ISOLATION AND IDENTIFICATION OF FUNGI FROM INFECTED MILK SAMPLES OBTAINED FROM CATTLES WITH MASTITIS AND STUDING THE ANTIFUNGAL ACTIVITY OF ROSEMARY ETHANOLIC EXTRACT AGAINST THE MAIN STRAINS .

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ABSTRACT

Objective: The purpose of this study was to isolate and identification fungi from the samples of cows milk with mastitis, the samples were collected during the period between February- April 2009 in the Abu-Ghraib zone.

Material: In the present study a 100 samples of mastitis cows milk was collected.

Results: Eighty samples (80%) were showed positive fungal infections, of which 78.75% (63 samples) was yeast and 21.25% (17 samples) was molds.

The main strains which were isolated and responsible about high percentage of infection were the followings, *Candida albicans*25%. *Geotrichumcandidum*20%, *Rhizopus*spp.17.5%, and other species which include, *Candida*spp.11.25%, *Sacchromycescerevisiae*11.25%, *Candida tropicalis*7.5%, *Cryptococcus neoformans*2.5%, *Penicillium* spp. 2.5%, *Rodotorula* spp. 1.25% and *Aspergillus terreus*1.25%, while 20 samples showed a negative results.

Rosemary ethanolic extract showed a different antifungal activity aginst the main strains of isolates (*C.albicans*, *Sacch. cerevisiae*, *Geot. candidum*, *Asp.terreus*, and *Rhizopus*spp). There was an inhibition zone of using extract in a concentration of 200mg/ml on the growth of *C.albicans*, *Geot. candidum*and *Sacch. cerevisiae*, while a concentration of l00mg/ml of the extract was effective only against *Sacch.cerevisiae*. The extract showed antifungal activity against the growth of *Asp. terreus*in all concentrations, with minimum inhibitory concentration (MIC) of l0mg/ml, in contrast, the extract had no effect on the growth of Rhizopusspp. in low concentration (10, 20 mg/ml) and only a concentration of 40, 80 mg/ml showed a minimal effect compared with the antifungal drug (Clotrimazole).

Conclusion: The presence of yeasts and molds in cows milk indicate that the mycotic mastitis may be occurred in association with bacterial mastitis. Rosemary ethanolic extract had an antifungal activity most probably due to the presence of some compounds (α-Pinene, Bornyl acetate, Camphor and 1,8-Cineole) that responsible about this property.

Key Words: Samples of mastaticcows milk, Fungi, Rosemary ethanolic extract.

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INTRODUCTION

Fungi are wide spread in nature, being noted in bedding and gear from the stables on milking machines. Mycotic mastitis also existed in cattle before the arrival of antibiotics, however, since then there has been an ever increasing number of cases reported, almost always associated with prior antibiotic treatment of suspected or proven bacterial mastitis (Lagneau *etal.*,1996).

In most cases, bacteria are recognized as the primary pathogens while, fungi particularly yeasts, have been regarded as secondary invading pathogens of mastitis (Costa *etal.*,1998).

Mycotic infections of the mammary gland usually occurs as sporadic cases affecting a small percentage of cows, or as outbreaks affecting the majority of animals. In both situations, however, the seriousness of infection depends on the number of organisms present in the glands and the species of yeast and mold involved (Farnsworth , 1977).

Yeasts are a group of unicellular organisms, ever present in the natural surrounding of dairy cattle and are normal inhabitants of the skin of the udder and teats. Yeasts are considered as opportunistic pathogens which colonize the cows udder (Richared *etal.*,1980).

The use and abuse of antibacterial drugs, treatment with contaminated antibiotic solutions, as well as syringes, or other materials brought in contact with the mammary gland may favor yeast colonization of cows udders(Stantos and Marin, 2005; Costa *etal.*,1998; Gibbony *etal.* , 1970).

Different fungi have been reported as a cause of mycotic mastitis such as *Aspergillusfumigatus*, *Aspergillusterreus*, *Candida*spp., *Cephalosporium* spp., *Coccidioides* spp., *Cryptococcus neoformans*, *Geotrichumcandidium*, *Histoplasma* spp., *Mucor* spp., *Rhizopus* spp., *Torulopsis* spp. and *Trichosporon* spp. (Krukowski *etal.*, 2000; Aalbaek *etal.*, 1994).

Furthermore other fungi such as *Cryptococcus* spp., *Rhodotorula* spp., *Trichosporumcutaneum*, *Aureobasidiumpullulans*and *pichiaohmeri*, have also been isolated from the milk of healthy glands (Lagneau *etal.* , 1996; Costa *etal.*, 1993).

There is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. The use of synthetic antioxidants in the food industry is severely restricted as to both application and level (Tawaha, 2007; Peng, 2005). Currently, there is a strong debate about the safety aspects of chemical preservatives, since they are considered responsible for many carcinogenic and teratogenic attributes, as well as residual toxicity (Moreira, 2005). Growth of microorganisms in food may cause spoilage or food borne disease (Delcampo *etal.* , 2000).

Rosemary (*Rosmarinusofficinalis*) is of Lamiaceae (Labiatae) family is a spice and medicinal herb widely used around the world. Of the natural antioxidant, rosemary has been widely accepted as one of the spices with the highest antioxidant activity (Peng, 2005). Rosemary extracts and essential oil is also used an antibacterial, antifungal (Oluwatuyi, *etal.*2004; Fernandes-Lopez, *etal.*2004), and anticancer agent (Leal, 2003). High percent of the antimicrobial

activity is attributed to carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries (Delcampo *et al*., 2000). The mechanism of action has not been studied in great detail considering the large number of different groups of chemical compounds present in essential oils.

The main compounds responsible for the antimicrobial activity are α-Pinene, Bornyl acetate, Camphor and 1,8-Cineole (Daferea**,** *et a.l*2000).

The aim of this study was to isolate and identify" fungi (yeast and molds) from cows milk with mastitis in veterinarian clinics of Abu-Ghraib zone and to investigate antifungal activities of rosemary ethanol extracts on the main and important isolates and to detect the minimum inhibitory concentration(MIC) value of the extract.

MATERIALS AND METHODS

Milk samples:

One hundred milk samples from quarters with clinical and subclinical mastitis were examined. The samples were collected between February- April 2009 from mastitis cows in veterinarian clinics of Abu- Ghraib zone and these samples were investigated in laboratories of zoonosis diseases unit in the Vet. Med. College /Baghdad University. The milk samples were always taken aseptically, kept at temperature of 4C° and plated, at the latest 24 h. after sampling.

Isolation of fungi:

Milk samples were also inoculated on Sabouraud dextrose agar (SDA) with chloramphenicol (0.05mg/ml), which then incubated at 28-30 C° for 2days-3weeks. The isolated fungi were classified according to the colony characteristic, microscopic examination after staining with lactophenol cotton blue stain (LPCB) in a wet mount, hyphae's size and shape of spores were noticed. The identification of the yeast growth based on the morphological colony, physiological and biochemical characteristic, by using Gram stain for staining, ability to growth at 37C° and 42C°, germ tube test, urease production, presence of capsule, caffic acid ferric citrate agar test, and carbohydrate fermentation tests for detection of *Candida*spp., *Cryptococcus neoformans*and other species of yeasts were also under taken.

Rosemary extract:

The leaves of rosemary (*Rosmarinusofficinalis*) plant were obtained from the local market and verified by Iraq National Herb, Agricultural Ministry. The leaves were cleaned, dried and then ground to powder, about 100 gram of powder was suspended in 500 ml of 70% ethanol and kept on shaker at room temperature over night, then separated using separator funnel, subsequently filtered through whatman filter paper No.l and filtrate dried(Harborne, 1984), the dried extract was weighted to prepare the stock solution by dissolving (4g) of extract powder in 20 ml of distaled water with 5% dimethyl sulfoxide (DMSO) to prepare a concentration of 200mg/ml, from which another concentration (100,50) mg/ml were prepare for yeasts and another concentration

(10, 20,40, 80) mg/ml for molds to determine the minimum inhibitory concentration (MIC) of the extracts on growth of yeasts and molds.
Note: MIC was measured by the lower concentration of extract which gave inhibition to
the growth of strains.
Fungal culture:

The antifungal activity of 70% ethanolic extract of rosemary was tested against (3) isolates of yeasts from milk samples (*Candida albi*cans, *Geotrichumcandidum*, *Saccharomyces cerevisiae*) and (2) isolates of molds(*Aspergillusterreus* and *Rhizopus*spp.), the antifungal effect of prepared extract was tested on SDA by using agar
geldiffusion method in case of yeasts and agar dilution method by adding the extract in different concentration to the SDA media in case of molds, by adding (1,2,4,8) ml of stock solution (200 mg ml) of extract, to (19, 18, 16, 12) ml Of SDA, to obtained the different concentration (10, 20, 40, 80) mg/ml and inoculated the molds on the surface by cut 5 mm diameter of each mold and inoculate it at the center of plate then incubated at 28-30 C° for 3-8 days. Diameter of growth inhibition zone for different molds were measured for each one in duplicate average of two perpendicular diameter and according to this equivalent (Lima *etal*., 1992). Compared with the control plate.

Inhibition% = [(C-T)/C] x 100
C=the colony diameter of the mycelium on the control petridish.
T=the colony diameter of the mycelium on the test petridish.

Agar gel diffusion method:-

The 3 isolates of yeasts cultured on a sabouraud dextrose broth and incubated at 25-30 C° for 48 hrs. the inoculums was standardized according Mcfarlands turbidity standard. The turbidity was compared with mcfarlands 0.5 standard which provide turbidity comparable to yeast suspension containing $1.5x10^8$CFU/ml (Lima *etal*., 1995). This test was performed using the standard procedure as described by (20) the inoculums suspension of each yeast strains was swabbed on the entire surface SDA, holes of 7mm in diameter were made with stainless steel cylinders and filled (60µl) microliter with the fluid extract in different concentration (200,100,50) and fourth hole filled with distal water as control, then left at ambient temperature for 15min. to allow excess per diffusion of extract prior to incubation at 25-30C^o for 48-72 hrs. Inhibition zones were measured and expressed in mm by notice the inhibited growth around the holes compared with the control plate. At that time the antifungal impacts of Rosemary ethanolic extract compared with Clotrimazole antibiotic (0.25 mg/ml)for yeasts and molds was studied.

RESULTS AND DISSCUSION

A total of 100 samples of cows milk with mastitis was studied mycologically for pathogenic fungi, from which 80 samples were positive for fungal infections and a different species of yeasts and molds were isolated .(Table 1)

*C. albicans*was the major cause of infections 20(25%), then *Geot. candidum*16(20%), and *Rhizopus*spp. 14(17.5%). While *Candida*spp. 9(11.25%), *Sacch. cerevisiae*9(11.25%) and *C.tropicalis*6(7.5%), and miner cause was *Crypt. neoformans*2(2.5%), *Penicillium* spp. 2(2.5%), *Rodotorula*spp. 1(1.25%) and *Aspergillusterreus*1(1.25%), whereas 20 milk samples shows a negative results for fungal infection.

The results revealed that the rosemary ethanolic extract showed an antifungal activity against the main pathogenic isolates from mastaticcows milk, (table 2) and the concentration of 200 mg/ml had the strongest effect by giving large zone of inhibition on *Geot. candidium*(19 mm), *Sacch. cerevisiae*(17mm), and small zone on *C. albicans* (12mm), while at the

Table 1:*Species of fungi (yeasts and molds)* **.)** *isolated from mastatic cows milk.*

Yeasts	**No. of isolates**	**Percent of isolation(%)**
C.albicans	20	25
Candida spp.	9	11.25
C.tropicalis	6	7.5
Sacch. cerevisiae	9	11.25
Geot. candidium	16	20
Crypt. neoformans	2	2.5
Rhodotorula. spp.	1	1.25
Total of yeasts	63	78.75
Fungi		
Rhizopus spp.	14	17.5
Penicillium spp.	2	2.5
Asp. terreus	1	1.25
Total of fungi	17	21.25
Total of all isolations	**80**	**80**

concentration of 100 mg/ml the inhibition zone was (11mm) in case of *Sacch. cerevisiae*, and the others (*C.albicans*, *Geot.candidium*) gave a negative results, whereas the concentration of 50 mg/ml had no effect on the three strains, compared with the antifungal drug (Clotrimazole) that give large inhibition zone in case of *Sacch. cerevisiae*(40mm), *C.albicans*and *Geot. candidum*(35mm). The zone of inhibition increased with increasing in concentration of ethanolic extract.

Table 2: *Antifungal activity of Rosemary ethanolic extract against some yeasts (growth inhibition zone mm.*

Conc. mg/ml	Zone Inhibition mm.		
	Geot. candidium	*C. albicans*	*Sacch. cerevisiae*
200	19	12	17
100	-	-	11
50	-	-	-
Clotrimazole 5mg/ml	35	35	40

Table (3) showed the effect ofethanolic extract of rosemary on molds (*A.terreus*and *Rhizopus*spp.), the MIC of 10 mg/ml concentration on *A. terreus*and the percentage of growth inhibition was 65%, whereas the concentration of 40 mg/ml record high percentage of growth inhibition 77.5 % compared with the effect of antifungal drug (Clotrimazole) 0.25mg/ml which give similar percent 79.4%. However, the conc. of 80 mg/ml of extract showed higher percentage 83.75% compared with Clotrimazole and at 20mg/ml which showed a low effect 71.25%. While there was no antifungal activity of the extract against *Rhizopus*spp. at the concentration 10,20 mg and shows low percentage of growth inhibition 11.8 %, 23.5% in the concentration 40, 10 mg/ml. and the MIC of it at 40mg/ml compared with the plate treated with Clotrimazole showed 83.5% compared with control plates.

(MIC was measured by the lower concentration of extract which gave inhibition to the growth of strains).

There is a reverse proportion between the mean of fungal growth and the concentration of extract,

Table 3 : Effect of different concentration of Rosemary ethanolic extract on the growth of *Asp. terreus* and *Rhizopus* spp.

Asp.terreus			***Rhizopus*spp.**		
Conc. of extract mg/ml	Mean of fungal growth	Percentage of inhibition %	Conc. of extract mg/ml	Mean of fungal growth	Percentage of inhibition %
control	80	0	control	85	0
10	28	65	10	85	0
20	23	71.25	20	85	0
40	18	77.5	40	75	11.8
80	13	83.75	80	65	23.5
0.25 Clotrimazole	17.5	79.4	0.25 Clotrimazole	14	83.5

the fungal growth decreased in the diameters when the conc. increased, in contrast; the percentage of growth inhibition was increased when the concentration increased.

Mastitis is an infection of the cows udder, caused by micro-organisms freely encountered in the environment. Fungi are opportunistic microorganisms which parasitized the animals weakened immunological system and develop by causing disease and impairing the normal flow of milk, with heavy loss for the farm owner (Wawron and Szczubial, 2001). In this study, yeasts were isolated in a percent 78.75% of all samples analyzed, *Candida*spp. were the predominant eukaryotes one isolated in 43.75%, and the most important species of *Candida*was gave the highly percentage of infection was *C.albicans*(25%), while other types of *Candida*reported 11.25% , and *C.tropicalis*7.5%. the present study was accordant with (Lagneau, *et al.*1996; Santos and Marin, 2005; Williamson and Dimenna, 2007 and Barros *et al.*,2011) who were reported that the predominant yeasts cause bovine mastitis are *Candida*spp., and*C.albicans* was isolated in a highpercent.

In several surveys of mycotic mastitis *C.tropicalis*was the most important one (Richared *etal.*,1980).

*C.albicans*has been reported as the most common species of yeast pathogen found and cause mastitis problems. *Candida*spp. normally lives in saprobiosis, although in favorable circumstances, it may develop its pathogenic potential. As a rule, *Candida*spp. occurs in milk without any associated pathogens, although it may cause mastitis in the sub-clinical, clinical or chronic modes (Wawron and Szczubial, 2001). High contamination index by Candida may be related to lack of hygiene during the milking process.

Geot.candidum is an opportunistic, keratinophilic yeast-like fungus. In the past, there are only a very few reports from around the entire world regarding its incrimination with bovine mastitis. Mishra and Panda, 1986 found only one case (0.7%) of *Geot.candidum*cause mastitis. In contrast, Costa *et al.*(1993) detected (6.4%) positive mastitis for*Geot. candidum*, and Chahota *et al.*(2001) isolated itonly from clinical mastitis in cow, this study was agreement with the previous study in which was isolated this species in 20% from bovine mastitis**.**

Another yeasts were reported in this study are *Sacch. cerevisiae*(11.25%), *Crypt. neoformans*(2.5%) and *Rodotorula*spp. (1.25%).There are many regional differences in yeast species and their percentage of its causing mastitis, Turkyilmaz and Kaynarca, 2010 have been reported *Crypt.neoformans*(2.4%) and *Sacch. cerevisiae*(2.4%), also Pengov, 2002 showed that the *Crypt. neoformans*revealed (2%) of isolated strains, while Costa, etal.1993 have been reported that the *Cryptococcus*spp. (71 strains), *Rhodotorula*spp. (40 strain).

The molds classified in following genera were also isolated in this Research *Rhizopus*spp.(17.4%) ,*Penicillium*spp.(2.5%), and *Asper.terreus*(1.25%). Costa *etal.*(1993) recorded that the molds were in (11.95%) classified in the following *Aspergillus*,*Penicillium*(1.2%) which similar to this study.

In Iraq الذهيبات (1992) detected molds and yeasts In Abu-Ghraib zone in winter *Aspergillus*88%, *Rhizopus*20%, *Penicillium*12% from cow milk and
yeasts*Sacch. cerevisiae*28%, *Candida* spp.20%, *Rodotorula*20%. This is reverse with this study in which the *Aspergillus*showed the high percent, while the *Rhizopus*and *Penicillium*record the lower, and *Sacch.cerevisiae*record the high percent while *Candida spp.*and *Rodotorula*the lower one.

Results show that although fungi may peacefully live with the host, found in a great variety of substrates, such as mammary gland, hands, soil and water, they may develop their pathogenic power and cause infections when they encounter favorable conditions such as environmental contamination associated with lack of hygiene during the milking, poor equipment's cleaning,some diseases that change the cell immunity and excessive or erratic use of antibioticsafter bacterial mastitis treatment
which represented the main factor that propitiatebecause they affect the microflora of the mammary glands which acts as an animal natural defense and this agree with Aboul- Gabal, *etal.*1977. In addition, large doses of antibiotics may cause reduction in the Vitamin A, leading to injury to the udders epithelium, facilitating the invasion of fungi (Krukowski *et al.*, 2000). Adequate management procedures, especially concerning the milk process, and the hygiene methods employed at this stage of milk production, may lessen the occurrence of fungus produced mastitis and decrease its contamination during the milking process.

The results indicated that the rosemary extract showed antifungal activity aginst many strains**,** and *Geot. candidium*gave high inhibition zone then *Sacch. cerevisiae*and the lower one is *C.albicans*at the concentration 200mg/ml and the MIC of *Geot. candidum*, *C. albicans*were detected 200mg/ml, while in case of *Sacch. cerevisiae*was l00mg/ml. However the plates treated with Clotrimzole gave high inhibition zone compared with plates which treated with extract.

On the study by هادي (2007) showed antifungal activity of rosemary ethanolic extract on*C.albicans*detect the zone of inhibition 12mm at a concentration 150 mg/ml and the MIC was 75mg/ml gave 9mm inhibition zone who agreement this study, while, the effectiveness of extract on molds (*A.terreus*and *Rhizopus*)more over than yeasts . The growth inhibition of *A.terreus*was in all concentrations and the MIC was l0mg/ml, in contrast, the *Rhizopus*spp. growth showed no inhibition on concentration 10, 20

mg/ml and the MIC was 40 mg/ml, Santoyo *et al.*(2005) found that the volatile oil of rosemary has inhibition effect on the growth of *C.albicans* and *Asp.niger* which agreement the present study. The results indicated that the rosemary ethanolic extract showed antifungal activity according to Moreno, *etal*. (2006) because contained many compounds which responsible for this property. And these compounds act by inhibiting cell wall synthesis of micro-organisms, decrease essential protein synthesis, composed of a complex which involved with the cell wall and retarded the permeability, inhibit enzymes metabolization which important in growth and reproduction and rupture the cell membranes or change the function of its (Cowan ,1999).

Identification of yeasts and molds in cows milk samples were analyzed demonstrate the occurrence of mycotic mastitis which lead to decrease in milk quality, and consumption milk contaminated by fungi or their toxins from human being which lead to human fungal infections or diseases, early diagnosis of mastitis by using California mastitis test (CMI) in
Iraqi farms, and antibacterial therapy with previous susceptibility tests may be recommended to decrease fungal infection or mycotic mastitis.

CONCLUSION

Cows milk may be obtained by human or it's used in food industry like cream, cheese, Butter, …etc. or other industrials which involved milk with their industerlization so these product should be free from any contamination.

Rosemary extracts may be promising with regard to their incorporation into various foods, pharmaceutical products and foods industry for which a natural aroma, colored and antioxidant/antimicrobial additive is desired. These properties are also needed by the food industry in order to find possible alternatives to synthetic preservatives.

Further studies are necessary to investigate the incorporation of extracts in to appropriate food, antioxidant and antimicrobial activities in the whole food system.

REFERENCES

الذهيبات،محمد شمخي جبر.1992. تلوث الحليب الخام بالفطريات وبعض الجراثيم الدالة.رسالة ماجستير.كلية الطب البيطري. جامعة بغداد.

هادي، سالي محفوظ.2007. الفعالية التثبيطية للزيوت الطيارة لنبات إكليل الجبل في بعض ألأحياء المجهرية الممرضة. رسالة ماجستير. كلية العلوم. جامعة بغداد.

Aalbaek B., J. Stenderup and H. E. Jensen.1994.Mycotic and Algal bovine mastitis in Denmark *APMIS*.102: 451-6.

Aboul- Gabal M., R.M. Hogle and J.K. West.1977.Pyometra in mare caused by *Candida rugosa. J. Am. Vet. Med. Assoc*.170: 177-178.

Barros L.S.S., S.L.O. Soglia, M.J. Ferreira, M.J. Rodrigues and M.P.C. Branco.2011. Aerobic andanerobic bacteria and *Candida* species in crude milk. *Journal of Microbiology and antimicrobials*.3(8): 206-212.

Chahota R., R. Katoch, A. Mahajan and S. Vermas .2001. Clinical bovine mastitis caused by *Geotricumcandidum* .*Vet. Arhiv*. 71(4): 197-201.

Connie R., I. Mahon and I.V. George Man voelis .1995. Text Book of Diagnostic microbiologyW.B. Saunder Company. Publication. U.S.A.

Costa E.O., A. R. Ribeiro, E.T. Watanable and P.A. Melvilla .1998. Infectious bovine mastitis caused by environmental organisms. *J.Vet.Med*. B.45: 65-71.

Costa E.O., C. R.Gandra, M.S. Pires, S.D.Coutinh, W**.** Castilho and CM. Teixeira.1993. Surveyof bovine mycotic mastitis in dairy herds in the state of SaoPaulo, Brazil. *Mycopathologia*.124: 13-17.

Cowan M.M. 1999. Plant products as antmicrobial agents.*Clinical microbiology reviews*.12 (4): 564-582.

Daferea D.J., B.N. Ziogas and M.G. Polissiou.2000. GC-MS analysis of essential oils from some Greek aromatic plants and their fungi toxicity on *Penicilliumdigitatum. Journal of Agricultural and Food chemistry*.Athens,48(6): 2576-2581.

Delcampo J., M.J. Amiot and C. Nguyen.2000.The Antimicrobial effect of rosemary extracts.*Journal of Food protection*.10: 1359-1368.

Farnsworth R. J. 1977.Significance of fungal mastitis, *JAVMA*.170: 1173-4.

Fernandes-Lopez J., N. Zhi, L. Aleson-Carbonell, J.A. Perez-Alvarez and V. Kuri.2004. Antioxidant and antibacterial activities of natural extracts: application in beef meat balls. *Meat Science*.69: 371-380.

Gibbony W. J., E.J. Catcott and J.F. Smithcory.1970.Bovine Medicine and surgery.*USA American Veterinary publications.*

Harborne J.B. 1984.Method of extraction and isolation, Phytochemical method. 2nd edition, London, New York Champar and Hall.

Krukowski H., M.Tietze, T. Majewski and P. Rozanski.2000. Survey of yeast mastitis in dairy herds of small-type farms in the Lublin region, Poland. *Mycopathologia*.150: 5-7.

Lagneau P.E., K. Lebtahi and D. Swinne.1996.Isolation of yeast from bovine milk in Belgium. *Mycopathologia*.135: 99-102.

Leal P.F. Functional properties of spice extracts obtained via supercritical fluid extraction.2003. *Journal of Agricultural and food Chemistry, Campinas*.51(9):2520-2525.

Lima E.O., O.F.Gornpertz, M.Q. Paulo and A.M.Giesbrecht.1992. In vitro antifungal activity of essential oils against clinical isolates of dermatophytes. *Rev. Microbial*.23(4):235-238.

Mishra P.R. and S.N. Panda. 1986.Some observations on the occurrence of mycotic mastitis in Orissa. *Ind.Vet.J*.63:886-888.

Moreira M.R.2005. Inhibitory parameters of essential oils to reduce a food borne pathogen. *LWT-Food Science and Technology*, *Mardel Plata*38(5):565-570.

Moreno S., T.Scheyer, C.S. Romano and A.A. Vojnov .2006. Antioxidant and antimicro- bialactive ties of rosemary extract linked to their polyphenol composition. *Free Radical Research*.40: 223-231.

Oluwatuyi M., G.W. Kaatz and S. Gibbons. 2004.Antibacterial and resistance modifying activity of *Rosmarinusofficinalis. Phytochemistry*, London/Detroit. 265(24):3249-3254.

Pengov A.2002. Prevalance of mycotic mastitis in cows, *Acta Veterinaria*.52 (2-3): 133-136.

PengY.2005. Determination of active components in rosemary by capillary electrophoresis with electrochemical detection. *Journal of Pharmaceutical and Biomedical Analysis*,Fujian/Shanghai.39(3-4): 431-437.

Richared J.L., J.C. Mcdonald, R.E. Fichtner and A.J. Anderson.1980. Identification of yeasts from infected bovine mammary glands and their experimental infectivity in cattle. *Amer. J.Vet.Res*.41:1991-4.

Santos R.C. and J.M. Marin.2005. Isolation of *Candida* spp. from mastitc bovine milk in Brazil. *Mycopathologia*.59:251-253.

Santoyo S., S. Cavero, L. Jaime, E.Ibaanez, F.J. Senorans and G. Reglero.2005. Chemical composition and antimicrobial activity of *Rosemarinus officinalis L.* essential oil obtained Via supercritical fluid extraction. *J. Food Prot*. 68(4): 790-5.

Strasbourg .2001.European pharmacopoeia, 4thed.Counical of Europe.93-99.

Tawaha K.2007. Antioxidant activity and total phenolic contents of selected Jordanian plant species. *Food Chemistry*,Amman /Irbid.104(4): 1372-1378.

Turkyilmaz S. and S. Kaynarca .2010. The slime production by yeasts isolated from subclinicalmastitic cows,CL4 *Vet. BRNO*.79: 581-581.

Wawron, W. and M. Szczubial.2001.Treating mastitis mycotica in cows. *Vet.Med.*57:863-866.

Williamson J.H. and M.E. Dimenna.2007. Fungi isolated from bovine udders, and their possible sources. *NZ Vet. J.*55: 188-90.

عزل وتشخيص الفطريات من عينات الحليب المصابة المأخوذة من التهاب الضرع في الأبقار ودراسة الفعالية الفطرية المثبطة للمستخلص الكحولي لنبات أكليل الجبل على أهم العتر.

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المستخلص

استهدفت هذه الدراسة عزل وتشخيص الفطريات الموجودة في عينات الحليب المأخوذة من ألأبقار المصابة بالتهاب الضرع، جمعت عينات الحليب خلال شهر شباط إلى نيسان لسنة 2009 من العيادات البيطرية في منطقة أبوغريب. تم جمع100 عينة حليب، أظهرت 80 عينة (80%) إصابات فطرية مختلفة ،عزلت الخمائر منها بنسبة 78.75% (63 عينة)، وعزلت الأعفان بنسبة 21.25%(17عينة)، أغلب العزلات كانت لجنس *Candidaalbicans* حيث عزلت بنسبة 25%، تليها عزلة *Geotrichumcandidum* بنسبة 20% ،ثم عزلة *Rhizopus* spp. بنسبة 17.5%.أما باقي الأجناس فكانت النسب كالأتي:*Candida spp.* 11.25%، جنس *Sacchromycescerevisiae* 11.25%، *Candida tropicalis* 7.5%، *Cryptococcusneoformans* 2.5%، *Penicillium* spp.2.5%، *Rodotorula*spp. 1.25% وأخيرا عزلة *Aspergillusterreus* بنسبة 1.25%، و 20 عينة (20%) لم تظهر إصابة فطرية. تم تحضير المستخلص الكحولي (70% من الكحول الأثيلي) لنبات إكليل الجبل لدراسة تأثيره على نمو أهم الفطريات المعزولة من عينات الحليب والتي هي *Candidaalbicans*،*Geot. candidum*، *Rhizopus* spp.، *Asp. terreus*، *Sacch. Cerevisiae*. أظهرت النتائج تأثير المستخلص بتركيز 200ملغم/مل على نمو الخمائر *Geot.candidum*، *Sacch.cerevisiae*، *Candida albicans*، وكانت أقطار التثبيط كالتالي 19،17،12 ملم بينما لم يظهر المستخلص أي تأثير مثبط بتركيز 100ملغم/مل إلا على خميرة *Sacch.cerevisiae* وبقطر تثبيطي 11ملم. أما بالنسبة للأعفان فكان التركيز ألأدنى للتثبيط هو 10ملغم/مل بالنسبة لعفن *Asp. terreus* عند إضافة المستخلص إلى الوسط الزرعي In vitro وأظهرت

التراكيز 80،40،20 ملغم/مل أعلى نسبة تثبيط مقارنة بالأطباق المعاملة بعقار Clotrimazole بينما كان التركيز ألأدنى للتثبيط لعفن .*Rhizopus*spp 40ملغم/مل وأظهر التركيز 80ملغم/مل نسبة تثبيط قليلة مقارنة بالطبق المعامل بالعقار. يتبين مما تقدم إن عزل الخمائر والأعفان من حليب ألأبقار يدل على إلتهاب الضرع الفطري وقد يحدث بعد ألإصابة بالتهاب الضرع البكتيري، وقد أظهر المستخلص الأثيلي لنبات إكليل الجبل فعالية مثبطة لنمو الخمائر والأعفان وذلك لإحتواءه على مركبات Pinene, (α-Bornyl acetate, Camphor and1,8-Cineole) تؤثر في نموها.

الكلمات المفتاحية: عينات الحليب من أبقار مصابة بالتهاب الضرع، الفطريات، المستخلص الكحولي لنبات إكليل الجبل.