In Vivo Efficacy of *Echinops spinosus* Decoction as a Therapeutic for Cows at Risk of Clinical Endometritis

Saleh Boudelal 1, Mounir Adnane 2,*, Abdelatif Niar 1,3 and Aspinas Chapwanya 4

**Abstract:** Clinical endometritis (CE) is a multifactorial disease of dairy animals. Retained fetal membranes (RFM) and metritis are the major risk factors of CE in dairy cows. Because uterine inflammation affects the profitability of the dairy industry, antibiotics and hormonal therapies are commonly used to mitigate against the disease. However, the One-Health concept aims to reduce antibiotic use in food animals to avoid the emergence of drug resistance or residues in milk or meat. Thus, phytotherapy may represent a good alternative to antibiotics in food animals. *Echinops spinosus* (*E. spinosus*) is a natural plant known to have therapeutic, anti-inflammatory, antimicrobial, and wound-healing properties in vitro. The aim of the present study was to investigate the efficacy of *E. spinosus* as a preventive strategy for CE in dairy cows with other postpartum complications. Holstein–Friesian cows (*n* = 36) diagnosed with RFM or metritis enrolled in the study were allocated into three groups. One group received antibiotic treatment. Another group received prostaglandin injection (PG). The experimental group received *E. spinosus* decoction orally. As a control group, eutocic cows (*n* = 36), without RFM and metritis were included in the study. The efficiency of the treatment was based on the occurrence of CE and improved reproductive outcomes. At 30 ± 2 DPP, CE was diagnosed in 25%, 58.34%, and 75% in antibiotic, PG, and *E. spinosus* groups, respectively (*p* < 0.05). There were no differences between the groups at 55 ± 5 DPP (16.67%, 33.44%, and 41.67% in antibiotic, PG and *E. spinosus* groups, respectively, *p* > 0.05). The *E. spinosus* group had the longest open days, lowest conception rate at 150 DPP, and highest number of services per conception. Oral *E. spinosus* extract is ineffective as a therapeutic for cows at risk of CE. These findings may pave the way for future innovative strategies employing *E. spinosus* to protect cattle against endometritis.

**Keywords:** *Echinops spinosus*; retained fetal membranes; metritis; phytotherapy; treatment; reproductive performances
1. Introduction

Subfertility in dairy cattle is often caused by reproductive disorders including retained fetal membranes (RFM) and metritis [1,2]. Uterine diseases result in considerable economic losses for dairy producers through increased veterinary bills, reduced milk yield, infertility, and prevalent reproductive diseases such as clinical endometritis (CE) [3]. CE is an inflammation of the superficial layer of the uterus, which is characterized by foul, odorous, or pyogenic vulval discharge [4]. CE is diagnosed after 21 days postpartum (DPP) by vaginal examination using vaginoscopy or Metricheck to collect and score vaginal secretions according to their aspect and odor [5].

In Algeria, the incidence of bovine CE is reported to be 37% and often associated with RFM (78.7%) and metritis (64.7%) [6]. If it is not diagnosed at an early stage, CE perturbs fertility [7]. Therefore, it is imperative that newer alternative therapeutics are developed to combat uterine disease. Recently, several herbal therapeutics were described for treating mastitis [8] and endometritis [9,10]. These have complemented other therapeutic strategies using local or systemic antibiotics, hormones (particularly prostaglandins), or homeopathic remedies [3,11]. Due to the increased concern about antibiotic residues and antibiotic-resistant strains, phytotherapy may play a role in reducing these risks [12]. In fact, herbal remedies were previously recommended to treat or prevent reproductive diseases in cattle [11,13,14]. For these alternative therapies, leaves, root, and bark are mixed with feed in concoctions and fed orally [13,15]. It was previously reported that Echinops spinosus (E. spinosus; Asteraceae) has medicinal properties beneficial for treating reproductive disorders in sheep, cattle, and humans [15,16]. In addition, in some regions of the world, E. spinosus is thought to accelerate parturition, reduce the risk of early postpartum complications, evacuate the uterus postpartum, and hasten the resumption of ovarian activity [15,17]. E. spinosus is an abortifacient herb used for voluntary termination of pregnancy [16,17]. E. spinosus has 42 metabolites of different phytochemical classes, among which quinoline alkaloids, sesquiterpenoids, flavonoids, and sterols are the most important [16,18–20]. The aerial parts contain 23 different flavonoids, which are known for their antioxidant activity [17,21,22]. A new apigenin derivative, named apigenin-7-O-ß-D-glucoside-(4′′-O-trans-p-coumaroyl), was identified [23]. Another two new sesquiterpenoids were identified and named echinopine A and echinopine B [19]. Thirteen sterols were characterized, among which ß-sitosterol and stigmasterol were the most abundant [20]. A new thiophene, known as the acetylene 2,2-dimethyl-4-[5′′-(prop-1-ynyl)-2,2′-bithiophen-5-yl]-1,3-dioxalane, was discovered in roots [24]. Furthermore, a new sesquiterpenoid, 11-hydroxyisocom-2-en-5-one, was identified in root extracts [19].

Antioxidants eliminate free radicals and protect the integrity of cell membranes, thereby boosting the immune system. Flavonoids and tannin extracts from E. spinosus remove free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) molecules while tannin-containing ethyl acetate extract reduces iron and free radical by scavenging [25]. E. spinosus aqueous, ethanol, and chloroform extracts exhibit anti-inflammatory activities [26]. However, they have low antibacterial properties, especially against microorganisms causing postpartum uterine disease, such as Staphylococcus aureus, Bacillus cereus, and Micrococcus luteus. Interestingly, E. spinosus extract were confirmed to be effective against diabetes and its complications [27]. The antifungal activity was not confirmed for the plant extracts [19]. In humans, Echinops spp. have anti-proliferative activity against tumors [17]. These biological properties of E. spinosus may be beneficial for treating reproductive diseases in cattle. However, the harmful effects, if any, of E. spinosus must be determined in order to identify the toxic level of the plant extract.

Despite the widespread use of plants for medicinal purposes in countries such as Algeria, there are few clinical studies documenting the benefits of these plants as alternative therapeutics in domestic animals. To the best of our knowledge, there are no studies on the clinical effectiveness of E. spinosus against CE in cows with RFM and metritis. The present study aims to investigate the therapeutic effect of E. spinosus, locally known as Teskra,
against RFM and metritis, the occurrence of CE, and on long-term reproductive performances, in comparison with standard treatment protocols using antibiotics or hormones.

2. Materials and Methods
2.1. Animals, Housing, Feeding, and Breeding

The study was conducted on four dairy farms located in Souqueur in the north-western part of Algeria. All animals were fed meadow fodder or corn silage and oat vetches hay and supplemented with commercial dairy concentrates (17% crude protein). Water was given ad libitum. Cow parity ranged from 1 to 5. At calving, the body condition score (BCS) range was 3.0 to 3.5 (1–5-point scale) [28]. Cows were milked twice a day with an annual milk yield of about 6500 kg. All farms used natural mating for breeding. All cows were dried off 60 days prior to their expected calving date.

2.2. Plant Materials and Decoction Preparation

The Teskra plant was collected during the bloom season and a botanical classification was performed. A voucher specimen was submitted to the herbarium of the Department of Ecology, University of Tiaret, Algeria (voucher number: AST/20/31). Plants were washed and dried and kept in a dark room at ambient temperature for 30 days. To prepare the Teskra decoction, plant roots were boiled for 30 min. The supernatant was then filtered and cooled at room temperature and preserved in a glass container in a refrigerator at 4 °C. The decoction was left at room temperature for 30 min prior to oral administration.

2.3. Experimental Design

General clinical examination was performed on all animals. Postpartum cows (n = 36) with RFM and/or metritis were selected. RFM was defined as failure to expel the placenta within 24 h after calving [29]. Metritis was defined as purulent and/or fetid-odor vaginal secretions, associated or not with clinical symptoms, diagnosed within 21 DPP [4,30]. The presence of abnormal vaginal discharge with or without a fetid odor after 21 DPP was considered as CE [3,4]. Females with vaginal laceration at calving, caesarean section, or those that received antibiotic treatment at least 15 days before the onset of the study were excluded. Any cows that received antibiotic treatment were removed from the study.

On each farm, cows with RFM and/or metritis were randomly assigned to three different treatment groups (12 cows in each). Group One (CCFA) cows received a single intramuscular injection of 6.6 mg/kg of Ceftiofur crystalline free acid (Naxcel®, Zoetis, 10 Sylvan Way, Parsippany, NJ 07054, USA). Group Two (PG) cows received intramuscular injections of 150 µg d-cloprostenol, (Dalmazin®, FATRO S.P.A. Veterinary Pharmaceutical Industry, Via Emilia, 285, 40064 Ozzano dell’ Emilia, Bologna, Italy) at 2 and 9 DPP. Group Three (Teskra) cows received oral administration of 4 L of E. spinosus root decoction daily for three days. A control group (animals with disease but not treated) was not included in the study for ethical reason.

Females that had an eutocia (normal delivery, no RFM or clinical signs of metritis or endometritis) at 30 ± 2 DPP were deemed a control group for reproductive performances. To evaluate the effectiveness of the treatment protocol at 30 ± 2 DPP, all treated cows were examined by transrectal palpation and vaginoscopy, as previously described [31,32]. The clinical response of cows was assessed by the absence of abnormal discharges in the vagina, subjective evaluation of uterine involution and resumption of ovarian activity at 30 ± 2 DPP. Clinical examinations were repeated at the end of voluntary waiting period fixed at 55 ± 5 DPP. Fertility parameters were determined (Table 1). After the end of the voluntary waiting period, cows were bred by natural mating. Pregnancy diagnosis was performed by transrectal ultrasonography five weeks post-mating.
Table 1. Description of fertility parameters adopted in the study.

| Parameters                          | Trait Description                                                                 |
|-------------------------------------|-----------------------------------------------------------------------------------|
| Occurrence of CE                    | Number of cows with clinical symptoms of CE divided by the number of treated cows in each group × 100 |
| First service conception rate (FSCR%) | Number of pregnant cows at first service divided by the number of mated cows × 100 |
| Conception rate until 150 DPP (CR150%) | Number of pregnant cows until 150 DPP divided by the number of cows enrolled × 100 |
| Days to first service interval (DFS) | Number of open days from calving to the first service                               |
| Days to conception interval (DC)    | Number of days from calving to successful service                                  |
| Number of services per conception (NSC) | Number of services divided by the number of pregnant service                       |

2.4. Statistical Analysis

Data were analyzed using IBM SPSS version 24 software. The occurrence of CE, FSCR%, and CR150% were tested using the Chi-square analysis and Fisher’s exact tests. The others reproductive parameters were expressed as means ± standard deviation (SD). Statistical analysis was performed using the Mann–Whitney test for intergroup comparisons. Statistical significance was considered as \( p < 0.05 \).

3. Results

The occurrence of CE at 30 ± 2 DPP was 25%, 58.34%, and 75% in the CCFA, PG, and Teskra groups, respectively (Table 2). The difference was statistically different between the CCFA and Teskra groups \(( p = 0.041)\). However, at 55 ± 5 DPP the CE occurrence was not significantly different between the three treatment groups, with 16.66%, 33.33%, and 41.66% for the CCFA, PG, and Teskra groups, respectively \(( p > 0.05)\) (Table 2). Reproductive performances of the control group and the three experimental protocols are summarized in Table 3. FSCR% and NSC were statistically similar between the control group and the experimental groups. CR150% was the highest in the control group (75%) and the lowest in the Teskra group (33.33%) \(( p < 0.05)\). DFS (mean ± SD) was the shortest in the control group (59.94 ± 11.64) and the longest in Teskra group (94.67 ± 11.44) \(( p < 0.05)\). Likewise, DC (mean ± SD) was the shortest in the control (76.50 ± 17.74) and the longest in the Teskra group (118.16 ± 20.03) \(( p < 0.05)\). Except for DFS, no significant differences were found between the control and CCFA groups for any of the defined parameters, and cows treated with CCFA had nearly identical reproductive performances to healthy cows without postpartum uterine diseases.

Table 2. Effect of treatment protocol on the occurrence of clinical endometritis at 30 ± 2 and 55 ± 5 days postpartum.

| Occurrence of Clinical Endometritis% (n) | CCFA  | PG  | Teskra |
|-----------------------------------------|-------|-----|--------|
| 30 ± 2 DPP                              | 25% (3/12) \(^a\) | 58.34% (7/12) | 75% (8/12) \(^b\) |
| 55 ± 5 DPP                              | 16.66% (2/12) | 33.33% (4/12) | 41.66% (5/12) |

Within each row, values with different superscript letters \(^{a,b}\) are significantly different \(( p < 0.05)\). Values without superscripts are not significantly different from any other values in the same raw.
Table 3. Effect of treatment on reproductive parameters, days to first service interval, days to conception interval and number of services per conception.

|                  | Control (n = 36) | CCFA (n = 12) | PG (n = 12) | Teskra (n = 12) |
|------------------|------------------|---------------|-------------|-----------------|
| FSCR% (n)        | 36.1% (13/36)    | 33.33% (4/12) | 33.33% (4/12)| 25% (3/12)      |
| CR150% (n)       | 75% (27/36) a    | 50% (6/12) b  | 41.66% (5/12)b | 33.33% (4/12) b |
| DFS (mean ± SD)  | 59.94 ± 11.64 a  | 71.58 ± 6.64 b| 76.75 ± 8.71 b| 94.67 ± 11.44 c|
| DC (mean ± SD)   | 76.50 ± 17.74 a  | 83.02 ± 11.39 a| 94.83 ± 15.09 b| 118.16 ± 20.03 c|
| NSC (mean ± SD)  | 1.75 ± 0.69      | 1.83 ± 0.71   | 1.9 ± 0.66   | 2.38 ± 0.42     |

Within each row, values with different letters (a,b,c) are significantly different (p < 0.05). Values with the same letters or without superscripts are not significantly different from any other values in the same row. SD: standard deviation.

4. Discussion

CE is a common problem on many dairy farms that lowers cattle fertility, thereby reducing profitability. Several risk factors for CE have been identified, among which RFM and metritis were the most common [6,33]. CE is a multifactorial disease that is traditionally treated with antibiotics, a practice that may lead to antibiotic resistance and residues in milk and meat. Therefore, there is a need to develop alternative therapies.

In Algeria and other regions of the world, farmers often use phytotherapy to treat gastrointestinal and reproductive diseases. In this study, we investigated the clinical outcome of using the Teskra plant to reduce the occurrence of CE. Ethnobotanical reports stated that *E. spinosus* hastens uterine involution and cures postpartum diseases [15,34]. Furthermore, *Echinops* species are frequently used for symptomatic treatment of inflammation, pain, and fever, and to resolve peripartum problems such dystocia and RMF [17].

Here, compared with Ceftiofur, Teskra did not reduce the incidence of CE. The observed differences may be because the oral route of administration did not promote an effective concentration of the active ingredients at the endometrial mucosa. Previous studies concluded that *E. spinosus* has low antimicrobial activity against Gram-positive and Gram-negative bacteria, including microbes causing postpartum uterine disease [19]. Flavonoids are more abundant in flowers and leaves of *E. spinosus* compared with roots [17,21]. The antioxidant activity of these flavonoids may boost bacterial clearance during uterine involution in postpartum cattle. Given that endometritis is a prolonged inflammatory response that is gradually resolved over time, at 55 ± 5 DPP there were no differences between the treatment groups regarding the occurrence of CE, possibly because some animals may have recovered spontaneously, without treatment [3,30,35,36]. There are reports of spontaneous recovery rates of 15.6% and 55% among all animals [30,36–38]. The spontaneous resolution of clinical symptoms associated with uterine diseases increases by 2.6% per day postpartum [39].

The FSCR% was similar between all animals. This could be explained by the fact that dairy cows experience negative energy balance postpartum, which would affect ovarian activity and the first service conception rate [40]. Furthermore, cows with postpartum problems such as metritis and RFM suffer from genital lesions and disrupted ovarian activity, which would impair conception [41,42]. In a meta-analysis study, Fourichon et al. [43] reported that cows with RFM or metritis were associated with lower FSCR% (4–10% for RFM and 20% for metritis). As expected, CR150% was significantly higher in the control group, compared with the PG and Teskra groups. Our results are similar to other studies where cows with puerperal metritis had lower conception rates at 100, 150, and 200 DPP [39]. However, CR150% was not different between the control and CCFA groups. Likewise, Piccardi et al. [41] reported similar conception rates between cows with metritis that were treated with Ceftiofur and healthy animals (35.5% and 34.5%, respectively). The absence of difference in our study would be due to the early application of treatment (second DPP) resulting in a better management of postpartum infections and reduced complications.

In the present study, the resumption of ovarian activity after calving was earlier in healthy cows compared with those having RFM or metritis. Fourichon et al. [43] reported
delayed onset of estrous behavior in cows with RFM or metritis, compared with cows without a history of RFM and metritis. There is a general agreement that both diseases negatively impact ovarian activity and ovulation, resulting in a delayed FSCR%, prolonged DFS, lower pregnancy rate, and increased NSC [1,29,41]. Compared with healthy animals, puerperal and clinical metritis delay the resumption of ovarian activity by 36 and 16 days, respectively (median = 140 vs. 120 vs. 104 days, respectively) [39].

In the present study, CE was the highest in animals treated with *E. spinosus*, suggesting that Teskra is not effective to prevent CE nor to improve reproductive performances in cows at risk of endometritis. Similarly, Yahyaoui et al. [44] reported low sensitivity of *Escherichia coli* to the essential oils of *E. spinosus* roots in vitro.

5. Conclusions

These findings are consistent with earlier research that concluded that conventional antibiotic and hormonal therapies are better options for treating endometritis. The preventive effects of oral administration of *E. spinosus* in vivo are not apparent. Further studies are required to investigate the beneficial effects, if any, of using other routes, including intrauterine infusion of plant extracts. Using the aerial parts or the whole plant may be another possible option to obtain the maximum benefits of the plant.

Author Contributions: All of the authors state that they contributed equally to writing and proof editing of the paper in its final form and approved it for submission. The authors state that the manuscript, including related results, has not been previously published and that the manuscript is not under consideration elsewhere. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Publication fees were provided by the One Health Research Center (C1), Ross University, School of Veterinary Medicine.

Institutional Review Board Statement: The experimental protocol was approved by the scientific committee of the Institute of Veterinary Sciences, University of Tiaret (protocol code DEPG 268, 2016), in accordance with the animal welfare standards.

Informed Consent Statement: Not applicable.

Data Availability Statement: All available data are included in the present paper.

Conflicts of Interest: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

References

1. Hossein-Zadeh, N.G.; Ardalan, M. Cow-specific risk factors for retained placenta, metritis and clinical mastitis in Holstein cows. *Vet. Res. Commun.* 2011, 35, 345–354. [CrossRef] [PubMed]
2. Boudelal, S.; Niar, A. Risk factors associated with reproductive disorders in dairy cows in Algeria. *J. Hell. Vet. Medical Soc.* 2020, 71, 2213–2218. [CrossRef]
3. LeBlanc, S.J.; Duffield, T.F.; Leslie, K.E.; Bateman, K.G.; Keefe, G.P.; Walton, J.S.; Johnson, W.H. The effect of treatment of clinical endometritis on reproductive performance in dairy cows. *J. Dairy Sci.* 2002, 85, 2237–2249. [CrossRef]
4. Sheldon, I.M.; Lewis, G.S.; LeBlanc, S.; Gilbert, R.O. Defining postpartum uterine disease in cattle. *Theriogenology* 2006, 65, 1516–1530. [CrossRef] [PubMed]
5. Williams, E.J.; Fischer, D.P.; Pfeiffer, D.U.; England, G.C.W.; Noakes, D.E.; Dobson, H.; Sheldon, I.M. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 2005, 63, 102–117. [CrossRef] [PubMed]
6. Boudelal, S.; Adnane, M.; Guidoum, A.K.; Niar, A. Risk factors of purulent vaginal discharge in Algerian dairy cows. *Veterinaria* 2020, 69, 153–164.
7. Sheldon, I.M.; Williams, E.J.; Miller, A.N.; Nash, D.M.; Herath, S. Uterine diseases in cattle after parturition. *Vet. J.* 2008, 176, 115–121. [CrossRef] [PubMed]
8. Akhtar, M.; Shaukat, A.; Zahoor, A.; Chen, Y.; Wang, Y.; Yang, M.; Umar, T.; Guo, M.; Deng, G. Hederacoside-C inhibition of *Staphylococcus aureus*-induced mastitis via TLR2 & TLR4 and their downstream signaling NF-κB and MAPKs pathways in vivo and in vitro. *Inflammation* 2019, 43, s10753–s010819.
9. Lv, X.; Fu, K.; Li, W.; Wang, Y.; Wang, J.; Li, H.; Tian, W.; Cao, R. TIIA attenuates LPS-induced mouse endometritis by suppressing the NF-κB signaling pathway. *Can. J. Physiol. Pharmacol.* 2015, 93, 967–971. [CrossRef] [PubMed]

10. Ibrahim, Z.; Shehi, A.; Murtezani, A.; Krasniqi, S.; Agani, Z. Kosovo’s public health damage from abusive use of antibiotics in dairy cattle. *Mater. Sociomed.* 2015, 27, 149–153. [CrossRef] [PubMed]

11. Arlt, S.; Padberg, W.; Drillich, M.; Heuwieser, W. Efficacy of homeopathic remedies as prophylaxis of bovine endometritis. *Theriogenology* 2018, 121, 67–71. [CrossRef] [PubMed]

12. Bitew, H.; Hymete, A. The Genus Echinops: Phytochemistry and Biological Activities: A Review. *Phytother. Res.* 2005, 19, 128–132. [CrossRef]

13. Bouzabata, A.; Mahomoodally, M.F.; Cordell, G.A. Microscopic identification of Echinops spinosus ssp. Boteri (Boiss.) Murb. using multivariate tests. *J. Res. Pharm.* 2019, 23, 797–803. [CrossRef]

14. Bouzabata, A.; Mahomoodally, M.F.; Cordell, G.A. Microscopic identification of *Echinops spinosus* ssp. Boteri (Boiss.) Murb. using multivariate tests. *J. Res. Pharm.* 2019, 23, 797–803. [CrossRef]

15. Huang, X.; Wang, S.; Wang, L.; Wang, H.; Li, X.; Cui, D. Administration of an herbal powder based on traditional Chinese veterinary medicine enhanced the fertility of Holstein dairy cows affected with retained placenta. *Theriogenology* 2018, 121, 67–71. [CrossRef] [PubMed]

16. Huang, X.; Wang, S.; Wang, L.; Wang, H.; Li, X.; Cui, D. Administration of an herbal powder based on traditional Chinese veterinary medicine enhanced the fertility of Holstein dairy cows affected with retained placenta. *Theriogenology* 2018, 121, 67–71. [CrossRef] [PubMed]

17. Miara, M.D.; Bendif, H.; Ouabed, A.; Rebbas, K.; Ait-Hammou, M.; Amirat, M.; Greene, A.; Teixidor-Toneu, I. Ethnoveterinary remedies used in the Algerian steppe: Exploring the relationship with traditional human herbal medicine. *J. Ethnopharmacol.* 2019, 244, 112–164. [CrossRef] [PubMed]

18. Miara, M.D.; Bendif, H.; Ouabed, A.; Rebbas, K.; Ait-Hammou, M.; Amirat, M.; Greene, A.; Teixidor-Toneu, I. Ethnoveterinary remedies used in the Algerian steppe: Exploring the relationship with traditional human herbal medicine. *J. Ethnopharmacol.* 2019, 244, 112–164. [CrossRef] [PubMed]

19. Boumaraf, M.; Benyahia, S.; Mekkiou, R.; Benayache, S.; Benayache, F. Flavonoids from ethyl acetate extract of *Aegle marmelos* with acute puerperal metritis. *Theriogenology* 2013, 79, 961–969. [CrossRef] [PubMed]

20. Bouattour, E.; Fakhfakh, J.; Dammak, D.F.; Jabou, K.; Damak, M.; Jarraya, R.M. Hexane extract of *Echinops spinosus* L. in North Africa: A Mini Review. *J. Complement. Med. Res.* 2018, 9, 40–50. [CrossRef]

21. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* L. in North Africa: A Mini Review. *J. Complement. Med. Res.* 2018, 9, 40–50. [CrossRef]

22. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

23. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

24. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

25. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

26. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

27. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

28. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

29. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

30. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

31. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

32. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

33. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

34. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]

35. Bouattour, E.; Fakhfakh, J.; Affes, M.; Chawech, R.; Damak, M.; Jarraya, R.M. Chemical constituents of *Echinops spinosus* from Tunisia. *Chem. Nat. Compd.* 2017, 53, 984–987. [CrossRef]
36. Ghanem, M.E.; Tezuka, E.; Devkota, B.; Izaike, Y.; Osawa, T. Persistence of uterine bacterial infection, and its associations with endometritis and ovarian function in postpartum dairy cows. *J. Reprod. Dev.* 2015, 61, 44–60. [CrossRef]
37. Sheldon, I.M.; Cronin, J.; Borges, A. The postpartum period and modern dairy cow fertility Part 1: Uterine function. *Livestock 2011*, 16, 14–18. [CrossRef]
38. Mc Laughlin, C.L.; Stanisiewski, E.; Lucas, M.J.; Cornell, C.P.; Watkins, J.; Bryson, L.; Tenia, J.K.S.; Hallberg, J.; Chenault, J.R. Evaluation of two doses of ceftiofur crystalline free acid sterile suspension for treatment of metritis in lactating dairy cows. *J. Dairy Sci.* 2012, 95, 4363–4371. [CrossRef]
39. Giuliodori, M.J.; Magnasco, R.P.; Becu-Villalobos, D.; Lacau-Mengido, I.M.; Risco, C.A.; De la Sota, R.L. Metritis in dairy cows: Risk factors and reproductive performance. *J. Dairy Sci.* 2013, 96, 3621–3631. [CrossRef]
40. Kim, I.H.; Jeong, J.K. Risk factors limiting first service conception rate in dairy cows and their economic impact. *Asian-Australas. J. Anim. Sci.* 2019, 32, 519–526. [CrossRef]
41. Piccardi, M.; Romero, G.; Veneranda, G.; Castello, E.; Romero, D.; Balzarini, M.; Bó, G.A. Effect of puerperal metritis on reproductive and productive performance in dairy cows in Argentina. *Theriogenology* 2016, 85, 887–893. [CrossRef]
42. Mellado, M.; Solano, R.; Veliz, F.; DE Santiago, A.; Gaytan, L.; Garcia, J. The effects of four protocols for the treatment of retained placenta on reproduction performance and milk yield in Holstein cows. *J. Hell. Vet. Medical Soc.* 2018, 68, 513–520. [CrossRef]
43. Fourichon, C.; Seegers, H.; Malher, X. Effect of disease on reproduction in the dairy cow: A meta–analysis. *Theriogenology* 2000, 53, 1729–2000. [CrossRef]
44. Yahyaoui, A.; Khedher, O.; Rigane, G.; Ben Salem, R.; Moussaoui, Y. Chemical analysis of essential oil from *Echinops spinosus* L. roots: Antimicrobial and antioxidant activities. *Rev. Roum. Chim.* 2018, 63, 199–204.