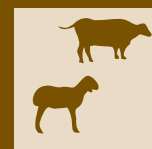


Antibacterial and repellent activities of *Hypericum perforatum* (St. John's Wort) on different bacterial strains and anatomical tissues of Ovine and Bovine species



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SUMMARY

St. John's Wort (*Hypericum perforatum*, SJW) is an herbaceous medical plant that grows abundantly and spontaneously in Mediterranean area. Since ancient times, SJW has been used to treat different kind of mental and physical diseases, and for its antiseptic, anti-inflammatory and antibacterial properties. In this study, we investigated the antibacterial and repellent activities of *Hypericum perforatum* oil on different animal samples *in vitro* and *ex vivo*. The flowering aerial parts of *Hypericum perforatum* were obtained from a cultivation Mediterranean area in Italy; fresh flowering aerial parts of *Hypericum perforatum* were left for maceration in a transparent jar inside extra virgin olive oil under daylight. The oily part was then removed by filtering and placed in dark brown jars with hermetic plastic stoppers. The inhibitory activity was evaluated on six different bacterial strains: 2 strains of *Staphylococcus aureus*, 2 strains of *Listeria monocytogenes*, 1 strain of *Salmonella tiphymurium* and 1 strain of *Escherichia coli* O157:H7. Agar gel diffusion technique was used with four different concentrations of the compound (5, 10, 15 and 20 µL). As a control, a sterile disc, not soaked with the oil, was placed on each plate. The effect of the oil against the selected strains was evaluated by measuring the halo of inhibition around the discs soaked with *Hypericum perforatum* oil. To establish the repellent activity, different animal samples were brushed once with the oil and kept outdoors under natural environmental conditions for one week. Only *Staphylococcus aureus* was susceptible to *Hypericum perforatum* oil, even at the lowest concentration used. For the other bacterial strains no inhibition was observed. A single application of the oil was effective against different species of insects, unlike untreated samples. The results achieved in this study confirm that *Hypericum perforatum* possesses antibacterial properties only against Gram positive bacteria and a strong repellent activity against insects.

KEY WORDS

Antibacterial activity, *Hypericum perforatum*, repellent activity, St. John's wort, *ovis aries*, *bos taurus*.

INTRODUCTION

St. John's Wort (SJW) is an herbaceous medical perennial plant, belonging to the family of Hypericaceae, genus *Hypericum* (APG III classification)¹; this common denomination is used both for *Hypericum perforatum* (*H. perforatum*) and *Hypericum perforatum* (*H. perforatum*). SJW grows on dry lands, as high as 1200-1600 meters above sea level, in almost all of Europe, in North Africa, West Asia and many other parts of the world. The flowering season is between May and August.

Since ancient times, SJW has been used to treat mild to severe forms of depression, anxiety and in psychiatric illness in general², as a topical remedy for skin wounds, abrasions and burns, and for its antiseptic³, antiviral, antitumor, antiangiogenic, anti-inflammatory⁴ and antibacterial properties. The most common side effects reported after using SJW are photosensitivity⁵ and chemical interaction with some drugs⁶. Even if sever-

al constituents of the aerial part have been recognized as therapeutic in these plants⁷ the greatest medical and therapeutic activity is mainly comprised in two substances: hyperforin and hypericin. Hyperforin is the major lipophilic constituent, even if it is unstable and susceptible to oxidative degradation. It seems to play the greatest role in the antidepressant effect of SJW². However, it also shows anti-inflammatory, anticarcinogenic⁸ and antimicrobial effects.

Hypericin is a lipophilic and hydrophobic naphthodianthrone, which is known to be a non specific protein kinase inhibitor, nontoxic and nonmutagenic, with antiviral, antineoplastic⁹, antidepressant¹⁰ and antimicrobial effects.

Furthermore, six *Hypericum* essential oils, in particular the monoterpene and sesquiterpene fractions, also showed antimicrobial effects against Gram-positive *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*)¹¹. No notable activity against Gram-positive *Enterococcus faecalis* (*E. faecalis*) and Gram-negative *Escherichia coli* (*E. coli*) was detected¹¹. In other studies, the antibacterial, against both Gram-positive and Gram-negative bacteria, and antifungal activities have been described^{3,12-13}. SJW has also shown antibacterial activity against different strains of mycobacteria¹⁴ and Methicillin-Resistant

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Staphylococcus aureus (MRSA)¹⁵. The different effectiveness against Gram positive and Gram negative bacteria could depend on the different structure of cell walls; the lack of an outer lipopolysaccharide membrane in the Gram-positive bacteria may allow increased permeability of *Hypericum* metabolites into cells¹⁶. Moreover, the alcoholic extracts were found to be more active than aqueous solutions³.

Regarding *Hypericum perforatum*, a recent study¹⁷ reported a high content in polyphenols (gallic, chologenic and caffeic acid, rutin, hyperoside and quercetin) and antimicrobial activity against *S. aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus cereus* (*B. cereus*), *E. coli* and *Chronobacter sakazakii* (*C. sakazakii*) of chloroform and chloroform-methanol extracts. Although the antibacterial activity against several bacterial pathogens of SJW is well known, few studies have been carried out on the antibacterial properties of *Hypericum perforatum*¹⁷⁻¹⁹ and, to the authors' knowledge, no scientific work exists on its repellent activity against insects in animal biological samples.

In view of such considerations, the aim of this study was to evaluate the *in vitro* antibacterial properties of a phytotherapeutic product from *Hypericum perforatum* against the most common agents, and the repellent activity against insects on different anatomical tissues of Ovine and Bovine species.

MATERIALS AND METHODS

Experimental material and preparation of SJW oil

The flowering aerial parts of *Hypericum perforatum* were obtained from a cultivation Mediterranean area of Sicily, Italy (37°43'N, 13°26'E) and the crude oily extract was prepared according to an ancient Sicilian recipe. Fresh flowering aerial parts of *Hypericum perforatum* (300 g) were left for maceration for 40 days in a transparent jar inside extra virgin olive oil (1 litre) (Biancolilla-Nocellara, Sicily) under daylight. The oily part was then removed by filtering through sterile gauzes to obtain a clear filtrate and placed in dark brown jars with hermetic plastic stoppers, kept in a dark place²⁰.

In vitro antibacterial activity

In vitro antibacterial activity was investigated by evaluating the inhibitory activity on different bacterial strains. 2 strains of *Staphylococcus aureus* (*S. aureus*), 2 strains of *Listeria monocytogenes* (*L. monocytogenes*), 1 strains of *Salmonella typhimurium* (*S. typhimurium*) and 1 strains of *Escherichia coli* (*E. coli*) O157:H7 were chosen. All the strains had been previously isolated from various food products. The strains were enriched in *Brain Heart Infusion* (BHI) (Oxoid Ltd., Basinkstoke, Hampshire, England) broth and incubated at 37° C for 24 hours. Each culture was adjusted in order to reach an optical density of 0.5 McFarland.

Agar gel diffusion technique was used to assess the effectiveness of SJW against the selected strains. Four different concentrations of the compound (5, 10, 15 and 20 µL) were tested. Muller Hinton Agar plates (MHA) (Oxoid Ltd., Basinkstoke, Hampshire, England) were inoculated with each strain suspension in BHI broth and sterile discs (Biolife, Milan, Italy), soaked with SJW oil were placed on the surface of MHA. Plates were then incubated at 37° C for 24 h. As a control, a sterile disc, not soaked with the oil, was placed on each plate.

The inhibitory activity was evaluated by measuring the halo of inhibition around the discs.

Repellent activity against insects

To establish the repellent activity of the same ointment, anatomical tissue (head, fetlock, muscle, testicle and mammary gland) of various species (ovine, bovine) were brushed once with the oil and, then, kept outdoors (sunrise at 04.33, sunset at 19.19 over the study period) uncovered, at natural environmental conditions. Thermal and hygrometric records were carried out for the whole study by means of a data logger (Gemini, UK), and they followed the normal seasonal pattern for the place (minimum and maximum mean temperature between 20.3° C and 27.3° C, mean relative humidity of 59.3%). Other samples, similar in dimension, without *Hypericum perforatum* oil treatment, were kept in the same condition. Samples were checked for one week, every hour during the first day and then three times a day.

RESULTS

In vitro antibacterial activity

The two strains of *S. aureus* were susceptible to *Hypericum perforatum* oil, with halos of inhibition from 3 mm, with the lowest concentration (5 µL), to 6 mm, with the highest concentration (20 µL) used. However, for the other strains evaluated in the study, no halo of inhibition was detected even with the highest concentration of the oil. In each plate, no halo of inhibition was found around the control disc (Figure 1).

Repellent activity against insects

A single application of the oil was effective in repelling various species of insects (Table 1). During the observation period, no insects had landed on the surface of the treated animal biological samples. On the other hand, the untreated samples were strongly targeted by various species of insects (flies, horseflies, bees, wasps and ants). Furthermore, it should be mentioned that the treated meat gradually dried out, forming a superficial crust, without signs of putrefaction, which however appeared in the untreated samples, from the second day of observation (Figure 2).

Table 1 - Visual inspection of the *Diptera* (flies and horseflies) presence on the anatomical tissue of Ovine and Bovine species, 8 hours after treated with *Hypericum perforatum* oil and without treatment.

Species	Anatomical tissue	Number of Diptera (flies and horseflies)	
		Treated with oil	No treated with oil
Ovine	Head	Absence	10
	Fetlock	Absence	15
	Muscle	Absence	7
	Testis	Absence	5
	Mammary gland	Absence	6
Bovine	Head	Absence	12
	Fetlock	Absence	9
	Muscle	Absence	14
	Testis	Absence	6
	Mammary gland	Absence	9

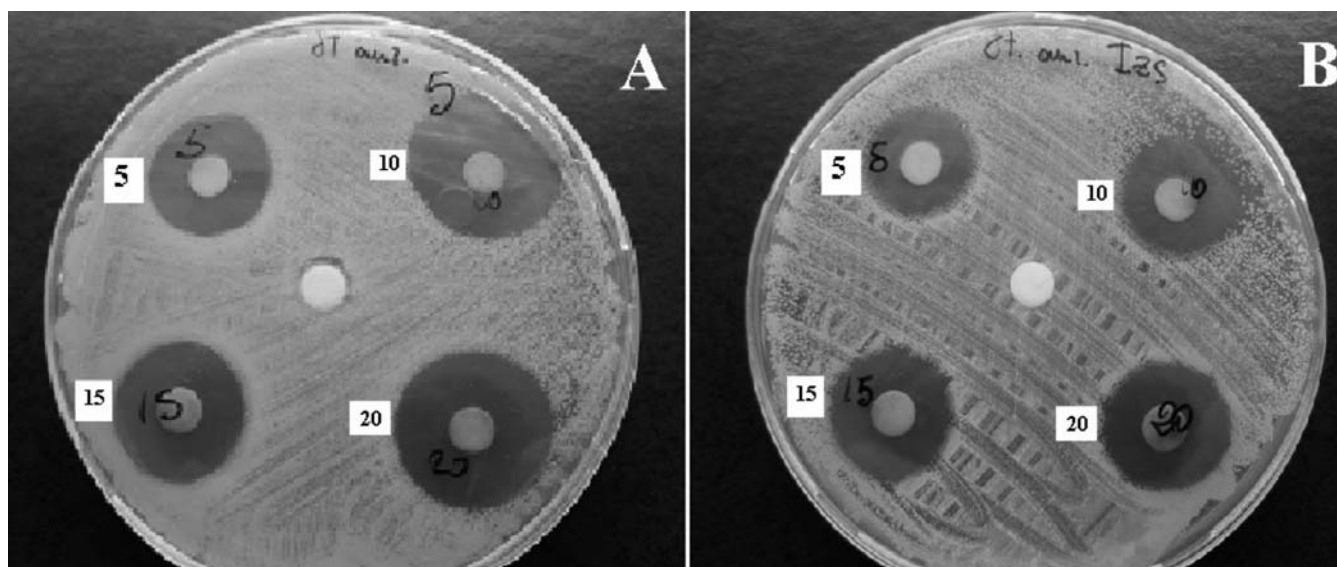


Figure 1 - Inhibitory action of the alcoholic extract of *Hypericum perforatum* on crops of two strains of *Staphylococcus aureus* (A and B): inhibition halo between 12 and 18 mm. Four different concentrations of the compound (5, 10, 15 and 20 µL) were tested.

DISCUSSION

The results obtained in the present study show that *Hypericum perforatum* possesses antibacterial properties against Gram positive bacteria (*S. aureus*), whereas no oil activity against Gram negative (*S. typhimurium* and *E. coli* O157:H7) and *L. monocytogenes* was observed. The results obtained are in line with previous studies on *Hypericum perforatum* antimicrobial activity^{17,19} regarding *S. aureus*. However, Del Monte et al.¹⁷ also demonstrated *Hypericum perforatum* antibacterial activity against *E. coli*. This could be connected to the different type of extract used or to a different sensitivity of *E. coli* O157:H7 compared to the other strains.

Considering that *Staphylococcus* spp. is one of the most common cutaneous bacteria and often responsible for skin infections, this could explain the effectiveness of SJW ointment in wound healing²¹⁻²⁴. Furthermore, the demonstrated and long lasting repellent activity against insects in general, and especially against *Diptera* (flies and horseflies), could represent a useful and cheap aid for skin lesion management, especially in

animals living outdoors, to prevent the onset of myiasis and to reduce the risk of Summer Seasonal Recurrent Dermatitis (SSRD, *sweet itch*) in horses.

Although the literature reports that the stability of SJW oil should last for an indefinite period, our product showed a reduction in effectiveness after one year, compared to fresh oil. It is advisable, therefore, to renew it annually to increase the effectiveness of the medication.

Considering the small number of bacterial strains tested in this study, further scientific work should be carried out on a larger and more varied number of bacterial species. Moreover, further evaluations should be made to evaluate the antibacterial activity of *Hypericum perforatum* oil *in vivo* skin infections caused by Gram positive bacteria, and to demonstrate the absence of interference with anti-doping investigation in sport horses and of those residues in food production animals that could be dangerous for human health. Finally, more studies should be carried out in order to establish the possible toxic effect of these extracts.

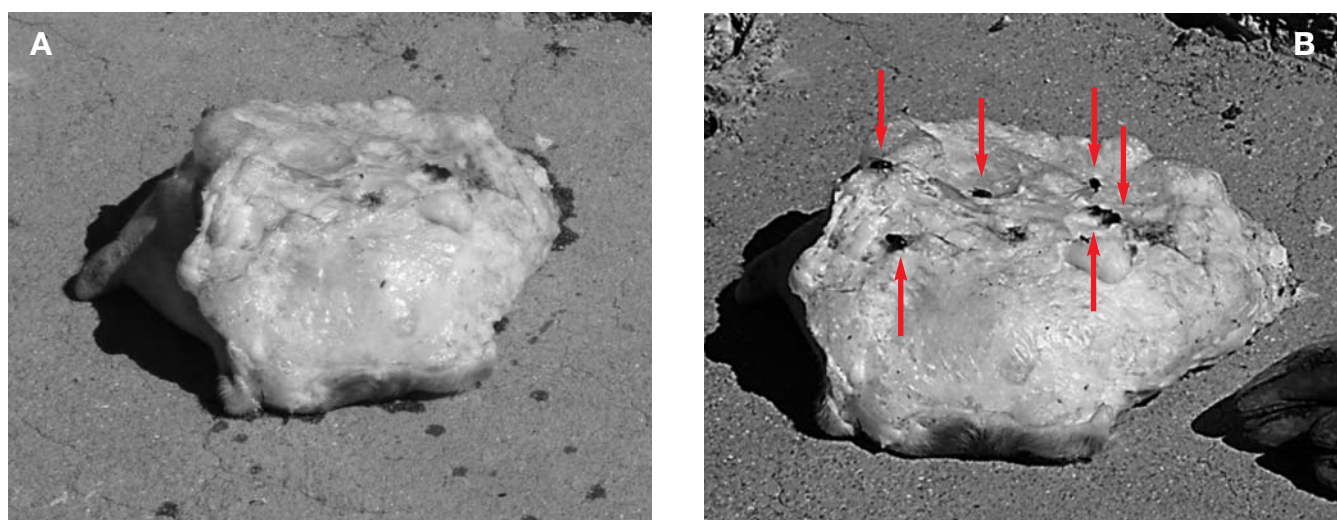


Figure 2 - Representative example of anatomical tissue (ovine mammary gland) 8 hours after treated with *Hypericum perforatum* oil (A) and without treatment (B). Red arrows indicate *Diptera* (flies and horseflies).

CONCLUSIONS

The use of SJW in veterinary practice should be better studied. Its extraction technique is economical and easy to perform and the extracted product shows a remarkable stability over time. For this reason, it could be a good remedy for use in biological farming, with a positive effect in terms of environmental impact and drug resistance. Our results improve the knowledge on the effects of *Hypericum perforatum* extract, in contrast to the more well-known *Hypericum perforatum*²⁵⁻²⁶, present in some commercial products. The findings of this study seem to suggest that SJW could be a good antibacterial and repellent remedy. In *in vivo* infections, it could be used to make treatment of infection easier, cheaper and less dangerous for human and animal health.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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