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Full Length Research Paper

An *in vitro* evaluation of Cinnamon (*Cinnamomum* spp.) and Siwak (*Salvadora persica*) extracts for controlling the foulbrood pathogens of honeybee

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Instead of the synthetic antibiotics, the antibacterial activity of the aqueous and ethanolic extracts of both Cinnamon (*Cinnamomum spp.*) and Siwak (*Salvadora persica*) plants was evaluated against the foulbrood bacteria under the laboratory conditions. To the study knowledge, it is the first time to evaluate Siwak plant against the foulbrood diseases. 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 plutonius*.

Key words: Siwak extracts (ASE & ESE), Cinnamon extracts (ACE and ECE), American foulbrood, 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) (Genersch, 2010). AFB is caused by *Paenibacillus larvae* spore_bacterium (Genersch et al., 2006), while EFB is caused by *Melissococcus plutonius* non spore_bacterium according to the study of Shimanuki and Knox (2000). The two foulbrood diseases are widespread in the world (Del Hoyo et al., 2001), and Since 1996, the known symptoms of these diseases had appeared in several Egyptian apiaries according to observations of several researchers such as Owayss (2007), Hashish et al. (2008), Ibrahim (2009) and Kamel et al. (2013).

Due to the seriousness and damages of these foulbrood diseases, many beekeepers pursued to fight the diseases by using several veterinary antibiotics such as oxytetracycline (OTC) and sodium sulfathiazole, but often the foulbroods were returned again after short

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Figure 1. AFB symptoms.

Figure 2. A ropy threadlike material AFB only.

periods, besides the bad effects of these chemicals' residues in the honeybee products according to Kamel et al. (2013). They also allow the creation of new *P. larvae* strains or varieties which are resistant to the cures like what happened with OTC antibiotic according to Miyagi et al. (2000) and Evans (2003).

The chemical antibiotics are forbidden in beekeeping in European Union (EU) and many other countries, that is why new (alternative) products effective in foulbrood control are necessary. For examples, *Cinnamomum celanicum* as ethanol and hexanol extracts (Alhojaymi, 2005), laurel leaf as extracts (Damiana et al., 2014) and

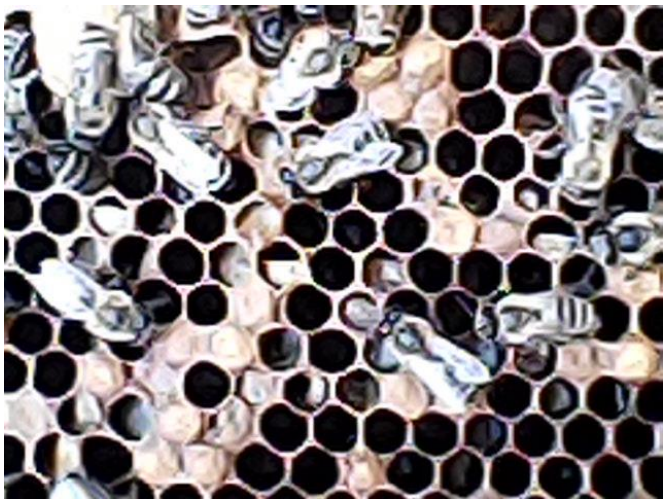

Figure 3. EFB symptoms.

Mentha spp. as essential oils (Gende et al., 2014). So, this study aimed to use extracts of two natural materials which are bark of Cinnamon plant (*Cinnamomum spp.*) and sticks of Siwak plant (*Salvadora persica*). This is in line with the later researchers of the same trend that aims to avoid using chemical antibiotics for controlling the foulbrood diseases.

MATERIALS AND METHODS

Microbial tests

These laboratory assays were carried out in 2011 under the suitable sterilized conditions at the specific micro. Labs, Fac. Agric., Moshtohor, Benha Univ., Egypt.

The isolated bacteria used

Two different bacteria were used for this assay, which were *P. larvae* spore-bacterium, the agent of AFB disease, and *M. plutonius* non spore-bacterium, the agent of EFB disease, whereas they were isolated according to De Graaf et al. (2013) on Sheep Blood Agar (SBA) medium and on Bailey medium respectively from samples appeared to have the disease symptoms whether in respect to AFB according to Scuch et al. (2001) (Figures 1 and 2), or in respect to EFB according to Shimanuki and Knox (2000) (Figures 3 and 4). For confirming the isolates, some tests were performed such as description of the bacterial colonies characters according to Djordjevic et al. (1998) and Del Hoyo et al. (2001), catalase production, Gram stain test and the negative stain according to Shimanuki and Knox (2000).

Preparation of aqueous and ethanolic extracts

According to the study of Almas (2001), Albayati and Sulaiman (2008), Mahfuzul et al. (2008), Muthiah (2008) and Prasad et al. (2011), the extracts were prepared as: 1 kg from each of Cinnamon bark and dried sticks of Siwak were purchased from a local retail

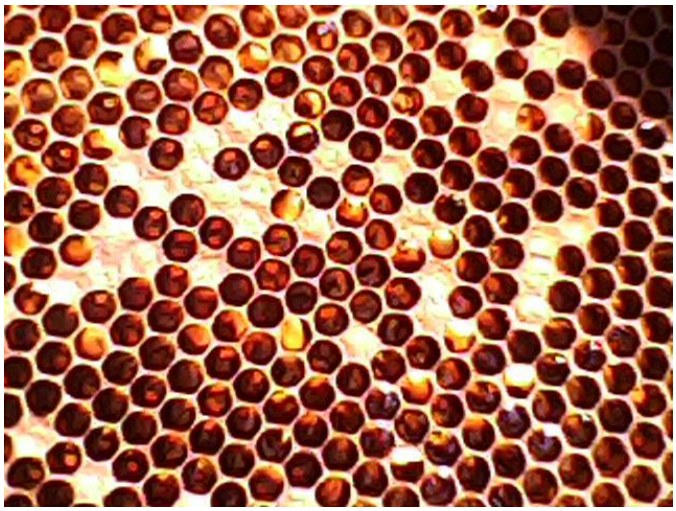

Figure 4. Conglobated and dried larvae.

market, each plant materials were cut into small pieces and were separately ground using a grinder (SONAI, Model MAR 3000) then both different powders were separately sieved in a screen cloth to obtain a very fine powder. For the preparation of the aqueous extracts, 200 g fine powder of each plant were separately mixed with 800 ml of deionized sterile distilled water, soaked in a 1000 ml sterilized beaker and were kept at 40°C for 48 h, then filtered by using 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 to 50°C by using an oven or incubator until thick pastes were formed which were considered as 100% concentration of extracts. The extraction was repeated by following the same procedure except for the solvent which was replaced with ethanol of 95% for preparation of the ethanolic extracts. Thus, plant extracts already had the presence of conc. as 100%, and they were Aqueous Cinnamon Extract (ACE), Ethanolic Cinnamon Extract (ECE), Aqueous Siwak Extract (ASE) and Ethanolic Siwak Extract (ESE). The pastes were stored in labeled sterile bottles, and kept in the freezer of a refrigerator until the time it was used.

Antibacterial activity assay

Firstly, three concentrations of 10, 30 and 50% (*w/v*) were prepared from each extracted paste (100% conc.) of the four extracts separately by mixing it with the appropriate volumes of the similar solvent (deionized sterile distilled water or ethanol 95%). For subculture and treating *P. l*. bacterium, Muller Hinton broth, Yeast, Phosphate, Glucose and Pyruvic acid (MYPGP) medium was used, whereas it was assembled and prepared as Dingman and Stahly (1983), but Bailey medium was used for subculture and treating *M. plutonius* bacterium according to the study of De Graaf et al. (2013).

The plant extracts were tested on the selected bacteria by the filled wells method (ditch plate method), using an aseptic tube of 4 mm in diameter, three circular wells were made in 5 dishes of each medium, then wells of 1 dish of each medium were pipetted and filled with 50 µl extract of the plant (ACE, ECE, ASE or ESE) in concentrations of 50, 30 and 10% separately, the results for 4 dishes of each medium were treated by the extracts. But wells of the 5th dish of each medium differed in the treatment, whereas in them, 1st and 2nd wells were pipetted and filled with 50 µl just from the two solvents, deionized sterile distilled water or ethanol 95% was pipetted as a negative control, while for comparison or as a positive control, the 3rd well was pipetted and filled with 50 µl from the antibiotic. Tylosin (ADWiA company) was diluted to concentration of 100 ppm according to the study of Mostafa (2009), and then a sterilized cotton swab was used to spread portions of a spore suspension from *P. l.* isolated bacterium streaky on surface of whole MYPGP dishes. Finally, the plates were left for 1 h at room temperature and then incubated at 37±1°C for 72 h.

While another sterilized cotton swab was used to spread portions of a cell suspension from *M. plutonius* isolated bacterium streaky on surface of whole Bailey's dishes, finally the plates were left for 1 h at room temperature, and then anaerobically incubated at 34±1°C 4 days. The experiment was triplicated, and then the whole values of the inhibition zones were measured, and statistically analyzed according to the study of Sendegor and Cochran (1980), and then compared to Duncan's multiple range test at 0.05 level.

Chemical analysis of the four extracts

These assays were performed in honeybee products analysis Lab., Apicultures Dept., Agric. Res. Cent., Egypt.

Determination of active compounds in the different extracts

Firstly, only 1 g of each extract pastes of 100% conc. resulted from the aqueous or ethanolic extraction of both the two plants which were separately dissolved and completed to 100 ml of suitable solvent (deionized sterile distilled water or ethanol 95%), thus four extracts of 1% conc. were obtained which are ACE, ECE, ASE and ESE.

Total phenolics content

This content was determined by colorimetric assay according to Shahidi and Naczk (1995) and Waterhouse (2002). An aliquot 200 µl of aqueous or ethanolic extract 1% of Cinnamon or Siwak, 800 ml deionized water and 100µl of Folin-Ciocalteu reagent were mixed and incubated for 3 min at room temperature. 300 µl of sodium carbonate (Na2CO3) 20% w/v were added and the mixture was incubated for 2 h at room temperature under dark condition. The absorbance was determined using biochrom Libra S12 UV-Vis spectrophotometer at 765 nm. A blank was prepared with distilled water instead of aliquot extract. Gallic acid standard curve was first prepared from 0 to 320mg/ l, and total phenolics (TP) were expressed in mg gallic acid equivalent (GAE) / g dry matter and calculated from the prepared standard curve with 0 to 100 mg/ gallic acid (GA).

Total flavonoids content

This content was determined by colorimetric analysis according to Proestos et al. (2006). A mixture of 200 µl from aqueous or ethanolic extract of 1% of Cinnamon or Siwak and 150 µl of sodium nitrite ($NaNO_2$) 5% w/v was first incubated for 6 min at room temperature. Then, 150 µl of aluminum chloride hexahydrate ($AlCl_3.6H_2O$) 10% w/v were added and incubated for 6 min at room temperature. 800 µl of sodium hydroxide (NaOH) solution 10% w/v were added and incubated at room temperature for 15 min. For blank, extract was replaced with deionized water. Absorbance was read by using biochrom Libra S12 UV-Vis spectrophotometer at 510 nm. A standard curve of quercetin dissolved in 80% ethanol was

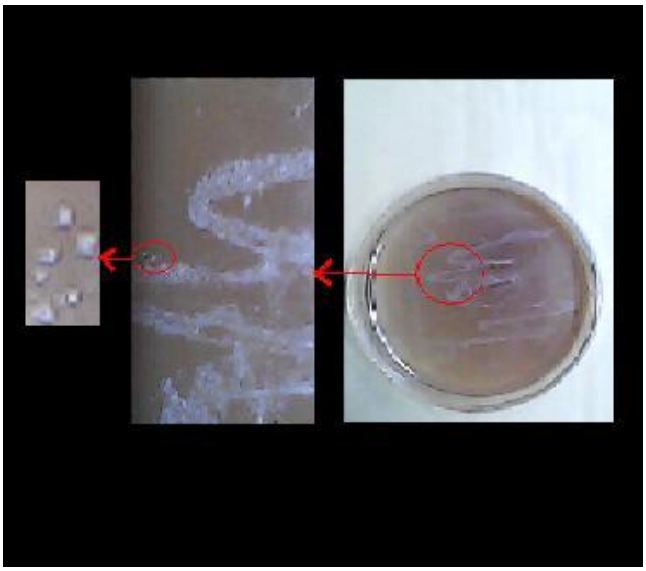

Figure 5. *P. larvae* colonies on SBA medium.

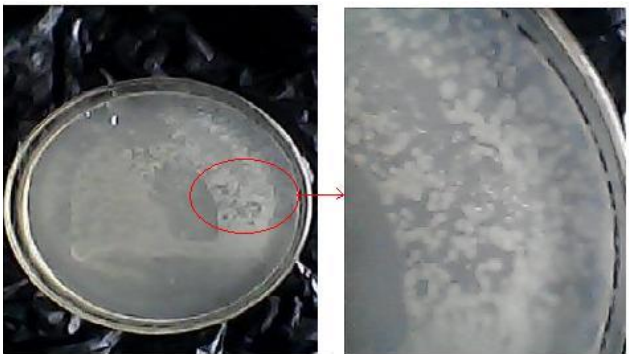

Figure 6. *M. plutonius* colonies on Bailey medium.

initially prepared from 0 to 1000 µg/ ml. Total flavonoids (TF) were expressed in mg Quercetin equivalent (QE)/ g dry matter.

Total tannins content

This content was determined by using Folin-Ciocalteu assay according to Tamilselvi et al. (2012). An aliquot 100 µl of aqueous or ethanolic extract 1% of Cinnamon or Siwak were added to 750 µl of deionized water, 500 µl of Folin-Ciocalteu reagent and 1000 µl of 35% Na_2CO_3. The mixture was shaken vigorously after diluting to 10 ml of distilled water. The mixture was incubated for 30 min at room temperature, and read at 725 nm using biochrom Libra S12 UV-Vis spectrophotometer. Distilled water was used as blank. Gallic acid standard solutions were prepared as described earlier. The total tannins (TT) were expressed as GAE/ g dry matter, as calculated from the prepared standard curve with 0-100 mg/ GA.
All assays of both samples and standard were triplicated, whole values were statistical analyzed (Sendegor and Cochran, 1980), and all means were compared by Duncan's multiple range test at0.05 level, whereas percentages of the value average were calculated according to the equation:

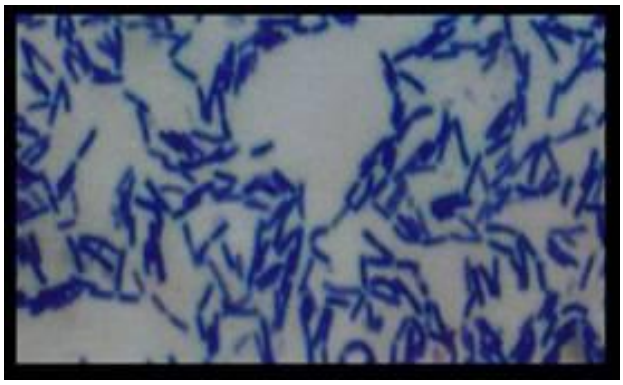

Figure 7. Gram +ve Rods of *P. larvae* stained with violet crystal dye, Magnify 1000 once

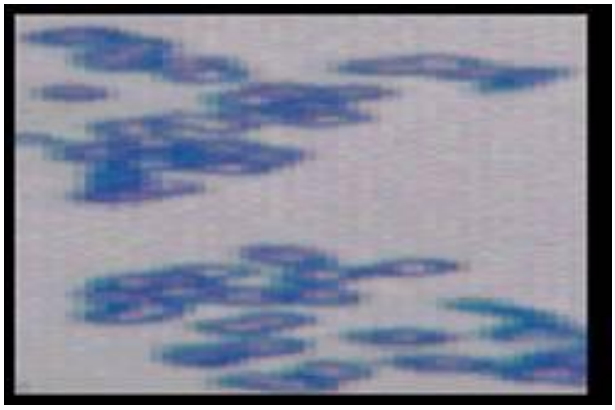

Figure 8. Rods of *P. larvae* turn to spores, magnify 1000 once.

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

Finally, the % mean of content ± standard error were presented.

RESULTS

Microorganisms

The colonies appeared slowly and singly on sheep blood agar (SBA) medium (Figure 5), measured about 4 mm in diameter, had irregular edges, flat, light grey, non-pigmented belonging to *P. l.* bacterium which is in line with the study of Chantawannakul and Dancer (2001). While the bacterial colonies which appeared very slowly on Bailey's medium belonging to *M. plutonius* bacterium (Figure 6) had the description of a single colony, very small measuring 1 to 2 mm in diameter, biconvex, shape circular, had regular edges, white of color and sometimes grow deeply into the medium, and this description agreed with the study of Djordjevic et al. (1998). Moreover, the used strains whether *P. larvae* or *M. plutonius* were catalase (-) and gram (+) (Figures 7 to 10) according to

Figure 9. *P. larvae* spores Magnify 1000 once.

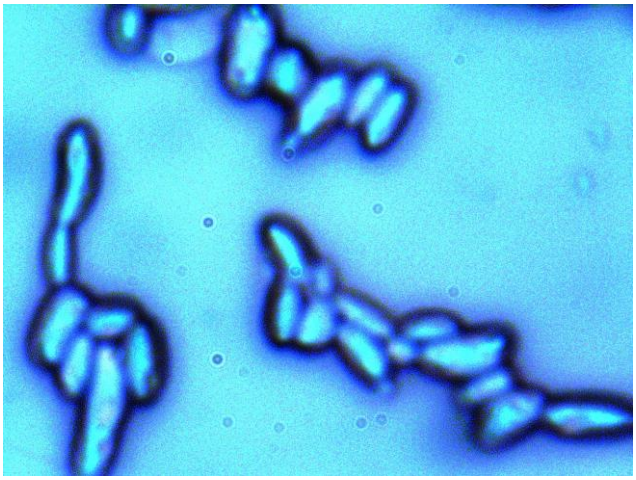

Figure 10. *M. plutonius* cells, lanceolate-cocci magnify 1000 once

Djordjevic et al. (1998) and Piccini and Zunino (2001).

DISCUSSION

It came to light from Table 1 that all used extracts of the two plants had antibacterial activities at their cleared concentrations against both of the two foulbrood bacterial growth on the culturing media (Figures 11 to 18) but with varying degrees and without inducing of a complete inhibition.

Cinnamon plant

With respect to *P. larvae,* the study found within each concentration that ECE had surpassed by its antibacterial act on ACE. Also, ECE had surpassed within each concentration on all other extracts by its ability for inhibition of *M. plutonius.*

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

In contrast with the study results, Goda (2011) examined the ethanolic extract of Cinnamon, but this extract wasn't successful for inhibiting *P. larvae* or *M. plutonius*, while Alhojaymi (2005) stated that both ethanol and hexanol extracts of *Cinnamomum celanicum* plant were examined for inhibition of *B. larvae* in the laboratory by loading method with disc diffusion assay, and the results appeared that hexanol extract of Cinnamon was the most effective on *B. larvae* at 2000 µg concentration whereas it completely inhibited the bacterial growth to area 50 mm, but the used concentrations of the ethanol extract wasn't stated. Furthermore the inhibition areas were induced by it.

Siwak plant

By scrutiny of the inhibition areas in Table 1 and among the different concentrations, the study found that ESE had preceded by its highest concentration which 50% on ACE in its two lower concentrations, while in the same concentration 50%, ESE had surpassed ASE in relation to its ability on inhibition of *P. larvae* also the same concentrations were used for extracts against *M. plutonius* but with higher inhibition zones.

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

Also Prasad et al. (2011) examined alcoholic extracts of *Salvadora persica* L. (fresh and dry plant) which were screened for *in vitro* 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 alcoholic extracts of herbaceous parts of *S. persica* L.

Table 1. Comparison between the different extracts.

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

IZD; Inhibition zone's diameter, Mm; Millimeter, $LSD_{0.05}$; low significant difference at alpha 0.05, P. l.; *Paenibacillus larvae* bacterium, *M. plutonius; Melissococcus* plutonius bacterium, ECE; Ethanolic Cinnamon Extract, ACE; Aqueous Cinnamon Extract, ESE; Ethanolic Siwak Extract, ASE; Aqueous Siwak Extract, (a, b, c …); Marks of the ranks.

Figure 11. Inhibition zones of *P. l.* by ACE

Figure 12. Inhibition zones of *P. l.* by ECE.

revealed a higher inhibition zone to *Proteus vulgaris* followed by other tested organisms which exhibited less zone of inhibition such as *B. subtilis*, *L. brevis*, *S. aureus*, *E. coli*, *C. albicans* and *A. niger*. And this result confirmed that *S. persica* L. contained substances with antimicrobial properties.

Noteworthy in this study, solvents used for extraction of both the two plants did not inhibit the bacterial growth of *P. larvae* or *M. plutonius* bacterium on the culturing media, and by comparison, the veterinary antibiotic Tylosin tartrate had inhibited *P. larvae* and *M. plutonius* areas to be 28.8 and 35.3 mm respectively (Figures 19 and 20). On basis of the study results and the previous presentation there were five facts:

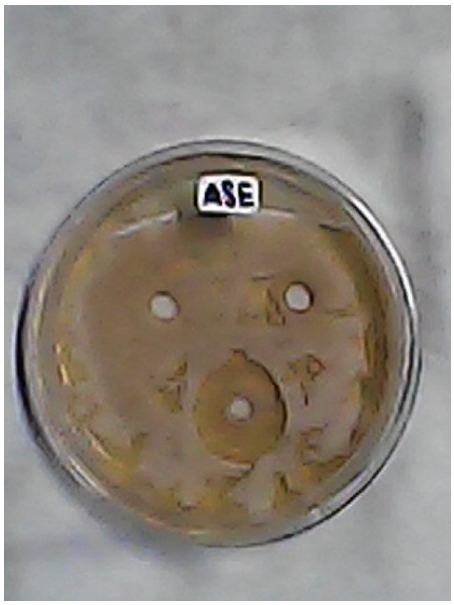

Figure 13. Inhibition zones of *P. l*. by ASE.

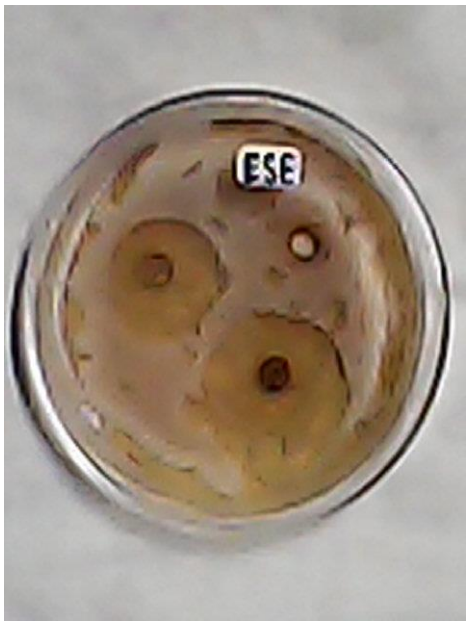

Figure 14. Inhibition zones of *P. l*. by ESE.

Figure 15. Inhibition zones of *M. plutonius* by ACE.

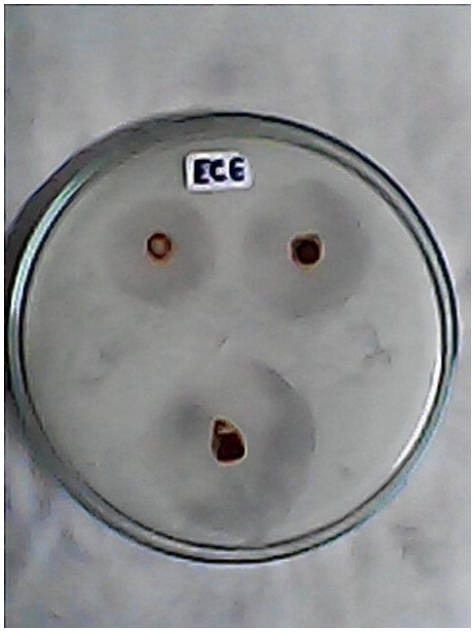

Figure 16. Inhibition zones of *M. plutonius* by ECE.

1st fact: The concentrations which are higher than or upper 50% conc. of Cinnamon or Siwak extracts have good antibacterial acts, 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 the inhibition of the foulbrood bacterial growths in Petri's dishes.

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

4th fact: Antibiotic Tylosin remains has a high effect against the foulbrood bacteria with full reservation and caution from using it because of its contribution in

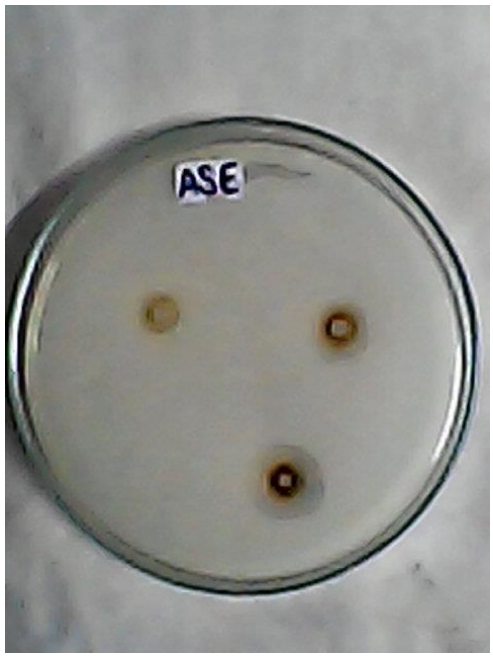

Figure 17. Inhibition zones of *M. plutonius* by ASE

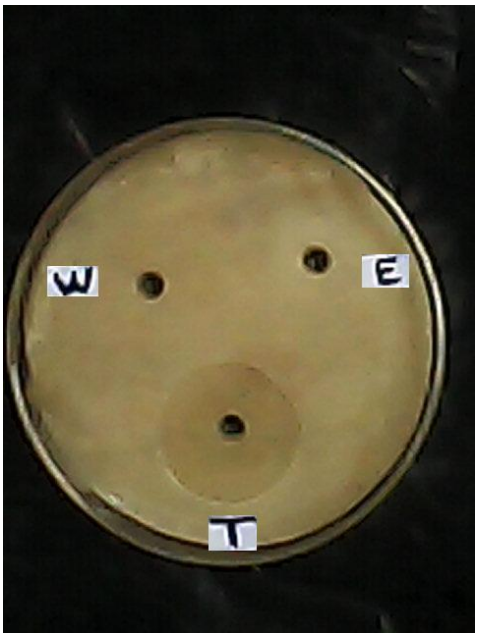

Figure 19. Inhibition zones of *P. l.* by W: deionized distilled water solvent; E: ethanolic alcohol 95% solvent; T: Tylosin antibiotic.

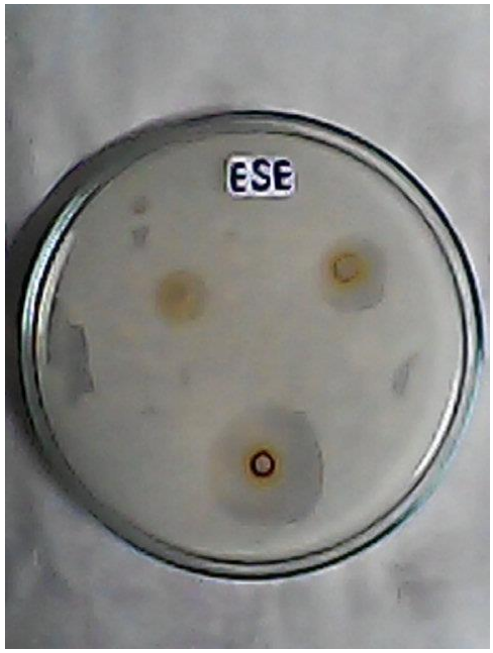

Figure 18. Inhibition zones of *M. plutonius* by ESE.

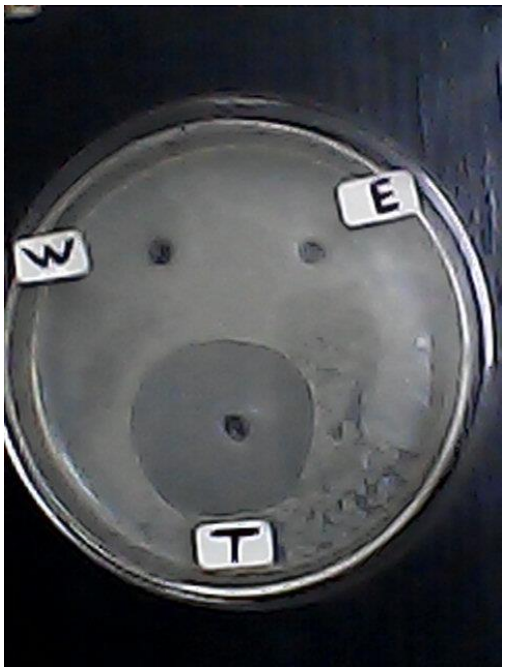

Figure 20. Inhibition zones of *M. plutonius* by W: deionized distilled water solvent; E: ethanolic alcohol 95% solvent; T:Tylosin antibiotic.

generation, and the development of foulbrood bacterial races are most resistances when controlling the hive bee according to Miyagi et al. (2000) and Evans (2003).

5th fact: Affecting power of the two plants' extracts on growths of the foulbrood bacteria exactly was due to these extracts that contained ingredients which had high antibacterial act, and the study tried to evidence this

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

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

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

thinking or reasoning by the chemical analysis which their results came up with Table 2.

Chemical analysis

The results tabulated in Table 2 are percentages of mean values of triplicate assay of some active ingredients which total phenolics, flavonoids and tannins compounds in the two plant extracts (Cinnamon and Siwak) 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 these results were supported by many researchers, whereas the 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, and the total phenolics content and 1,1- Diphenyl - 2- picryl hydrazyl (DPPH) radical scavenging activity from the organic solvent extraction (50% methanol) were 139±6mg GAE/ g dry sample (Khuwijitjaru et al., 2012). On other hand, *Salvadora persica* was known to containing on several biologically active chemical constituents such as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates (Kamil et al., 1999; Garboui et al., 2009; Abdillahi et al., 2010).

The highest value in total phenolics compounds was in ECE, and the lowest value was recorded in water extract of Siwak (ASE), and data of total phenolics compounds showed a significant difference between the two plants (cinnamon and Siwak), also between the two extract solvents in the one plant. Flavonoids content in the same table showed a high significant difference in total flavonoids, and the extract ASE showed the lowest value, on other hand ECE extract showed the highest value. Experimental data of total tannins generally revealed statistically differences between Siwak and Cinnamon plants, whereas they ranged as ECE, ACE, ESE then ASE respectively.

All previous measures were close to other results, whereas respecting Cinnamon, Muchuweti et al. (2007) said that concentration of the phenolics compounds in the following spices ranged from 6.90 to 15.83 mg GAEg-1 and they were in the order of oregano > cinnamon > sweetbasil > bay leaves > mint > sage > rosemary > parsley > marjoram. In addition to the concentration of tannins after treating the sample with polyvinyl-polypyrolidone (PvPp), the result 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. The total phenolics content ranged from 1.12 mg GAE/g DW in bay leaves to 5.82 mg GAE/g DW in cinnamon (Vallverdu et al., 2014). The total phenolics content in cinnamon was 279.64mM GAE/g (Deepshikha, 2013). Different organic solvents such as methanol, ethanol, ethyl acetate and water were used 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 23.625 and 9 mg/L respectively, then ethanol showed best results in extraction of eugenol and its concentration was 12.4 mg/L (Sree et al., 2012). While showing respect to Siwak, Ibrahim et al. (2015) said that total phenolics content for *S. persica* collected from the southern region was 794.6 mg as D-catechin equiv/100 g Miswak, and the total flavonoids for the same Miswak samples was 503.8 as quercetin equiv/100 g Miswak, on other hand, the ratio of total flavonoids/total phenolics was 0.63 to 0.68 in the present samples indicating 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 10.1 mg GE/g dry weight respectively.

Generally, this study draws a conclusion that ethanolic extracts of Cinnamon and Siwak plants exhibited high phenolics, flavonoids and tannins contents as compared to water extraction method of the same plants which can be reflected in the biological activity of these plants because of the phenolic compounds, flavonoids and tannins which play an important role in the main part of antimicrobial activity of these plants (Cinnamon and Siwak). 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, 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 ethanolic extract showed comparatively better results (Mukhtar and Ghori, 2012). Odhav et al. (2002) suggested the mechanism of antibacterial action of spices involved in the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, this mechanism is named membrane destruction and cell wall disruption of electron transport systems. The 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 (Darout et al., 2000).

The ethanolic extracts showed better results as compared to aqueous as being organic dissolved more in organic compounds resulting in the release of greater amount of active antimicrobial components (Cowan, 1999). In addition, 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 (Wendakoon and Sakaguchi, 1995). There were several studies which focused on chewing stick Miswak, *Salvadora persica* L. and on the chemical components which had antimicrobial activity (Saleh and Khan, 2013).

Conclusions

It was confirmed by all researchers that each of American and European foulbrood diseases are caused by only one and definitive bacterium which are *P. larvae* and *M. plutonius* for the mentioned foulbroods respectively. The aqueous and ethanolic extracts of each of the Cinnamon and Siwak 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 act which was exhibited by these extracts against *P. larvae* and *M. plutonius* bacteria on the special media (*in vitro*).

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

Conflict of Interests

The authors have not declared any conflict of interests.

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