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In Vitro Antibacterial Effect of Four Medicinal Plant Extracts on *Brucella Abortus* Isolated from Cattle

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Abstract

Background: Brucella abortus is the main causative agent of an infectious zoonotic disease known as brucellosis in both human and livestock. The most commonly used vaccine, B. abortus S99 for brucellosis has often given false positive results in mature animals which has brought a high risk for the bacteria vaccine to humans, thus the option for new antibacterial compounds are becoming necessary for efficient brucellosis treatment. This study aimed to investigate the antibacterial activity of extracts of *Lepidium sativum*, Solenostemma argel, Organum marjoram and Eucalyptus camaldulensis.

Methodology: Aqueous, hexane and alcohol extracts of *Lepidium sativum*, *Solenostemma argel*, *Origanum marjoram* and *Eucalyptus camaldulensis* were used to determine the antibacterial activity using well diffusion test; inoculation broth method and disc diffusion method at different concentrations, using Streptomycin as a positive control.

Results: The results obtained showed different inhibition zones with different herbs, *Lepidium sativum* (46mm/20%/ hexane extract /well test), *Solenostemma argel* (36mm /20%/hexane extract /disc test) and *Marjoram origanum* (20 mm /20%/ethanol , hexane extracts /well test). No inhibition was observed in case of *Eucalyptus camaldulensis* extracts. Streptomycin reached inhibition (56mm) in *Lepidium sativum* hexane extract dish;(56mm) in the *Solenostemma argel* ethanol extract dish and extract (52mm) in the *Marjoram origanum* ethanol extract.

Conclusion: Both aqueous and hexane extracts could act as good anti- bacterial agents against Brucella abortus.

Keywords: *Brucella abortus; Lepidium sativum; Solenostemma argel; Marjoram origanum; Eucalyptus camaldulensis*; antibacterial activity

Introduction

Brucellosis is an infectious disease which has high morbidity in livestock caused by Brucella abortus [1]. Humans generally acquire the disease through direct contact with infected animals, by eating, drinking contaminated animal products, or by inhaling airborne agents, however majority of cases are caused by ingesting unpasteurized milk or cheese [2]. There has been a fall in trade for livestock especially cattle due to the escalated infections, unpreceded abortions, low lactation leading to reduced production of milk and giving birth to unhealthy offspring [3]. Both tetracyclines and streptomycin are being used in treatment of brucellosis, irrespective of their high failure rates and resistance which leaves it as an outstanding challenge in livestock., hence the need to look out for novel strong phytomedicines with high antimicrobial activity [4]. Thus, the need for this study was to screen for antibacterial activity of aqueous, hexane and alcohol extracts of *Lepidium sativum*, *Solenostemma argel*, *Origanum marjoram* and *Eucalyptus camaldulensis* on *Brucella abortus* isolated from cattle in Sudan.

Materials and Methods

Plant collection and identification

Lepidium sativum, *Solenostemma argel*, *Origanum marjoram* and *Eucalyptus camaldulensis* were collected from Alsoog alkabeer in Wad Medani the capital of Gezira State. The taxonomic identification of these plants was done by the Department of botany, University of Gezira.

Preparation of plant extracts

The seeds of *Lepidium sativum*, leaves of *Solenostemma argel*, leaves of *Origanum marjoram* and leaves of *Eucalyptus camaldulensis* were air dried at room temperature and later crushed into powder. *Lepidium sativum* seed extracts of 5%, 10%, 15% and 20% concentrations (w/v) were prepared by using distilled water, ethanol (98%) and hexane (95%) solvents. *Solenostemma argel* leaf extracts of 2%, 4%, 10%, 15% and 20% concentrations were prepared (w/v) were prepared by using the above-mentioned solvents with continuous agitation using an electric shaker every 30 minutes for six hours. After 24 hours, the prepared extracts were filtered and then immediately used for testing their inhibition activities against Brucella abortusS99.

Source of culture

The culture of (Brucella abortus) s99 was obtained from Department of Brucella, Veterinary Research Institute (VRI), Soba, Khartoum, Sudan. All microorganisms were maintained at 4°C on tryptose agar. The culture of (B. abortus) was offered by Animal &Plant Health Agency (APHA, UK, Surrey)

Data Analysis

Statistical analysis was done by using descriptive One-way ANOVA test.

Agar well diffusion method

A loop full of bacterial strain was inoculated in 30 nutrient broth in a conical flask and incubated for 72 hours to get active strain by using agar well diffusion method. Treptose Agar was poured into Petri dishes. After solidification 0.25 ml of test strains were inoculated in the media separately. Care was taken to ensure proper homogenization. Experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5 mm). The extract compound (50 µl) was introduced into the well and plates were incubated at 37°C for (72 hours up to ten days). All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition. A control with standard antibiotic (Streptomycin) was kept for all test strains and the control activity was deducted from the test as was recommended by, the results were recorded after 7 to 10 days [5].

Disc diffusion method

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by [8] to assess the presence of antibacterial activities of the plant extracts. A bacteria culture (which has been adjusted to 0.5 McFarland standards) was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of plant extracts were placed on the Mueller-Hinton agar surface. Each test plate comprises of six discs. One positive control, which is a standard commercial antibiotic disc, one negative control, and four treated discs. The standard antibiotic disc was Streptomycin 30 µg. Besides the controls, each plate had four treated discs placed about equidistance to each other. The plates were then incubated at 37°C for three to ten days depending on the species of bacteria used in the test. After the incubation, the plates were examined for inhibition zone. The inhibition zones were then measured using calipers and recorded. The tests were triplicate to ensure reliability.

Results and Discussion

Table 1: The initial Agar well diffusion test results by showing inhibition zones (mm) of different concentrations, of each extract.

Herpes	*Lepidium sativum*		*Origanum Majorana*		*Eucalyptus Camaldulensis*		*Solenostemma Argel*	
Conc.	5%	10%	1%	2%	3%	6%	2%	4%
Water	Resist	resist	Resist	conta	conta	conta	conta	conta
	0-4	0-4	0-4	-	-	-	-	-
Hexane	Inhib	Inhib	Resist	conta	conta	Resist	conta	Inhib
	Oct-35	Oct-35	0-4	-	-	0-4	-	Oct-35
Ethanol	Inhib	Inhib	conta	conta	conta	Resist	conta	conta
	Oct-35	Oct-35	-	-	-	0-4	-	-

Table 1 shows that, the inhibition (descriptively) of the aqueous, ethanol and hexane extracts (at two initial concentrations for each extract of *Lepidium sativum*, Solenostemma argel, Origanum marjoram and *Eucalyptus camaldulensis* on Brucella

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abortus. Concerning the aqueous extracts, all plants presented no obvious inhibition to B. abortus at each tested concentration. (1%, 2%, 3%, 4%, 5%, 6%and10%). Hexane extracts, L.sativum showed obvious inhibition to B. abortus at each tested concentration (5%and10%), S argel at concentration4% has also inhibition, while the rest of the plants hexane extracts showed no inhibition action. Ethanol extracts, only L. sativum appeared inhibition to B [6,7]. abortus at the tested concentrations (5%and10%), while the rest of the plant's ethanol extracts showed resistance action. The positive control (streptomycin) that used for brucellosis (Malta fever) in human showed total inhibition to B. abortus at the concentration (200mg /ml) .in volume of 0.05 ml = 50 µl. The degree of inhibition adversely related to the color depth of the obtained culture.(NCCLS)National Committee of Clinical Laboratory Standards-Quinn 1994 ,who illustrated that the range of zones occurred,(0-4)mm regarded resistant, while(5-9)mm regarded as moderate, whereas(10-35)mm concerned sensitive. The results obtained from the study revealed a number of facts. On the basis of the primary screening as presented in Initial well –test, L. sativum showed no inhibition to B. abortus at both tested concentrations (5%,10%) while the rest of the tested herbs, although, no inhibition was observed, some fungal contamination occurred. This could be due to the lower concentrations used (maximum6%) and the type of extract as well as the activity agent according to [8]. The overall results ensured that, the concentration and the type of extract plaid a vital role in the efficiency of the herpes against different pathogens. In a study conducted by [9], L. sativum seed extracts possessed antifungal activity that can be exploited as an ideal treatment for future fungal disease. However in this study , L. sativum inhibited the growth of B. abortus in both hexane and ethanol extract (66.7 %) in contrast to the positive control (streptomycin) ,that used for brucellosis (Malta fever) in human, which showed absolute inhibition to B. abortus at the concentration (200mg /ml) .in volume of 0.05 ml = 50 µl. Nevertheless [10]. Reported that L. sativum ethanolic extract showed inhibition activity against P. aeruginosa while, hexane extract exhibited the maximum zone of inhibition against C. albicans and moreover, it has potency against gram positive and negative bacteria.

Table 2: The statistical analysis of Initial well –test/ percentage.

Type of Herb	Result			Total n (%)
	Conta n (%)	Inhibition n (%)	Resistant n (%)	
Lepidium sativum				
Concentration 5%	0	2 (66.7%)	1 (33.3%)	3 (100%)
Concentration 10%	0	2 (66.7%)	1 (33.3%)	3 (100%)
Organum margoram				
Concentration 1%	2 (66.7 %)	0	1 (33.3 %)	3 (100%)
Concentration 2 %	3 (100 %)	0	0	3 (100%)
Solenostemma argel				
Concentration 2%	3 (100 %)	0	0	3 (100%)
Concentration 4%	2 (66.7%)	1 (33.3 %)	0	3 (100%)
Eucalyptus camaldulensis				
Concentration 3 %	3 (100%)	0	0	3 (100%)
Concentration 6%	1 (33.3%)	0	2 (66.7%)	3 (100%)

Table 3: The Broth test results shows the inhibition zones (mm) of different concentration, in descriptive terms of each plant with water, hexane and ethanol extract.

Herpes	*Lepidium sativum*		*Origanum majorana*		*Eucalyptus camaldulensis*		*Solenostemma argel*	
Conc.	5%	10%	1%	2%	3%	6%	2%	4%
Water	Inhib	Inhib	Resis	Inhib	Resis	moderate	moderate	Inhib
	10-35	10-35	0-4	10-35	0-4	5-9	5-9	10-35
Hexane	Resis	Inhib	Inhi	Inhib	Resis	Resis	Resis	Inhib
	0-4	10-35	10-35	10-35	0-4	0-4	0-4	10-35
Ethanol	Resis	In hib	Resis	Resis	Resis	Resis 0-4	moderate	moderate
	0-4	10-35	0-4	0-4	0-4	-	5-9	5-9

*Streptomycin (control) represented obvious inhibition activity.

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(Table 2 & 3) shows that, the inhibition activity (descriptively) of the aqueous, ethanol and hexane extracts for each plant (at two initial concentrations; that ranged from 1% and 10% concentrations) of *Lepidium sativum*, Solenostemma argel, Origanum marjoram and *Eucalyptus camaldulensis* on Brucella abortus S99.The aqueous extracts *Lepidium sativum* had high inhibition activity in both (10%) and (5%) tested concentrations; Origanum marjoram represented inhibition activity against Brucella with(1% and2%) concentrations of aqueous extract also *Solenostemma argel* showed high inhibition activity in high concentration (4%)on Brucella abortus but, moderate inhibition effect in (2% and 6%) for both .*Eucalyptus camaldulensis* had moderate inhibition effect in(6%) concentration. Hexane extracts, L. sativum showed high inhibition to B. abortus at tested concentration (10%); O majoram appeared inhibition effect in (1%) and inhibition in (2%) concentration. S. argel at concentration 4% show high inhibition effect, in contrast *Eucalyptus camaldulensis* hexane extracts in both (3% and 6%) concentrations showed resistance. Ethanol extracts, L. sativum showed inhibition to B. abortus at tested concentration (10%); S. argel showed inhibition for B. abortus at both tested concentrations (2%, 4%), while the rest extracts were of no effect. The degree of inhibition was related to the color depth of the obtained culture according to the National Committee of Clinical Laboratory Standards guidelines which illustrated that the range of zones occurred (0-4) mm regarded resistant, while (5-9) mm regarded as moderate [11]. Whereas (10-35) mm concerned sensitive. Table 4 depicts the inhibition zones quantitatively (mm) of the aqueous, hexane and ethanol extracts, at (10%, 15% and 20%) concentrations for the tested plant (*Lepidium sativum*), against B. abortus. The aqueous extract of *Lepidium sativum* had inhibition activity (32mm) at 10% conc, (30 mm) at 15% conc and (26mm) at 20% conc, whereas the control streptomycin gave (40mm) inhibition zone. Hexane extracts of *Lepidium sativum* had inhibition activity (40mm) at10% conc, (40mm) at 15% conc and (46mm) at 20% conc, while the control streptomycin measured (56 mm) inhibition zone. The ethanol extract of *Lepidium sativum* had inhibition activity (14mm) at 10% conc, (10mm) at 15% conc and(12mm) at 20% conc, while the control streptomycin gave(18mm) inhibition zone [12,13].

Table 4: Shows the inhibition zones (mm) for the tested plant (*Lepidium sativum*) with different extracts on *B. abortus*- well diffusion test.

Herbs	Extract	Conc %	Zone(mm)	Streptomycin
L.sativum	Aqueous	10	32	40
		15	30	
		20	26	
	Hexane	10	40	56
		15	40	
		20	46	
	Ethanol	10	14	18
		15	10	
		20	12	

Table 5: Shows the inhibition zones (mm) for the tested plant (*Origanum marjoram*) with different extract on *B. abortus*- well diffusion test.

Herbs	Extract	Conc %	Zone(mm)	Streptomycin
O. majorana	Aqueous	10	12	38
		15	14	
		20	16	
	Hexane	10	18	-
		15	16	
		20	20	
	Ethanol	10	20	52
		15	18	
		20	-	

Table 5 depicts the inhibition zones quantitatively (mm) of the aqueous, hexane and ethanol extracts at (10%, 15% and 20%) concentrations for the tested plant (Origanum marjoram), against B. abortus. The aqueous extract Origanum marjoram gave (12mm) inhibition zone at (10%) conc., (14mm) inhibition zone at (15%) conc and (16mm) inhibition zone at (20%) conc,

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while the control streptomycin gave (38 mm) inhibition zone. However, the hexane extract of Origanum marjoram gave (18mm) inhibition zone at (10%) concentration, (16mm) inhibition zone at (15%) concentration and (20mm) inhibition zone at (20%) concentration. Concerning the ethanol extract, Origanum marjoram gave (20mm) inhibition zone at (10%) concentration, (18mm) inhibition zone at (15%) concentration, while the control streptomycin gave (52mm) inhibition zone.

Table 6: Shows the inhibition zones (mm) for the tested plant (*Solenostemma argel*) with different extract on *B. abortus*- well diffusion test.

Herbs	Extract	Conc %	Zone(mm)	Streptomycin
Solenostemma argel	Aqueous	10	contamination	39
		15	contamination	
		20	contamination	
	Hexane	10	17	
		15	contamination	-
		20	contamination	
	Ethanol	10	24	
		15	16	56
		20	10	

Table 6 depicts the inhibition zones quantitatively (mm) of the aqueous, hexane and ethanol extracts at (10%, 15% and 20%) concentrations for the tested plant (Solenostemma argel) against B. abortus. The aqueous extract of *Solenostemma argel* showed contamination, the control streptomycin gave (39 mm) inhibition zone. Also hexane extract of *Solenostemma argel* was contaminated at (15%, 20%) conc, but had inhibition activity (17 mm) at 10% concentration .With ethanol extract , *Solenostemma argel* had inhibition activity with respective concentrations (24mm) at 10% , (16mm) at 15% and (10mm) at 20% while the control streptomycin gave (56 mm) inhibition zone. Table 7 represents the inhibition zones quantitatively (mm) of the aqueous, hexane and ethanol extract at (10%, 15% and 20%) concentrations for the tested plant (Eucalyptus camaldulensis), against B. abortus. *Eucalyptus camaldulensis* represented no inhibition effect in all concentrations with all types of extracts. The control measured (30mm) inhibition zone.

Table 7: Shows the inhibition zones (mm) for the tested plant (*Eucalyptus camaldulensis*) with different extract on *B. abortus*- well diffusion test.

Herbs	Extract	Conc %	Zone(mm)	Streptomycin
Eucalyptus camaldulensis	Aqueous	10	-	30
		15	-	
		20	-	
	Hexane	10	-	-
		15	-	
		20	-	
	Ethanol	10	-	-
		15	-	
		20	-	

Conclusion

Lepidus sativum was the best one and had strong anti-Brucella effect. *Marjoram origanum* and *Solenostemma argel* also they acted as anti- brucella. Aqueous and hexane extracts of these plants had efficiency than its ethanol extract against B. abortus. In general, the results revealed significant anti-brucella activity of medicinal plants, which could be a potential source of new antibacterial agents.

Recommendation

The results of this study are recommended to detect the active ingredients of *Lepidium sativum*, Origanum marjoram and *Solenostemma argel* which can act as anti-brucella agents. In addition, phytochemical screening should be carried out for all the plants. Further study should be done to know the Minimum inhibition concentration (MIC). The study should be continued in vivo by experimental animals or in tissue cultures.

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The synergistic effect and antagonist effect of *Lepidium sativum*, organum marjoram and *Solenostemma argel* should be considered for further study. All the above recommendations will justify the reason as to why the above herbs as depended on by most people from Sudan.

References

1. Grilló MJ, de Miguel MJ, Moñoz PM, Marín CM, Ariza J, et al. (2006) Efficacy of several antibiotic combinations against Brucella melitensis Rev1 experimental infection in BALB/c mice. Journal of Antimicrobial Chemotherapy 58(3): 622-626.
2. Lopez Goni IO, Callaghan D (2012) Brucella: Molecular Microbiology and Genomics. Caister Academic Press, p. 93-96.
3. Gul ST, Khan A (2007) Epidemiology and epizootiology of brucellosis: A review. Pakistan Veterinary Journal 27(3): 145-151.
4. Aboaba OO, Smith SI, Olude FO (2006) Antibacterial effect of edible plant extract on Escherichia coli O157:H7. Pakistan Journal of Nutrition 5(4): 325-327.
5. Seleem MN, Boyle SM, Sriranganathan N (2010) Brucellosis: a re-emerging zoonosis. Veterinary microbiology 140(3-4): 92-398.
6. Barrow P, Lovell M, Berchieri A (1998) Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin Diagn Lab Immunol 5: 294-298.
7. Barrow GI, Feltham RKA (1993) Characters of Gram-positive bacteria. Cowan and steel's manual for the identification of medical bacteria, (3rd edn). Cambridge University Press, Cambridge, USA.
8. Adam SI, Salih SA, Abdelgadir WS (2011) *In vitro*" Antimicrobial Assessment of" *Lepidium sativum*" L. Seeds Extracts. Asian Journal of Medical Sciences 3(6): 261-266.
9. Sharma RK, Vyas K, Manda H (2012) Evaluation of antifungal effect on ethanolic extract of *Lepidium sativum* L. seed. Int J Phytopharmacol 3(2): 117-120.
10. Bhasin P, Bansal D, Punia A, Sehrawat AR (2012) Antimicrobial activities of *Lepidium sativum*: Medicinal plant used in folklore remedies in India. Journal of Pharmacy Research 5(3): 1643-1645.
11. Karazhiyan H, Razavi SM, Phillips GO, Fang Y, Al Assaf S, et al. (2009) Rheological properties of *Lepidium sativum* seed extract as a function of concentration, temperature, time. Food hydrocolloids 23(8): 2062-2068.
12. Ashraf Z, Muhammad A, Imran M, Tareq AH (2011) In vitro antibacterial and antifungal activity of methanol, chloroform and aqueous extracts of *Origanum vulgare* and their comparative analysis. International Journal of Organic Chemistry 1(4): 257-261.
13. Zain ME, Awaad AS, Al Outhman MR, El Meligy RM (2012) Antimicrobial activities of Saudi Arabian desert plants. Phytopharmacology 2(1): 106-113.

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