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Short communication

GC–MS analysis of the fixed oil from *Sus scrofa domesticus* Linneaus (1758) and antimicrobial activity against bacteria with veterinary interest

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ABSTRACT

The bioprospection of zootherapeutic products can be a source of new drugs and to the creation of new strategies of natural resources conservation and management of endangered species. This fact is supported by ethnobiological studies indicating that the usage of zootherapeutic products can be replaced by the use of natural products isolated from plants and domestic animals. The emergence of antibiotic-resistant bacteria has increased the need for research for new active principles. Ethnoveterinary studies in Brazil have shown that *Sus scrofa domesticus* fat is used for diseases associated with bacterial pathogens. The objective of this study was to identify the chemical composition and to evaluate the antibacterial activity of the fixed oil of *Sus scrofa domesticus* (OFSC) when used alone or associated with antibiotics. In the analysis of the oil composition, there were 4 constituents identified, with oleic acid being the major constituent. The OFSC did not present antibacterial activity when tested alone; however, it showed synergism in the modulating activity when associated with antibiotics Amikacin and Amoxicillin.

1. Introduction

The advancement of resistance from bacterial strains to antibiotics is a source of concern for human and veterinary medicine. Its inappropriate and abusive use of antibiotics is a major factor in the emergence and selection of resistant bacteria (Poeta and Rodrigues, 2008; Arias and Carrilho, 2012). Enterobacteriaceae and the *Staphylococcus* and *Streptococcus* genera are examples of microorganisms in veterinary medicine that are resistant to antibiotics (Oliveira et al., 2005; Contreras et al., 2007). The resistance of these microorganisms is also of concern for human beings because Enterobacteriaceae and *Staphylococcus* are known for the possibility of zoonotic transmission (Oliveira et al., 2005; Horn et al., 2005).

Faced with this problem, many research have been looking for new molecules or new therapeutic options as alternatives to the types of treatments already established (Haida et al., 2007). The ethno-guided method is one of the alternatives for the discovery of new drugs, which consists in the investigation of natural products based on traditional knowledge (Ferreira et al., 2009). According to Albuquerque and Hanazaki (2006), information on therapeutic properties in animals and plants can be considered as a shortcut for the discovery of new drugs by the pharmaceutical industry.

Natural resources derived from ethno-guided information have been tested as possible antimicrobial agents. In this context, natural products of plant origin have been highlighted because they present antibacterial activity and because they potentiate the activity of antibiotics (Tintino et al., 2013, 2015). However, although there is a predominance of studies with plants, we also find papers with animal products that seek to analyze these same activities (Ferreira et al., 2009; Dias et al., 2013; Oliveira et al., 2014; sales et al., 2017).

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Even after the discovery of new drugs, the animal-derived natural products with medicinal properties (named as zootherapic procusts), the comprehension of the social context and the traditional knowledge that use these natural resources is a millestone to the elaboration of conservation and management strategies of these exploited species, as by as the determination of public policies aiming the sustainability of these animal populations (Albuquerque et al., 2007). The work of Ferreira et al. (2016), performed in public markets in the brazilian Northeastern demonstrated the complete possibility to replace the usage of this natural resource in some places.

Ethnobiological surveys show that components derived from wild or domestic animals are used to treat diseases that affect human beings and other animals (Barboza et al., 2007; Ferreira et al., 2012). In the northeast of Brazil, the body fat of the domestic pig, *Sus scrofa domesticus* is used in the treatment of diseases such as mastitis, furunculosis and dermal nodules that affect domestic animals (Souto et al., 2012).

The objective of this study was to verify if the OFSC has antibacterial and modulatory activity in front of bacteria of veterinary interest.

2. Materials and methods

2.1. Zoological material

Body fat from 4 female and 4 adult and unmixed males of *Sus scrofa domesticus* was extracted for this test. All the fat obtained was mixed and crushed before sending for the extraction of the fixed oil. From this mix, 60 g of body fat was analysed in the Soxhlet using hexane as solvent. After separation with the solvent, the yelding obtained was 10.815 g (18.025%). The adipose tissue was donated by the Municipal Slaughterhouse of Barbalha, located at Rua P-25, s/n, Malvinas, Barbalha-Ceará (Brazil). This work was approved by the Commission of Experimentation and Use of animals of the Regional University of Cariri (CEUA - URCA) with protocol number: 0300/2015.1.

2.2. Obtaining fixed oil and determination of the fatty acids of Sus scrofa domesticus

The extraction of fixed oil from *Sus scrofa domesticus* (OFSC) was performed according to Dias et al. (2018). Subsequently, there was 0.2 g of OFSC weighed and saponified for 30 min under reflux with potassium hydroxide solution in methanol, following the methodology described by Hertman and Lago (1973). After suitable treatment and pH adjustment, the free acids were methylated with methanol through acid catalysis to obtain the respective methyl esters, used as a parameter to identify the original fatty acids. The identification of OFSC constituents occurred by gas chromatography coupled to mass spectrometry (GC–MS).

2.3. Gas chromatography-mass spectrometry (GC–MS)

GC–MS analyses were performed on a Shimadzu GC–MS QP2010 series fitted with a fused silica Rtx-5MS (30 m × 0.25 mm I.D.; 0.25 m film thickness) capillary column and temperature programmed as follow: 60–240 °C at 3 °C/min, then to 280 °C at 10 °C/min, ending with 10 min at 280 °C. The carrier gas was He at a flow rate of 1.5 mL/min and the split mode had a ratio of 1:50. The injection port was set at 220 ∘C. Significant quadrupole MS operating parameters: interface temperature 240 °C; electron impact ionization at 70 eV with scan mass range of 40–350 *m/z* at a sampling rate of 1.0 scan/s. Injected volume: 1 μL of 5 μg/mL solution in dichloromethane. Constituents were identified by computer search using digital libraries of mass spectral data (NIST 08) and by comparison of their authentic mass spectra (Adams, 2001). The GC–MS analyses revealed peaks corresponding to elution and molecular mass of saturated fatty components and unsaturated often found in oils fixed (Table 1).

2.4. Antibacterial activity and Modulation of drug action

The assays of antibacterial and modulatory activities of drugs, the drugs and strains choosen to be part of this work and the statistical analysis were performed as described in the work of Dias et al (2018).

3. Results

The methyl esters of the OFSC fatty acids were analyzed by GC/MS and the results are demonstrated in the Table 2. In the OFSC, the saturation percentage found was 42.34% and 57.67% of saturated and unsaturated methyl esters, respectively, with oleic acid as the major constituent (42.08%).

When adding resazurin to evidence MIC, there was a value of ≥1024 μg/mL established for OFSC against all strains evaluated here. The results of this MIC showed that although there is an indication of the ethnoveterinary use of OFSC for the treatment of infections that affect domestic animals, there was not any clinically relevant activity.

In the subinhibitory concentration (MIC/8) of the antibiotic modulation (graphs 1,2,3,4,5 and 6), there was synergism for EC 06, PM 01, SA 10, SE ATCC and SE 01 when associated with or Amikacin antibiotic. At the same sub-inhibitory concentration when associated with Amoxicillin, it presented synergism to SE 01. The main synergism can be observed using amikacin with OFSC against the strain SE ATCC (graph 5).

Antagonistic effects occurred in the association of OFSC with Norfloxacin against EC 06, PA 24, PM 01, SA 10, SE ATCC and SE 01, with Amoxicillin against PA 24 and PM 01, with Amikacin against PA 24, with Oxytetracycline versus EC 06 (Figs. 1–6). The more evident antagonistic effects can be observed in the association between OFSC with amikacin and amoxicillin against the MDR strain PA 24 (graph 2).

4. Discussions

Researches demonstrating the antimicrobial activity of body fat from animals have been performed, aiming specially the human health promotion (Ferreira et al., 2009; Dias et al., 2013; Oliveira et al., 2014). However, there are many convergent points between the traditional medicine for human treatment and the ethnoveterinary, involving not only the healthcare but also the administration of medical care, techniques and behavior (McCorkle, 1986; Souto et al., 2011). About the use of zootherapeuthic drugs, this superposition is very commom between the ethnomedicine and ethnoveterinary (Almeida and Albuquerque, 2002; Souto et al., 2011).

The analysis of the chemical composition of the OFSC by GC/MS allowed the identification from the fatty acid methyl esters of four constituents. From the result found, a prevalence of unsaturated fatty acids occurred, representing a total of 57.67%, with oleic acid as the major component (42.09%). The proportions found between saturated and unsaturated fatty acids follow a trend with previous studies that prospect the antibacterial activity of body fat of other species of animals (Ferreira et al., 2009; Cabral et al., 2013). The presence of palmitic, linoleic, oleic and stearic acids in OFSC has also been evidenced in other studies on the bioprospection of the antibacterial activity of fixed oils (Cabral et al., 2013; Oliveira et al., 2014).

From the clinical point of view, this study showed the ineffectiveness of OFSC, against bacterial diseases when administered alone. For all strains analyzed here, the Minimum Inhibitory Concentration value was ≥ 1024 μg/mL. This concentration is considered to be ineffective because a very high dose would have to be delivered to achieve this plasma concentration (Houghton et al., 2007). These data corroborate with the study by Silva et al. (2011) who also verified that there is no antibacterial action of swine fat for clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

When we evaluated the OFSC at the subinhibitory concentration (MIC/8) associated with antibiotics, our results demonstrated a

Table 1
Strains of bacterial clinical isolates used for testing with their antibiotic resistance and origin profile.
Source: Laboratório de Microbiologia e Biologia Molecular - LMBM - Universidade Regional do Cariri-URCA and Laboratório de Microbiologia LB – UFC – Universidade Federal do Ceará.

Bacteria	Origin	Resistance profile
Escherichia coli 06	Urine culture	Cephalothin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicilin-sulbactam
Proteus mirabilis 01	Urine culture	Colistin, nalidix acid, Nitrofurantoin, imipenem
Pseudomonas aeruginosa 24	Urine culture	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacilin-tazobactam, ceftazidime, merpenem, cefepime.
Staphylococcus aureus 10	Rectal swab culture	Cephalothin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicilin-sulbactam
Staphylococcus epidermidis 01	Surgical wound	Benzylpenicilin; ciprofloxacin; Moxifloxacin; sulfamethoxazole-trimethoprim; gentamicin; norfloxacin

Table 2
Methyl esters and their equivalent fatty acids from *Sus scrofa* fixed oil (OFSC) identified using gas chromatography coupled to mass spectrometry (GC–MS).

Fatty acids	OFSC % (SD)
Palmitic acid	28.21±0.26
Linoleic acid	15.58± 0.20
oleic acid	42.08± 0.03
Stearic acid	14.13± 0.25
Saturated fatty acids	42.34
Unsaturated fatty acids	57.67
TOTAL	100

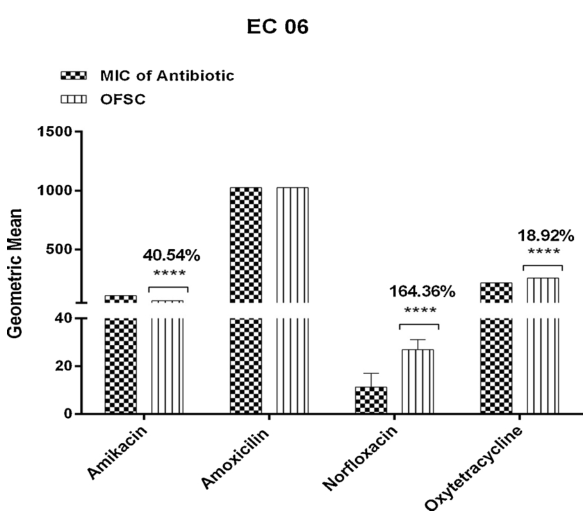

Fig. 1. *Sus scrofa* fixed oil (OFSC) effect of modulation in combination with antibiotics (Amikacin, Amoxicillin, Norfloxacin, and Oxytetracycline) against strains of *Escherichia coli* (EC06).
The columns represent the Minimal Inhibitory Concentration (MIC) expressed in Geometric Mean (MG) ± Standard Error of the Mean (S.E.M.), analyzed through the two-way ANOVA (Two-Way Analysis of Variance) followed by the *Bonferroni* test and multiple 't' post hoc. The significance level for rejection of the null hypothesis was $p < 0.05$ (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; **** - $p < 0.0001$).

synergistic effect in association with the Amikacin and Amoxicillin antibiotics. Fatty acids may inhibit bacterial activity (Agoramoorthy et al., 2007). Huang et al. (2010) found that the antibacterial action of n-6, n-7 and n-9 fatty acids on bacterial strains may vary according to the type of microorganism and the concentration of the fatty acid. The answer about OFSC on the strains analyzed here was different. The major fatty acid was oleic acid, an omega-9, corroborating the study by Huang et al. (2010).

Antagonistic effects have also been observed in OFSC associations with antibiotics. Previous studies that aimed to investigate the antibacterial and modulatory activity of body fat from other species of animals also report the occurrence of antagonistic effects when we associate the fixed oils with antibiotics, being attributed to a mutual

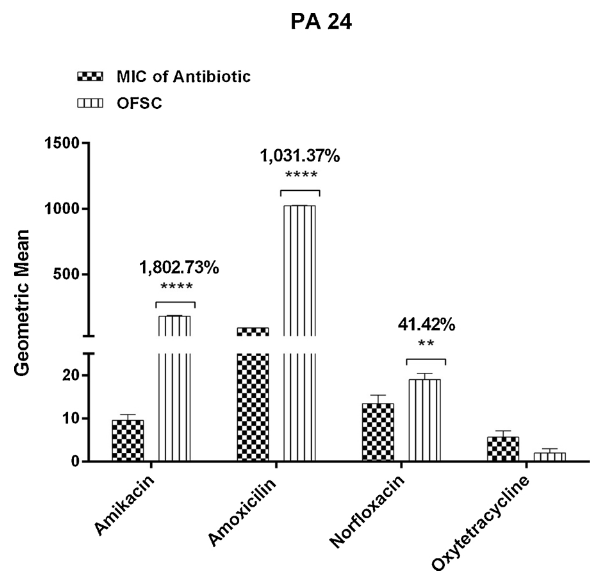

Fig. 2. *Sus scrofa* fixed oil (OFSC) effect of modulation in association with antibiotics (Amikacin, Amoxicillin, Norfloxacin, and Oxytetracycline) against *Pseudomonas aeruginosa* strains (PA24).
The columns represent the Minimal Inhibitory Concentration (MIC) expressed in Geometric Mean (MG) ± Standard Error of the Mean (S.E.M.), analyzed through the two-way ANOVA (Two-Way Analysis of Variance) followed by the *Bonferroni* test and multiple 't' post hoc. The significance level for rejection of the null hypothesis was $p < 0.05$ (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; **** - $p < 0.0001$).

chelation of the fatty acids present in the species analyzed and in the bacterial walls of the strains analyzed (Ferreira et al., 2009; Dias et al., 2013; Oliveira et al., 2014; Sales et al., 2017). Similar effect can be attributed here to the antagonistic effects evidenced here in the association of OFSC with the Amikacin, Amoxicillin, Norfloxacin and Oxytetracycline antibiotics.

5. Conclusions

Our data indicate that from the clinical point of view, OFSC does not have antibacterial activity when tested alone against the strains used here. However, when in subinhibitory concentration combined with the antibiotics, it demonstrated synergistic action for Amikacin and Amoxicillin.

This study evaluated the effects of OFSC in vitro. Therefore, we recommend the development of additional in vitro and in vivo studies to highlight the more detailed mechanisms by which the fatty acids present in OFSC modified the action of antibiotics.

Conflict of interests

The authors declare that there is no conflict of interest.

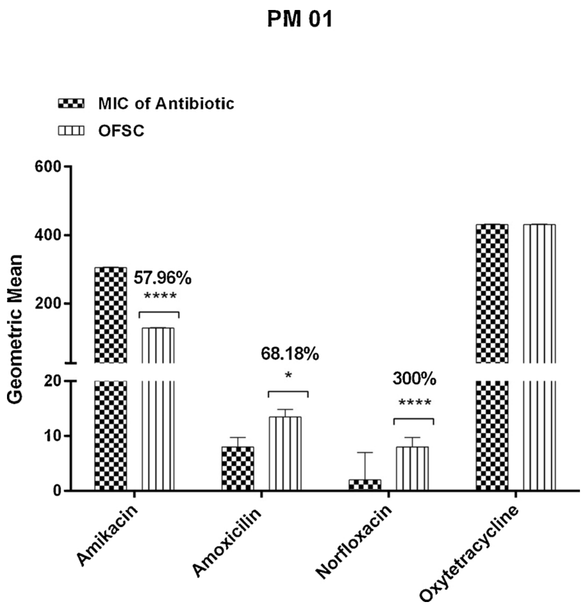

Fig. 3. *Sus scrofa* fixed oil (OFSC) effect of modulation in association with antibiotics (Amikacin, Amoxicillin, Norfloxacin, and Oxytetracycline) against *Proteus mirabilis* strains (PM01).
The columns represent the Minimal Inhibitory Concentration (MIC) expressed in Geometric Mean (MG) ± Standard Error of the Mean (S.E.M.), analyzed through the two-way ANOVA (Two-Way Analysis of Variance) followed by the *Bonferroni* test and multiple 't' post hoc. The significance level for rejection of the null hypothesis was $p < 0.05$ (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; **** - $p < 0.0001$).

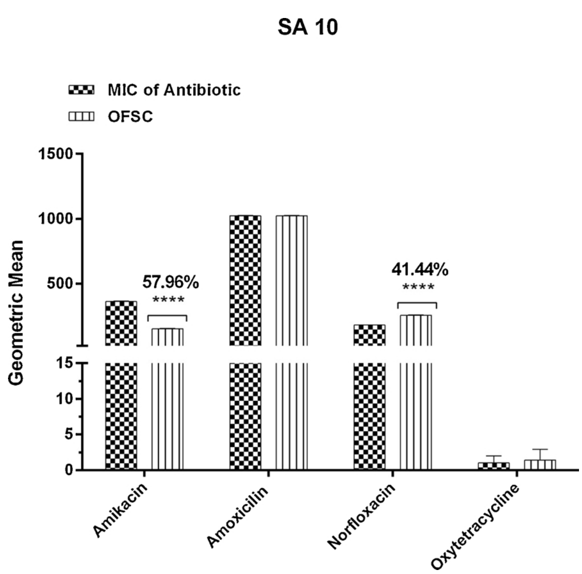

Fig. 4. *Sus scrofa* fixed oil (OFSC) effect of modulation in association with antibiotics (Amikacin, Amoxicillin, Norfloxacin, and Oxytetracycline) against strains of *Staphylococcus aureus* (SA10).
The columns represent the Minimal Inhibitory Concentration (MIC) expressed in Geometric Mean (MG) ± Standard Error of the Mean (S.E.M.), analyzed through the two-way ANOVA (Two-Way Analysis of Variance) followed by the *Bonferroni* test and multiple 't' post hoc. The significance level for rejection of the null hypothesis was $p < 0.05$ (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; **** - $p < 0.0001$).

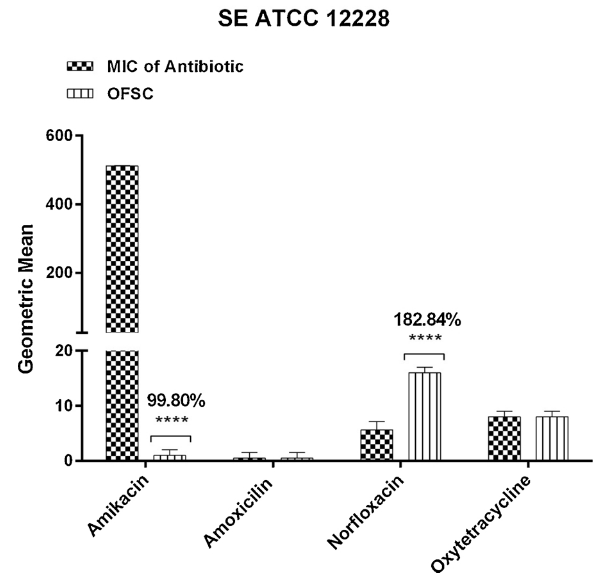

Fig. 5. Efeito da modulação do óleos fixos de *Sus scrofa* (OFSC) em associação com antibióticos (Amicacina, Amoxicilina, Norfloxacino e Oxitetraciclina) frente as cepas de *Staphylococcus epidermidis* ATCC 12,228 (SEATTC).
The columns represent the Minimal Inhibitory Concentration (MIC) expressed in Geometric Mean (MG) ± Standard Error of the Mean (S.E.M.), analyzed through the two-way ANOVA (Two-Way Analysis of Variance) followed by the *Bonferroni* test and multiple 't' post hoc. The significance level for rejection of the null hypothesis was $p < 0.05$ (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; **** - $p < 0.0001$).

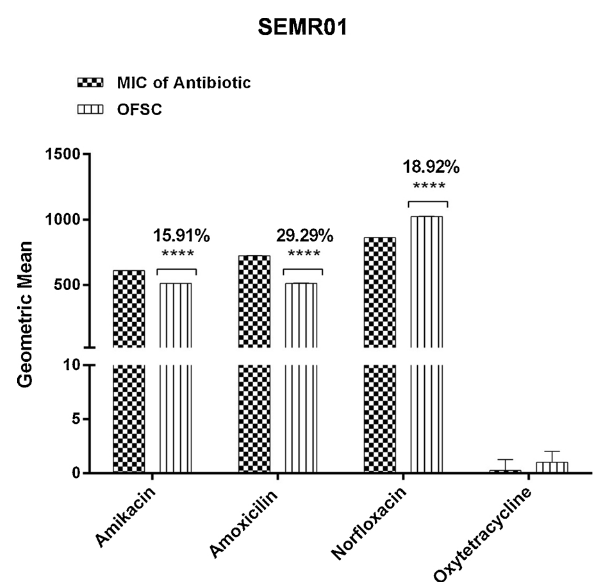

Fig. 6. *Sus scrofa* fixed oil (OFSC) effect of modulation in combination with antibiotics (Amikacin, Amoxicillin, Norfloxacin, and Oxytetracycline) against *Staphylococcus epidermis* strains multiresistant (SEMR01).
The columns represent the Minimal Inhibitory Concentration (MIC) expressed in Geometric Mean (MG) ± Standard Error of the Mean (S.E.M.), analyzed through the two-way ANOVA (Two-Way Analysis of Variance) followed by the *Bonferroni* test and multiple 't' post hoc. The significance level for rejection of the null hypothesis was $p < 0.05$ (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; **** - $p < 0.0001$).

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