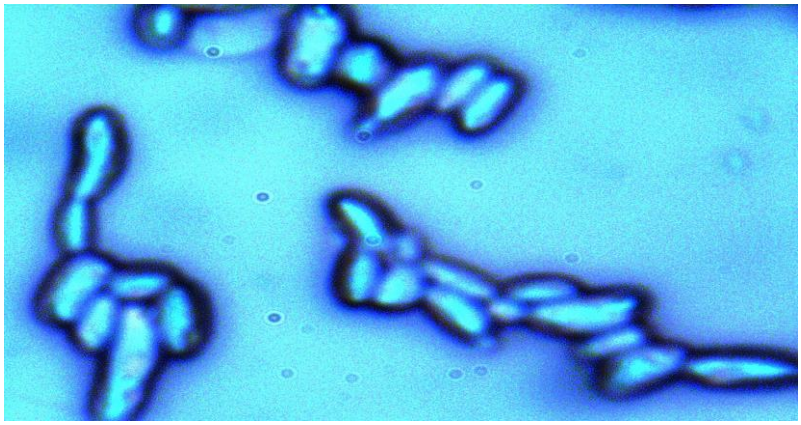


Fig. 11; *M. pluton* cells, lanceolate cocci stained by negative dye, Magnify 1000 once.

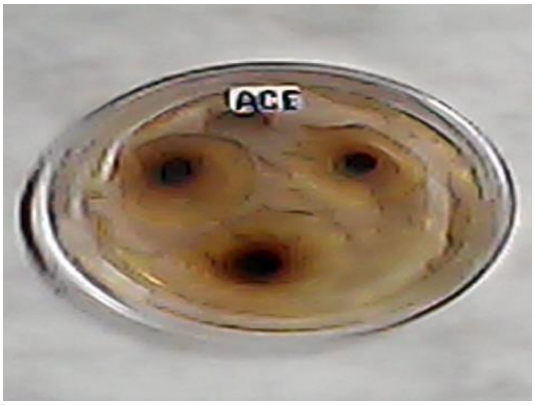


Fig. 12; Inhibition zones of *P. l. l.* by ACE

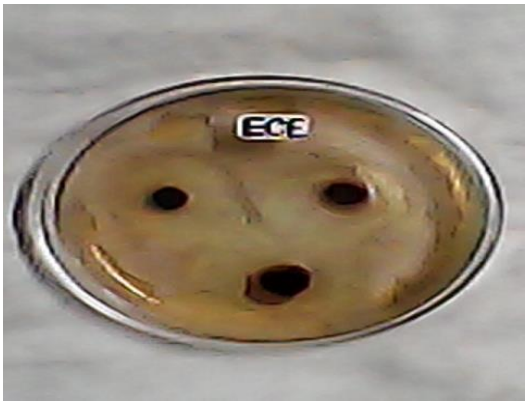


Fig. 13; Inhibition zones of *P. l. l.* by ECE

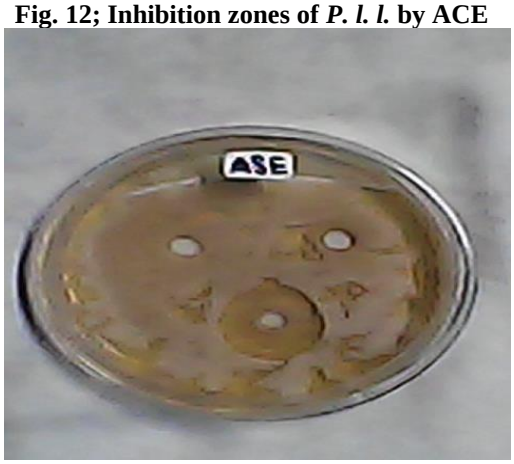


Fig. 14; Inhibition zones of *P. l. l.* by ASE

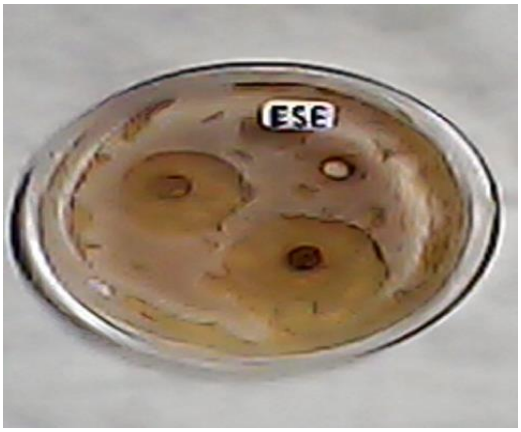


Fig. 15; Inhibition zones of *P. l. l.* by ESE

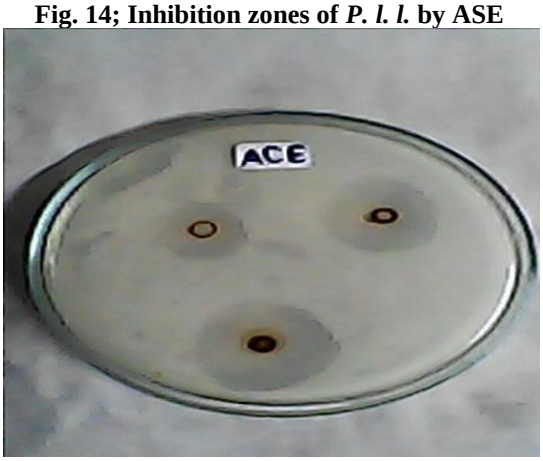


Fig. 16; Inhibition zones of *M. pluton* by ACE

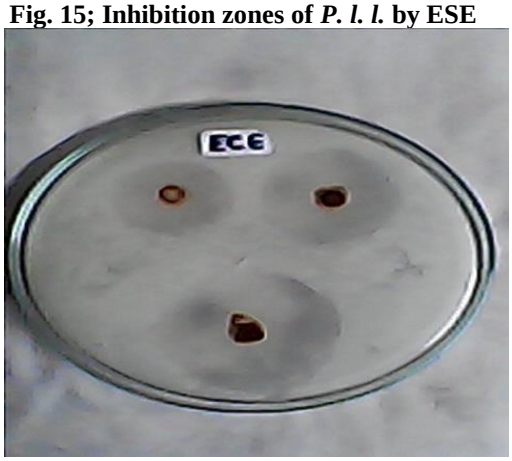


Fig. 17; Inhibition zones of *M. pluton* by ECE

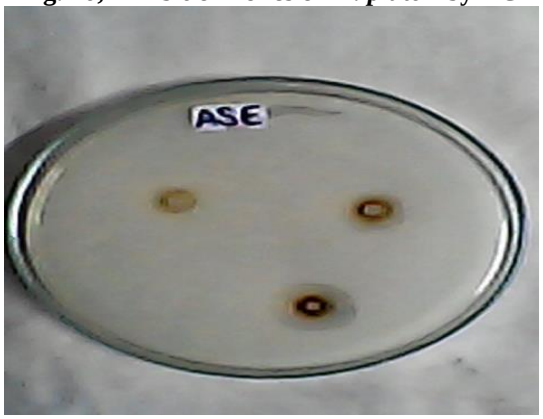


Fig. 18; Inhibition zones of *P. l. l.* by ASE

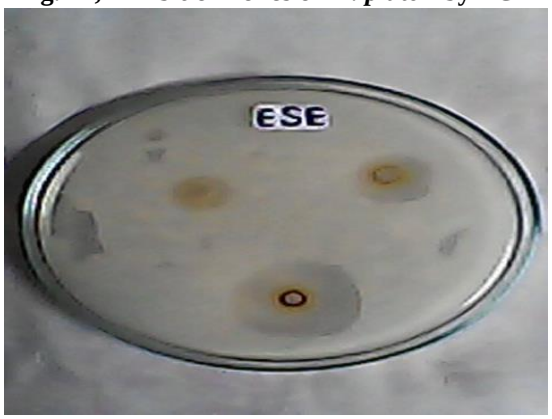


Fig. 19; Inhibition zones of *P. l. l.* by ESE

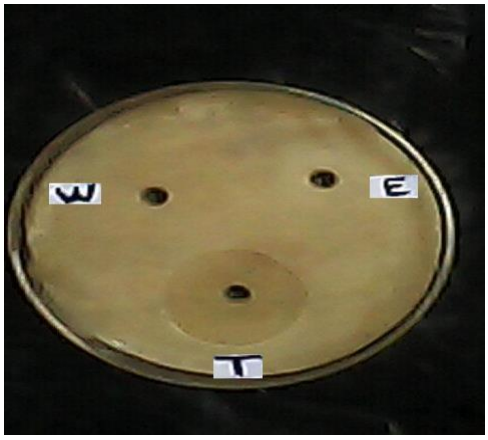


Fig. 20; Inhibition zones of *P. l. l.* by W; deionized distilled water solvent & E; ethanolic alcohol 95% solvent & T; Tylosin anti

Fig. 21; Inhibition zones of *M. pluton* by

CONCLUSIONS & RECOMMENDATIONS

It had confirmed as by all researchers that each of American and European foulbrood diseases were caused by only one and definitive bacterium which were *Paenibacillus larvae* and *Melissococcus pluton* for the mentioned foulbroods respectively.

The aqueous and ethanolic extracts of each of Arak (Siwak) and Cinnamon plants had considerable concentrations from the phenolics, flavonoids and tannins compounds, but ethanolic extracts were higher than the other in these compounds, and these contents certainly played an important role in the antibacterial activity which was appeared by these extracts against *P. l. l.* and *M. pluton* bacteria on the special media (*in vitro*).

Perhaps in the nearby future, it is advised by using of some or all extracts of these two plants for controlling AFB and EFB diseases after examination of them through field trial inside the honeybee colonies.

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"تأثير مستخلصات الآراك والقرفة على بكتيريات تعفن الحضنة داخل المعمل"

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في هذا العمل تم عزل وتنقية بكتيريا كل من مرضي تعفن الحضنة الأمريكي والأوروبي من عينات مرضية (أقراص حضنة) على البيئات المتخصصة داخل المعمل الميكروبيولوجي لتشخيص أمراض نحل العسل بقسم وقاية النبات كلية الزراعة بمشتهر، ثم نفذت بعض الإختبارات التأكيدية لهذه العزلات مثل وصف النمو البكتيري، إنتاج الكتاليز، الصبغ بالجرام، الصبغ السالب وذلك قبل إجراء إختبارات الحساسية بالمواد المختلفة. تلاه إختبار النشاط ضد بكتيري للمستخلصات المائية والكحولية لأعواد نبات الآراك (*Salvadora persica*) وقلف نبات القرفة (*Cinnamomum spp.*) كلاً على حده نحو تثبيط بكتيريات التعفن في أطباق بتري مقارنةً بإستخدام المضاد الحيوي البيطري تيلوزين بتركيز ١٠٠ جزء في المليون (ppm) وفقاً لإستخدام (Mostafa, 2009)؛ حيث تم الحصول على مستخلص مائي وآخر كحولي من كل نبات على حده وذلك بإستخدام المذيبين الماء منزوع الأيونات، والكحول الإيثيلي تركيز ٩٥٪ وجهزت المستخلصات الأربعة بالتركيزات ١٠ ، ٣٠ ، ٥٠٪ (وزن/حجم) / مستخلص، بالإضافة إلى إستخدام المذيبين مجردين كلاً على حده (كمقارنة سالبة)، فكانت النتائج كالتالي: أ - بوجه عام وضح أن لمستخلصات نبات الآراك تأثيراً مثبطاً لبكتيريات التعفن في أطباق بتري. ب- أظهر نبات القرفة في مستخلصاته المذكورة نشاطاً ضد بكتيري تجاه بكتيريات التعفن أكثر فعالية من نبات الأراك. ج - المستخلصات الكحولية متفوقة على المائية في تثبيط النموات البكتيرية للتعفنات في أطباق بتري. د- المستخلصات المائية أظهرت نوعاً نشاطاً ضد بكتيري تجاه كلٍ من بكتيريا *P. larvae* ، *M. pluton* وإن كان أقل. ذ- أحرز نبات القرفة تفوقاً معنوياً عالياً عندما إستخدم كمستخلص كحولي بتركيز ٥٠٪ حيث أعطى منطقة تثبيط لكلٍ من بكتيريا *P. larvae* ، *M. pluton* في أطباق بتري تساوي ٣٣,٣ ± ٠,٨٨٢ ، ٣٥,٨ ± ٠,٨٨٣ مم على التوالي. ه- كذلك أحرز نبات الأراك تفوقاً معنوياً جيداً عندما إستخدم كمستخلص كحولي بتركيز ٥٠٪ حيث أعطى منطقة تثبيط لكلٍ من بكتيريا *P. larvae* ، *M. pluton* في أطباق بتري تساوي ٢٤,٠ ± ١,٠٠١ ، ٢٥,٧ ± ٠,٨٨٣ مم على التوالي. و- ظهر التيلوزين ذو نشاط ضد بكتيري عالي تجاه بكتيريا التعفنات حيث جاء معنوياً في المركز الثاني بمساحات تثبيط (IZDs) لكلٍ من بكتيريا *P. l. l.* ، *M. pluton* تساوي ٢٨,٨ ± ١,٢٠٣ ، ٣٥,٣ ± ٠,٦٦٧ مم على التوالي. ى- أظهرت بكتيريا *M. pluton* حساسيةً أكثر من بكتيريا *P. l. l.* عند المعاملة بالمستخلصات المختلفة لنباتي القرفة والآراك كلٍ على حده حيث إتضح ذلك جلياً في مساحات التثبيط الأكبر نوعاً في حالة بكتيريا التعفن الأوروبي بأي من المستخلصات عنها في حالة بكتيريا التعفن الأمريكي. قدرت بعض المركبات الفعالة في المستخلصات المجربة عن طريق قياس إجمالي كل من الفينولات والتانينات مقدرةً بعدد الجرامات من المادة القياسية المكافئ حمض الجاليك (GAE) وكذلك قياس إجمالي الفلافونويدات مقدرةً بعدد الجرامات من المادة القياسية المكافئ كيرسيتين (QE) وذلك بإستخدام جهاز سبكتروفتوميتر (biochrom Libra S12 UV-Vis) بمعمل تحليل وقياس مواصفات أعسال النحل بقسم بحوث النحل، حيث تم حساب النسب المئوية لمتوسطات المكونات الفعالة ووفقاً للتحليل الإحصائي أثبتت المستخلصات المجربة إحتوائها على نسب معتبرة من المركبات المذكورة والتي يعزى إليها النشاط ضد بكتيري الحاصل؛ أ- بالنسبة لإجمالي الفينولات≺ المستخلص الكحولي من القرفة تفوق معنوياً على باقي المستخلصات بـ ١١,٠١٥ ± ٠,٠١٥ ٪ ثم جاء المستخلص المائي للقرفة في المركز الثاني ثم تلاه المستخلص الكحولي للأراك وأخيراً المستخلص المائي للأراك وذلك بالنسب ٩,٣٤٥ ± ٠,١٢٩ ٪ ، ٨,٢٧٣ ± ٠,٢١٦ ٪ وأخيراً ٥,٣٦٢ ± ٠,٠٤٣ ٪ على التوالي وذلك عند أقل فرق معنوي $LSD_{0.05}$ = ٠,٤٣٨ ٪. ب- بالنسبة لإجمالي الفلافونويدات≺ المستخلص الكحولي من القرفة تفوق معنوياً على باقي المستخلصات بـ ٠,٥٥٠± ٠,٠١٦ ٪ ثم جاء المستخلص المائي للقرفة في المركز الثاني ثم تلاه المستخلص الكحولي للأراك وأخيراً المستخلص المائي للأراك وذلك بالنسب ٠,٣٩٩ ± ٠,٠١٢ ٪ ، ٠,٢٦٠ ± ٠,٠١٧ ٪ وأخيراً ٠,٢٠٣ ± ٠,٠٠٦ ٪ على التوالي وذلك عند أقل فرق معنوي $LSD_{0.05}$ = ٠,٠٤٤ ٪ . ج- بالنسبة لإجمالي التانينات≺ المستخلص الكحولي من القرفة تفوق معنوياً على باقي المستخلصات بـ ١٣,١٣٠ ± ٠,٠٣٩ ٪ ثم جاء المستخلص المائي للقرفة في المركز الثاني ثم تلاه المستخلص الكحولي للأراك وأخيراً المستخلص المائي للأراك وذلك بالنسب ٨,٢٥٠ ± ٠,٠٢١ ٪ ، ٧,٣٥٨ ± ٠,٣٥٥ ٪ وأخيراً ٣,٧٦١ ± ٠,١٠٥ ٪ على التوالي وذلك عند أقل فرق معنوي $LSD_{0.05}$ = ٠,٦٠٢ ٪