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Isolation and Identification of *Pasteurella multocida* and *Mannheimia haemolytica* and the Antimicrobial Effect of *Plantago lanceolata* Extract

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Abstract | The present study was conducted to isolate and identify *Pasteurella multocida* and *Mannheimia haemolytica* and then estimation of the antimicrobial impact of alcoholic extract of *Plantago lanceolata* against these microbes. For this purpose, samples from nasal swabs and blood samples were collected from 150 cattle, 150 sheep and 150 goats. In addition, a total of 50 lung specimens were collected from infected 15 cattle, 20 sheep and 15 goats from slaughter houses. Results showed the isolation of three isolates of *P. multocida* and three isolates of *M. haemolytica*. Susceptibility test showed that all isolates of *P. multocida* were susceptible to Gentamycin (100%), while two (66.7%) isolates were susceptible to Azteronam, Clindamycin and Colistin sulfate and there was only one (33.3%) isolate that was susceptible to Erythromycin Doxycycline, Amoxicillin + Clavulanic acid. All isolates of *M. haemolytica* were susceptible to Gentamicin and Clindamycin while two (66.7%) were susceptible to Penicillin, Erythromycin, Doxycycline, Rifampin, Amoxicillin + Clavulanic acid, whereas only one isolate was susceptible to Aztreonam. The results showed that the inhibition zone was increased with the increasing the concentration of plant extract, *P. multocida* was appeared to be more susceptible than *M. haemolytica* to the *Plantago lanceolata* leaves alcoholic extract where the mean of inhibition zone diameter were 17.22, 19.11, and 21.94 mm against *P. multocida.* while the inhibition zones were 13.00, 14.55, and 15.55 mm against *M. haemolytica* at the concentration of 100, 150, and 200 mg/ml of the extract, respectively. These findings indicate that *P. multocida* and *M. haemolytica* are common microbes of Livestock and alcoholic extract of the leaves of *Plantago lanceolata* carry antimicrobial potential.

Keywords | *P. lanceolata*, *M. haemolytica, Plantago lanceolata*, Antibiotics

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INTRODUCTION

The *Pasteurella multocida* are small, Gram-negative rods or coccobacilli bacteria and occur singly, or in pairs or short chains, bipolar staining may be seen and capsules may be present, they are non-motile, non-sporing, grow on MacConkey agar (Quinn *et al.*, 2004; Momin *et al.*, 2011; Dabo *et al.*,2007; Hala *et al.*,2020). *Mannheimia haemolytica* (formerly known as: *Pasteurella haemolytica*) has been the subject of extensive reclassification in the past: first called Bacterium *bipolare multocidum* by Zecchinon et al. (2005), it wasrenam ed *Pasteurella haemolytica* in 1932 (Zecchinon *et al.*, 2005). The nomenclature of the diseases associated with infections with *Pasteurella* spp. in farm animals has been indefinite and confusing. A suggested nomenclature is based on the clinical findings and on the bacteria that

are commonly associated with each entity (Radostits *et al*., 2010). Therefore, there are three main diseases caused by *P. multocida* and *M. haemolytica* The first disease form is septicemic pasteurellosis of cattle (hemorrhagic septicemia or barbone), commonly associated with infection by *P. multocida* type 1 or B, is the classical disease of southern Asia, while the second disease is pneumonic pasteurellosis of cattle, which commonly associated with infection by *M. haemolytica* biotype A serotype 1, and *P. multocida* biotype A, is a common disease in Europe and the western hemisphere. *M. haemolytica* tends to cause a fulminating fibrinous lobar pneumonia, and *P.multocida* causes a fibrinopurulent bronchopneumonia, whereas the last disease called pasteurellosis of pigs, sheep, and goats, in pigs and this is usually associated with infection by *P. multocida* and is mainly pneumonic in form, pasteurellosis of sheep and goats is usually associated with infection by *M. haemolytica*. Although it is often pneumonic in form, a septicemic form of the disease is not unusual, especially in lambs (Sayyed *et al*., 2024).

The study on the distribution of the isolates in relation to the health status of the examined sheep, 7.45% (n = 7) and 29.6% (n= 16) were isolated in the asymptomatic and symptomatic sheep, respectively indicating that the isolation rate of the bacteria is more than double in the symptomatic sheep as compared to that of the asymptomatic sheep, and from the total of 66 young aged animals (≤ 6 months) a total of 10 (15.15%) and 2 (3.03%), *Manhaeimia haeimolytica* and *Pasteurella multocida* species were isolated respectively, both of which accounted 52.2% (12/23) of the total isolates showing relatively greater proportion (except for *P. multocida)* as compared to the older age category (>6 months of age) in which 9.76% *M. haemolytica*, 3.66% *P. multocida* and 47.8% of the total isolates recovered (Fentahum and Tsegaw 2023). *Plantago lanceolata*, narrow-leaved plantain is one of over 250 species of genus *Plantago* L. (Plantaginaceae). It is common in roadside grassland, with a world wide distribution, perennial, rarely annual or biennial plant, with a mass of surface fibrous roots (5–10cm long) and often a few deep roots, and is wind-pollinated (Hassemer, 2019; Hassemer *et al*., 2019; Drava *et al*., 2019).

This plant is mostly grown in grasslands, rarely in arable land, and its secondary metabolites are used in medicine (Hassemer, 2019). It is very rapid growth and deep rooting in the soil, which results in high drought tolerance and uptake of valuable nutrients from deep soil layers An intensive symbiosis with various mycorrhizal fungi is characteristic of plantain for a high capacity for nutrient and water appropriation (Michal *et al*., 2021). The use of *P. lanceolata* is discussed on permanent and non-permanent grasslands where agriculturally specific varieties have been developed for grazing animals showing positive health effects in them (Michal *et al*., 2021).

The unusual properties were also observed in veterinary medicine, Studies showed the possibility of using this plant as an additive to rainbow trout (*Oncorhynchus mykiss*) feed, after 90 days of basic diet with the addition of plantain's methanol extract and fulfillment of required breeding and experimental criterias, positive health effects were noted (Olumi *et al*., 2011).

The present study was aimed to investigate the prevalence of *Pasteurella multocida* and *M. haemolytica* in nasal swab and blood sample collected from different animals (cattle, sheep and goat) and study the antibacterial effect of alcoholic extract of *Plantago lanceolata* leaves against these bacterial isolates and compare it with different antibiotic.

MATERIALS AND METHODS

Collection of the samples

A total of four hundred and fifty of blood samples and four hundred and fifty nasal swab samples were collected from flock of Veterinary Medicine College, Baghdad University, Flock of Veterinary Medicine College, Fallujah University, flock of Agricultural College, Baghdad University and flocks around Al-Fallujah city.

The blood samples were collected in test tube with anti-coagulant The nasal swabs samples were collected from 150 cattle, 150 sheep and 150 goat respectively with different age and sex and 15, 20 and 15 samples were taken from infected lung of slaughtered cattle, sheep and goats respectively in abattoir and transported in cool box to the laboratory in order to use for bacterial isolation.

Plant material

Fresh Plantago (*Plantago lanceolata*) leaves were collected from a garden of Veterinary Medicine College, Baghdad University, which were classified in the Agriculture College, Baghdad University.

Pasteurella isolation

Bacterial isolation

Two methods were used to isolate Pasteurella from collected samples as follow:

Direct method

The samples of blood and nasal swabs were inoculated directly on the blood agar containing 5-7% sheep blood for recording the growth and type of hemolysis, samples were plated also on nutrient agar and MacConkey agar and colonies were tested for Gram stain. All plates were incubated aerobically at 37°C and examined for growth at 24- 48 hour. Then bacterial growth was purified on the same media. Infected lung samples sterilized by hot spatula, then incise was formed by a sterile incisor and samples take

were by loop and culture directly on nutrient agar, blood agar and MaCconky agar.

Indirect method

Nasal swabs samples were cultured on test tubes containing 5ml of nutrient broth and incubated at 37C° for 24-48 h, then plated on blood and MaCconky agar media. Blood samples: Five ml of blood samples were added to 25ml Tryptic soya broth, incubated at 37 °C for 24-48 h, then plated on blood agar, nutrient agar and MaCconky agar. Infected lungs: A small piece of infected lung were placed in nutrient broth and incubated at 37 °C for 24-48 h then plated on blood agar and MaCconky agar media (Quinn *et al*., 2004).

Microscopical examination of gram and giemsa stain

a. Gram stain: It was conducted according Quinn *et al*. (2004).
b. Bipolar stain: It was conducted according Baron and Feingold (1990).
c. Capsule stain: It was conducted according Baron and Feingold (1990).

Biochemical tests

A. Catalase test it was conducted according to Mcfaddin (2000)
B. Oxidase test it was conducted according to Forbes *et al*. (2002)
C. KOH test it was conducted according Baron and Feingold (1990)
D. Oxidation-Fermentation (O-F) test according Quinn et al. (2004)
E. Sugar fermentation test according Ronald and James (2006).
F. Urease test according to Colle *et al*. (1996).
G. Indol test according Macfadden (2000).
H. Motility test according to Colle *et al*. (1996).

Identification by the VITEK 2 system

The VITEK 2 system is a new fully automated system to provide rapid and accurate identification and susceptibility testing results for the most clinical isolates, Identification was made on the bases of biochemical reactions, and MIC determinations were made by applying an algorithm to the growth kinetics monitored by VITEK 2 system (Funke *et al*., 1998; Bassel *et al*., 1997; Graf *et al*., 2000). These procedures were performed according to the manufacturer's instructions.

Preservation of bacterial isolates

All isolates after definitive identification were cultured on sterile brain heart infusion broth and glycerol 20 % and incubated at 37C for 24 hrs, then after the occurrence of turbidity was stored in a deep freezing (Quinn *et al*., 2004).

Antibiotic sensitivity test

Five colonies of the same morphological type which were diagnosed previously were selected from nutrient agar, and a loopful was transferred to a tube containing 5ml of nutrient broth. The inoculated broth was incubated at 37 °C for 18 hrs turbidity appeared. A sterile swab was dipped into the standardized suspension of bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube above the fluid level. Then, the swab was streaked in three directions over the entire surface of the Muller Hinton-agar. The inoculated plates were allowed to dry for 5 minutes, then the discs of antibiotics were placed onto agar surface using a sterile forceps. after that the plates were inverted and incubated aerobically at 37C for 18-24 hrs (Quinn *et al*., 2004).

After incubation, the zones of inhibition were measured by using electronic vernea depending upon a zone of inhibition reported.

Collection and preparation of plant samples

Fresh *Plantago lanceolata* leaves were collected from a garden of Veterinary Medicine College, Baghdad University. Plant leaves were washed under tap water and then dried at room temperature at shade. The dried leaves were crushed by a laboratory blender. The plant classification was performed in the Ministry of Agriculture, State Board for seeds testing and certification SBSTC in Abu Graib, Baghdad.

Preparation of alcoholic extract of *Plantago lanceolata*

Organic solvents were used for the extraction of *P. lanceolata* leaves by using absolute ethanol (Ethyl alcohol) which was considered as very effective in extracting the active ingredients of the plant according tothe method described by (Effrain *et al*., 2000). This was performed by using soxhlet apparatus, which around bottom glass flask placed that fitted to an extraction unit. The extracting unit contained the solvent and cellulose (thumble) located inside it that contained the dry plant powder. A distiller unit was fitted on to the extraction unit. For condensation of Vapor solvent, 30 g of plant powder were placed inside the thumble and 300 ml of absolute ethanol were placed inside the flask. The extraction was carried out for 24 hrs. by heating temperature that kept the solvent at 50-60 co until a clear and colorless solvent appeared in the extracting unit. After that, the extract was dried by using a rotary evaporator 40-45 co. The dry extract was placed in an incubator under 38-40 co for complete dryness. The final extract was kept frozen at -20 co until use.

Determination the activity of *Plantago lanceolata in vitro*

Agar well diffusion method

Agar well diffusion method was used to check the activity of plant extract *in vitro* (Kavanagh, 1972). To achieve this purpose, pure colonies of *pasteurella multocida* and *Mannheimia hemolytica* were selected .Such colonies were inoculated into 4 ml of nutrient broth and incubated for 2-8 hrs at 37co, the turbidity of inoculums compared with standardized MacFaralnd tube number (one) containing (1.5 ×108) cfu/ ml, with a sterile cotton swab, the inoculum was spread evenly on the surface of Muller Hinton agar in pertridish, A five wells were made in Muller Hinton agar plates using a sterile cork borer (6 mm), 0.1 ml of different concentrations of plant extract (100, 150, 200mg /ml) were poured in the three wells while the other two wells were filled with 0.1 ml of DMSO and with ethyl alcohol as a control, the plates were incubated up down at 37 co for 24 hrs. Three replicates were carried out for each concentration extract, the diameter of inhibition zone was measured and the average values were recorded. The results and standard errors mean values were tabulated (Mahmood *et al*., 1989).

Statical analysis

The data obtained were expressed as Mean and Standard errors (Mean±SE) and subjected to statistical analysis using one-way and 2-ways analysis of variance (ANOVA) for comparison between the different groups. Also post hoc test was used when appropriate to find the least significant differences (LSD) between groups and ($P < 0.05$) was considered statistically significant. All statistical analyses were done using the Statistical Package for the Social Sciences program (SPSS) version 20 packages (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Percentage of infection with pasteurella

As shown in Table 1 the *Pasteurella multocida* was isolated from 2 out of 150 blood samples of cattle with infection rate of 1.33% and 1 out of 150 nasal swab of sheep with rate of infection of 0.66%. All other samples from different animal species were appeared to be free from this bacteria, The total infection rate with *Pasteurella multocida* were 0.66%. *Mannheimia haemolytica* was isolated from 3 out of 150 blood samples of cattle with infection percent of 2%, while all other samples of sheep and goats were not revealed infection with this bacterium (Table 1).

This rates of infection with *P. multocida* and *M. haemolytica* in the present study were less than what revealed in other studies, where Radostits *et al*. (2010) have mentioned that both morbidity and case-fatality rates vary between 50% and 100%. This decrement in the morbidity rate may be attributed to the use of vaccine against these bacteria in Iraq during the last years and the constant climate during the period of the study which lead to absent or less stress on animals of the study (Personal contact), This may also be due to adaptation of the animals to these bacteria because Iraq is endemic with pasteurellosis. The researchers found that morbidity depended on the immune status of the herd, either acquired naturally or induced by vaccination, the greater the percentage of immune to non-immune animals, the lower will be the morbidity and in endemic areas, adult animals developed a naturally acquired immunity and large outbreaks no longer occured in these areas (Radostits *et al*., 2010).

In Iraq, Mubarak (2000) has isolated *M. haemolytica* at a percent of 2.94% from different animals, this result in agreement with our result.

Isolation and identification of *Pasteurellac*

The identification of the bacteria was ensured according to the (Quinn *et al*., 2004) by the following steps:

Cultural characteristics

The isolated colonies of *Pasteurella multocida* appeared as grey and viscous, with a strong mucinous odour,rough, irregular and with no hemolysis on blood agar and have no ability to grow on MacConkey agar. These characters are in agreement with those mentioned by Quinn *et al*. (2004).

The colonies of *Mannheimia haemolytica* on blood agar appeared as small, and greyish colonies produced beta hemolysis and grown on MacConkey agar with pink colour. These characters are in agreement with those mentioned by Quinn *et al*. (2004).

Table 1: The prevalence level of *Pasteurella multocida* and *M. haemolytica* in nasal swab and blood samples from cattle, sheep and goats.

Sample		*Pseurella multocida*			*Mannhaimia haemolytica*		
		Cattle	Sheep	Goat	Cattle	Sheep	Goat
Blood	450	2(1.33%)	0	0	3(2%)	0	0
Nasal swab	450	0	1(0.66%)	0	0	0	0
Lung	50	0	0	0	0	0	0
Total	950	2(1.33%)	1(0.66%)	0	3(2%)	0	0

Microscopical examination of Gram stain

Gram stain and KOH test

Staining of suspected colonies with Gram stain showed a pleomorphic gram-negative coccobacillus which occurred singly, in pairs or short chains. These characters are in agreement with those mentioned by Jawetz *et al*. (2007). The results of Gram stain were confirmed by KOH test, where all the isolates of *P. multocida* and *M. haemolytica* in the present study revealed a positive result to KOH test. This result of KOH test were inversely related to the Gram stain result (Quinn *et al*., 2004).

Geimsa stain

Staining with Geimsa stain revealed the presence of colors only of two opposite poles of the bacterium and leaving the rest of the bacterium unstained or of a lighter color.

Results of biochemical test

The results of most important biochemical tests that were used to differentiate between *P. multocida*, and *M. haemolytica* and with other types of bacteria shown in Table 2.

The results of identification which were obtained by biochemical tests that mentioned above were confirmed by using VITEK 2 system for one isolate only (Table 3).

The results of the *P.multocida* diagnosis by Vitek 2 system showed that these bacteria were positive for some biochemical tests including Dmannose, Tyrosine Aryl Aminidase, ELL MAN, Coumarate and phosphatase andnegative for the remaining biochemical tests (Table 3).

Antibiotic susceptibility test

Antibiotic susceptibility test was conducted for 3 isolates of *P. multocida* and 3 isolates of *M. hemolytica* against different antibiotics to assess the prevalence of antibiotic resistance of each isolate that was tested against ten antibiotics and inhibition zone of bacterial growth around the antibiotic disc were measured to check the sensitivity of the isolate against these ten antibiotics. The results are shown in Table 4.

Table 2: The results of biochemical tests of the *Pasteurella* isolates.

Tests species	Catalase	Oxidase	O/F	Beta haemolysis	Motility	Growth on macconkey	Sugar fermentation				
							Indole	Urease	Glucose	Lactose	Sucrose
P. multocida	+	+	F	-	-	- +	+	+	-	+	-
M. haemolytica	+	+	F	+	-	+ -	-	+	+	+	+

Table 3: The results of VITEK2 system.

APPA	-	**ADO**	-	**PYrA**	-	**IARL**	-	**dCEL**	-	**BGAL**	-
H_2S	-	PNAG	-	AGLTp	-	dGLU	-	GGT	-	OFF	-
BGLU	-	Dmal	-	dMAN	-	dMNE	+	BXYL	-	BALap	-
ProA	-	LIP	-	PLE	-	TYrA	+	URE	-	dsOR	-
SAC	-	dTAG	-	dTRE	-	CIT	-	MNT	-	5KG	-
ILATK	-	AGLU	-	SUCT	-	NAGA	-	AGAL	-	PHOS	+
GLYA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
OI29R	-	GGAA	-	IMLTa	-	ELLM	+	ILATa	-		

Table 4: Antibiotic sensitivity test of each *P. multocida* and *M. haemolytica* to different antibiotics.

Antibiotics	*P. multocida*		*M. haemolytica*	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Penicillin	0	3(100)	2(66.7)	1(33.3)
Erythromycin	1(33.3)	2(66.7)	2(66.7)	1(33.3)
Gentamycin		0	3(100)	0
Aztreonam	2(66.7)	1(33.3)	1(33.3)	2(66.7)
Doxycycline	1(33.3)	2(66.7)	2(66.7)	
Rifampin	0		2(66.7)	1(33.3)
Amoxicillin + Clavulanic	1(33.3)	2(66.7)	2(66.7)	1(33.3)
Clindamycin	2(66.7)	1(33.3)	3(100)	0
Colistin sulfate	2(66.7)	1(33.3)	0	3(100)
Cloxacillin	0	3(100)	0	3(100)

Results showed that, Gentamycin was found to be the most effective antibiotic with 100% for both *P. multocida* and *M. haemolytica*,this result were in agreement with Naz *et al.* (2012) who haves found that *P. multocida* were sensitive to Gentamycin, and disagree with her result and the result of Sabiel *et al.* (2011) who found that Gentamycin was resistant, but disagree with those result for *M. heamolytica* that were found resistant to Gentamycin, Erythromycin and Penicillin. The results of Krisztina *et al.* (2024) showed that Atotal of 131 (84.5%) strains of *Pasteurella multocida* were susceptible to penicillin, and the same number of strains proved to be susceptible to enrofloxacin while A 100% resistance rate was observed for clindamycin. For erythromycin, 81.3% of strains showed reduced sensitivity while 9.7% were resistant. The result of Marru *et al.* (2013) were in disagreement with our results, who Have found that both *P. multocida* and *M. haemolytica* were resistant to Gentamycin. A study of ShivaChandra *et al.* (2004) that tested twenty antibiotics against *P. multocid* found highly susceptible to doxycycline, and their results was incompatible with our results. *Pasteurella multocida* is a widespread facultative pathogenic bacterium, which causes a wide range of diseases in both mammals and birds, the antibiotic susceptibility of 155 *P. multocida* strains were tested using the broth microdilution method to obtain the minimum inhibitory concentration (MIC) values for 15 antibiotics, The most effective antibiotics against pasteurellosis were ceftiofur, tetracycline, doxycycline, florfenicol and tilmicosin. Of the strains, 12 proved to be multi-drug resistant (MDR) (Krisztina *et al.*, 2024), some of these results were somewhat consistent with what we obtained. The present study showed that Erythromycin was not very effective antibiotic. These observations are in accordance with Watts *et al.* (1994) who have frequently encountered resistance of *M. haemolytica* and *P. multocida* of bovine origin to erythromycin. Our results were in agreement with Mohammadi *et al.* (2006) who concluded that *M. haemolytica* were sensitive to Penicillin and disagree with his result that *P. multocida* were resistant to Penicillin. The result of de Jong *et al.* (2014) showed that both *P. multocida* and *M. haemolytica* were susceptible to amoxicillin/clavulanic acid, and these finding were with our result for *M. haemolytica* and compatible with result of Nedbalcova *et al.* (2010), but disagree with our result for *P. multocida* which show resistant of this bacteria. Klima *et al.* (2014) have reported that both *P. multocida* and *M. haemolytica* were show resistant for to most antibiotics. Zaheer *et al.* (2012) demonstrated that both *M. haemolytica* and *P. multocida* were erythromycin resistance, and such result agree with our result that showed resistance of *P. multocida*, but disagree with the result of *M. haemolytica* sensitive.

Yield extract of *Plantago lanceolata*

The leaves of the *Plantago lanceolata* which were extracted with 95% ethanol yielded deep green extract with plant powder of 24.7 %. This was determined by using the following equation:

Percentage yield of the extract = weight of extract (gm)/weight of plantago powder (gm) × 100 (Banso and Adeyemo, 2006)

Example: 7.41 g /30 g × 100 = 24.7 %

The effect of *Plantago lanceolata* extract against the *P. multocida* and *M. haemolytica* growth *in vitro*

The results showed that the concentration of the extract played an important role in determining the diameter of inhibition zone, as the diameter was increased when a higher concentration of the extract was used. The mean of inhibition zone diameter of the alcoholic extract of the *P. lanceolata* against *P. multocida* were 17.22, 19 .11, 21.94 mm while were 13.00, 14.55, 15.55 mm against *M. haemolytica* at the concentration of 100, 150, 200 mg/ml of the extract, respectively as shown in Tables 5 and 6.

The low concentration (100mg/ml) showed a low inhibition zone of 17mm and a high inhibition zone of 18mm for *Pasteurella multocida*, while a low inhibition zone of 11mm and a high inhibition zone of 16mm in *Mannheimia haemolytica*. At concentration of 150 mg/ml a low inhibition zone was a 18mm and a high inhibition zone was 20 mm for *Pasteurella multocida*, but for *Mannheimia haemolytica*a low inhibition zone was 14mm and a high inhibition zone was 15 mm. At a high concentration of (200mg /ml) the low inhibition zone was 19.5mm and a high inhibition

Table 5: Sensitivity (inhibition zone mm.) of *P. multocida* against different concentrations of *Plantago lanceolata* extract.

No. of isolate	Concentration			DMSO	Alcohol
	100 mg/ml	150mg/ml	200mg/ml		
P1	17.00 0.00C	18.33 0.57B	19.83 0.28Ac	0.00 0.00D	0.00 0.00D
P2	17.00 0.00C	20.00 0.00B	23.66 0.57Aa	0.00 0.00D	0.00 0.00D
P3	17.66 0.57C	19.00 0.00B	22.33 0.57Cb	0.00 0.00D	0.00 0.00D
Total mean	17.22	19.11	21.94	0.00	0.00

P=*Pasteurella multocida* The different uppercase letters refer to significant differences between differentcolumn at (P<0.05). The different lowercase letters refer to significant differences between different row at (P<0.05).

Table 6: Sensitivity (inhibition zone mm) of *M. haemolytica* against different concentrations of *Plantago lanceolata* extract.

No. of isolate	Concentration			DMSO	Alcohol
	100 mg/ml	150mg/ml	200mg/ml		
M1	11.33 0.57Cb	15.00 0.00Aa	14.00 0.00Bc	0.00 0.00D	0.00 0.00D
M2	16.00 0.00Aa	14.00 0.00Cb	14.66 0.57Bb	0.00 0.00D	0.00 0.00D
M3	11.66 0.57Cb	14.66 0.57Bc	18.00 0.00Aa	0.00 0.00D	0.00 0.00D
Total mean	13.00	14.55	15.55	0.00	0.00

M= *Mannheimia haemolytica*. LSD=0.55.

zone was 24mm for *Pasteurella multocida* while for *Mannheimia haemolytica* a low inhibition zone was 14mm and a high inhibition zone was 18 mm; but no inhibition zone was recorded for DMSO and alcohol. In this regard the study of Alla *et al.* (2023) showed that Different concentrations of the ethanolic extract of *P. major* leaves exhibited different zones of inhibition against *P. aeruginosa* from 9.93 mm to 22.18 mm in diameter.

The results were showed the superiority of the concentration 200 mg/ml of plant extracts which could be attributed to a high amount of soluble active ingredients which decrease or prevent the bacterial growth. The antibacterial actions of the *P. lanceolata* extract could be attributed to their metabolites such as flavonoides, phenol, tannins, saponins, steroids, glycosides and resins were characterized by their antibacterial actions (Cowan, 1999). Different concentrations of the ethanolic extract of *P. major* leaves exhibited different zones of inhibition against *P. aeruginosa* from 9.93 mm to 22.18 mm in diameter. The ethanolic extract of *P. major* was able to inhibit the growth of *S. pyogenes* ATCC 19615 at concentrations of 250 mg/mL, 500 mg/mL, and 750 mg/mL, and 1000 mg/mL. Based on the thin layer chromatography test, the ethanolic extract of *P. major* contains a class of flavonoid compounds, alkaloids, steroids, phenols, tannins, and terpenoids. The mechanisms of action of active ingredients of *P. lanceolata* to impair the bacterial growth are different, such as tannic acid which have the ability to precipitating the gelatin of solution in a process known as astringency. Therefore, precipitating the protein and thus deprive the bacteria from the nutritional proteins (Giessman, 1963). On the other hand, the phenolic compounds exert their antibacterial action through the hydroxyl group (OH-) (Cowan, 1999). The flavonoids which can be synthesized by plants in response to the microbial infections (Dixon *et al.*, 1983) could be able to make a complex with the intracellular proteins and cell wall, and disrupting the lipophilic compounds of the bacteria (Chabot *et al.*, 1992).

The substitution of the ester or carboxylic acid functional group on the coumarine ring gave it a potent inhibitory action against both gram positive and gram negative bacteria (Kawase *et al.*, 2001; Kairat *et al.*, 2023). The superiority of the use of the plant extract, compared with the antibiotics, could be attributed to ability of the plant extract to attenuate of the pathogen virulence by allowing the immune system to participate in the elimination process of the pathogen, while the bactericidal antibiotics kill the bacteria directly, thus the anti-pathogenic ingredients in the *P. lanceolata* prevented the production of toxins or abolished the bacterial capability to adapt the host environment and would give a competitive advantage to the host immune system for clearance of the pathogens (Gonzalez-Lamothe *et al.*, 2009).

In addition to what mentioned above, the acidity of the alcoholic extract also inhibited the bacterial growth through their free hydrogen ions which banded to molecules and changed the microbial environment (Joklik *et al.*, 1992). The biostatistics analysis revealed significant differences ($P < 0.05$) between *P. multocida* and *M. haemolytic*, where as *P. multocida* isolates appeared significantly more sensitive to *P. lanceolata* extract than *M. haemolytica* isolates.

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NOVELTY STATEMENT

This study has proven the therapeutic importance of Plantago lanceolata extract with the in cooperation between authors and through their own efforts with the assistance of specialized laboratories at the university of Baghdad and Fallujah University.

AUTHOR'S CONTRIBUTION

HAA-R: Conceived of the presented idea, investigate and supervised the findings of this work and supervised the project.
TMN: Verified the analytical methods, carried out the experiment.
YK: Laboratory work assistant.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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