Essential oil from *Salvia officinalis* L. and its effect on microbial parameters of piglets in a model experiment

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Summary

Plant essential oils and their active components have shown a large scale of activities: as antimicrobials, anti-oxidants, digestive stimulators, anti-inflammators, appetisers and performance enhancers. The antimicrobial properties of the essential oil from sage (*Salvia officinalis* L., family *Lamiaceae)* were evaluated against selected bacteria in a model experiment in crossbred piglets (Slovak White x Pietrain) weaned at 10 days of age. The essential oil was applied daily in a dose of 0.05% into the commercial feed mixture ČOS 1 and ČOS 2 for a period of three weeks, starting at the age of 21 days to 7 piglets in an experimental group and pathogen concentrations were compared with a control group of 3 piglets. Faecal samples of piglets were analyzed on the 21st, 35th and 42nd days of age and counts of anaerobes, *Escherichia coli*, enterobacteria and enterococci were performed. The differences in counts of selected bacteria within the control group during the experiment were not statistically significant. The counts within the experimental group showed statistically significant differences ($p<0.05$) in number of all selected bacteria between 1st and 2nd samplings. The anaerobic bacteria count was also significantly different on 21st and 42nd day of age. Comparing the control and the experimental group counts of *Escherichia coli* at the age of 35 days were significantly lower in the experimental group. All statistically significant differences observed showed decrease in selected bacteria counts. No adverse effects on animal health were noticed when using sage essential oil. Therefore, sage oil may be recommended to be an alternative fytoaditive antimicrobial supplement to conventional additives used in animal feed. (Because of the essential oil is inhibitory to selected pathogenic microorganisms it may provide alternative and supplement to conventional antimicrobial additives in foods.)

Key words: *animal health, essential oil, microflora, piglets, sage*

INTRODUCTION

The increasing use of herbs, herbal products and essential oil is not limited to Europe and North America, but has an enormous significance and potential all over the world especially in the place where ethnoveterinary knowledge still exists. Hovewer, it is not only due to an ethnoveterinarian revival but also based on the fact that a) antibiotic growth promoters are banned in more and more countries all over the world due to the risk of resistances, b) in organic livestock production the use of synthetic drugs is very restricted, and c) many pet animal and horse owners prefer natural products and “soft medicine”. Phytochemical as well as *in vivo* and *in vitro* studies will help to exploit these resources for the benefit of humans and animals [1].

Furthermore, among scientists and the general public there is a growing interest in finding alternatives to the use of feed-grade antibiotics to promote growth and prevent disease in animal food production systems [2]. Antibiotics and antimicrobial compounds are given to starter piglet diets for their health and growth-promoting properties, but continued use of subtherapeutic levels of antibiotics in animal feeds may contribute to antibiotic resistance in humans [3]. Weaning remains a critical phase in pig production and is associated with digestive disorders causing reduced growth and diarrhea [4]. Plant essential oils have been reported to have health benefit properties and their preventive and therapeutic use in animals is expected to increase in the future [5]. Essential oils are important anti-infectious and antimicrobial agents whose importance grows as microbial resistance to antibiotic and antiviral drugs increases. The aim of the present model experiment was to assess the antimicrobial activities of the essential oil from sage (*Salvia officinalis*, L., family: Lamiaceae) against selected bacterial groups in piglets.

MATERIALS AND METHODS

Essential oil and its analysis

Established growers in the Western part of Slovakia supplied the herb sage for essential oil isolation. Essential oils are distilled in the large-scale distillation apparatus specifically designed for aromatic and medicinal plants. There are two types: Type HV-3000' (height: 5.250 mm, width: 2.180 mm, with a container for 200 or 250 kg of dried matter of 400 or 500 kg of fresh matter of plant material) and Type` HV-300' (height: 3.400 mm, width: 1.300 mm, with container for 40 or 50 kg of dry matter and 100 or 120 kg of fresh matter of plant material). Both types were developed in 1986–1996 and successfully proved by the Calendula Co., in Nova Lubovna, Slovakia.

Total composition of sage essential oil was carried out using a HEWLETT-PACKARD 5890/5970 GC/MSD system with Split-splitless system for injection, MSD detector, column BPX – 5 (SGE Ltd., Melbourne), fused capillary column, 50 m long x 0.25 mm i.d., film thickness 0.25 mm. The following experimental conditions were observed: carrier gas UHP helium, column pressure 21 p.s.i (flow velocity 250 $mm.s^{-1}$), injection temperature 240°C, detector temperature 290°C, oven temperature was programmed, 50 - 250°C at 3°C.min^{-1}, and then held for 15 minutes. Sample sizes were 1.0 μl and the injection was of manual type. Components of essential oil were identified by Kovats samples (C_5 to C_{22} alkalane mixture was injected during a sample analysis) and Kovats indices calculated from retention time using a third order polynomial. Fourty authentic compounds were purchased from different companies (Extrasynthese, Merck, Fulka, Sigma and Roth).

Model experiment schedule

The essential oil was applied daily to 7 piglets in the experimental group (Slovak White x Pietrain, from the age of 21 days), at a dose of 0.05% in the commercial feed mixture ČOS 1 and ČOS 2). Model experiment was performed using conventional piglets in the farm (Čaklov, Slovakia). The control group included 3 piglets. All piglets were housed in pens, which contained one self-feeder and one nipple water bottle to provide *ad libitum* access to feed and water. To check antimicrobial activity of sage, faeces of piglets were sampled on days 21 (start of sage application), 35 (2 weeks after sage application) and 42 (3 weeks after sage essential oil application).

Microbial analysis

The samples were determined by applying the standard microbiological dilution method. All samples were collected in the same way and for their determination the same methodology was also used.

The appropriate dilutions were plated on the following media: the counts of anaerobes were determined on VL Agar (VL) in the anaerobic atmosphere. Endo Agar and Violet Red Bile Glucose Agar (VRBG) were used to determine *Escherichia coli* and the other enterobacteriae. The number of enterococci was determined on Slanetz-Bartley Agar. All media were supplied by Imuna Pharm a.s., Šarišske Michaľany, Slovakia. The plates were incubated at 37°C for 24 hours, in the case of enterococci for 48 hours. The bacterial counts are expressed in colony-forming units per gram.

Statistical Analysis

Statistical analysis was performed using the UNISTAT 4.53 system. Wilcoxon test was used for the statistical analysis of data within the control group. The means within the experimental group were analyzed by one-way analysis of variance (ANOVA). Differences between the control and experimental groups were analyzed using the Mann-Whitney U test. Results were expressed as the mean ±SD of individual samples. P-values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

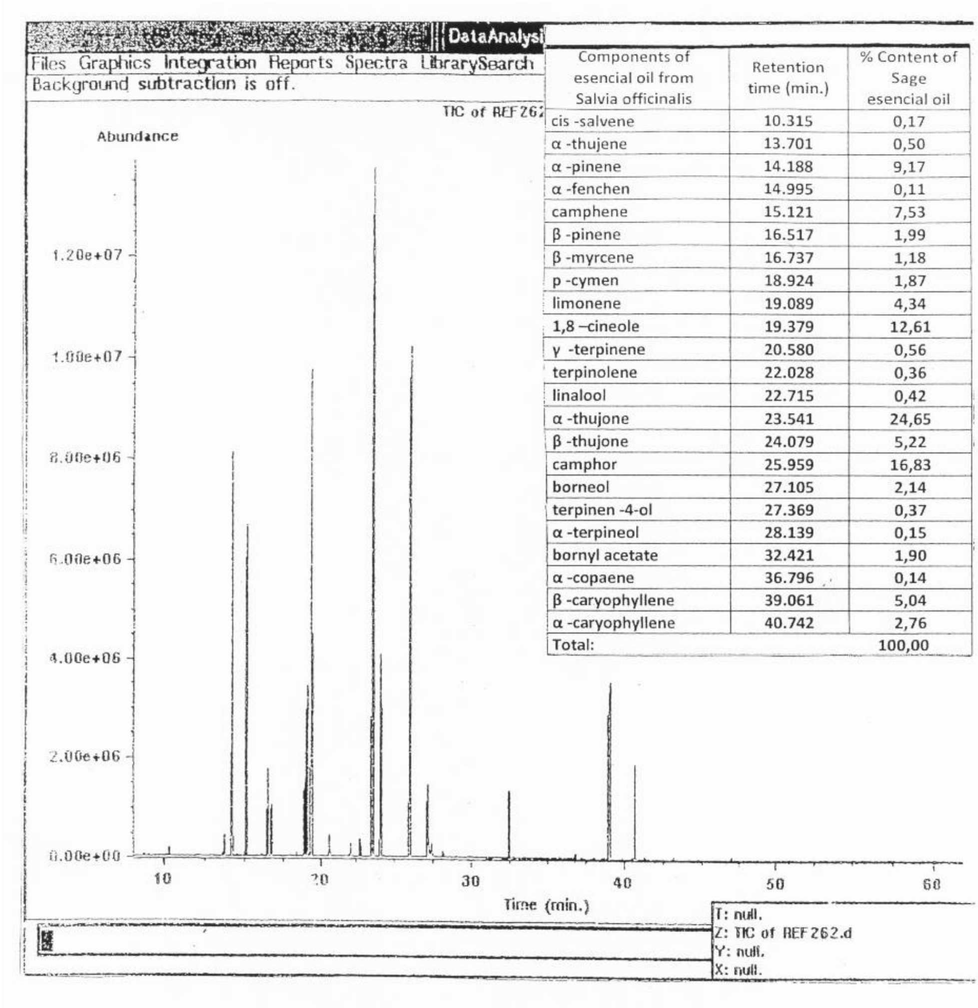
Sage is native to the Balkan area of the Mediterranean as well as adjacent parts of the Adriatic Sea. The plant has become naturalized throughout Eastern Europe. In regard to the phytomedicine programme of the former Slovakofarma, Co. in Hlohovec, the large-scale cultivation of this special crop in Slovakia was introduced in the late 1980s. Steam distillation of the freshly harvested herbs produces pale-yellow or almost colorless oil with a strong aroma and spicy, camphorous, slightly bitter flavor. The yield is about 2.5 %. The sage essential oil shows tremendous variability in the quantity of its principal components [6]. According to the GC/MS analysis, the essential oil consisted of 9.1% α-pinene, 7.5% camfene, 2.0% β-pinene, 4.3% limonene, 24.6% α-thujone, 5.2% β-thujone, 16.8% camphor, 2.1% borneole, 12.6% 1,8 cineole, 5.0 β-cariophylene (fig. 1).

Sage essential oils with a combined α- and β-thujone content of ≥30% and a camphor ≤20% are most valued [7].

The results of sage essential oil antimicrobial properties showed that although the mean counts of bacterial populations did vary over the course of the study in both the experimental group (EG) and the control group (CG) of piglets, no significant differences in numbers of selected bacteria within the control group were observed. The counts within the experimental group (EG) showed statistically significant differences ($p<0.05$) in number of all selected bacteria between 1st and 2nd samplings. The anaerobic bacteria count was also significantly different in 21st and 42nd day of age. When comparing the counts of *Escherichia coli* in piglets from the CG and EG on day 35, the counts were significantly lower in the experimental group ($p<0.05$, tab. 1).

The use of sage essential oil made statistically significant differences ($p<0.05$) on the count of enterobacteria after 35 days of application (treatment). Counts of enterobacteria and enteroccoci were reduced in the EG after 42 days of application of the sage essential oils (tab. 2). Obtained results refer to higher (better) antimicrobial effects of sage essential oils after a longer application (42 days and more).

The collection of faeces was done in a relatively short time interval. Therefore, no changes in the number of observed microorganisms in the 21st and 35th week were noticed in the control group in which sage essential oil was not applied.



Components of esencial oil from Salvia officinalis	Retention time (min.)	% Content of Sage esencial oil
cis -salvene	10.315	0,17
α -thujene	13.701	0,50
α -pinene	14.188	9,17
α -fenchen	14.995	0,11
camphene	15.121	7,53
β -pinene	16.517	1,99
β -myrcene	16.737	1,18
p -cymen	18.924	1,87
limonene	19.089	4,34
1,8 –cineole	19.379	12,61
γ -terpinene	20.580	0,56
terpinolene	22.028	0,36
linalool	22.715	0,42
α -thujone	23.541	24,65
β -thujone	24.079	5,22
camphor	25.959	16,83
borneol	27.105	2,14
terpinen -4-ol	27.369	0,37
α -terpineol	28.139	0,15
bornyl acetate	32.421	1,90
α -copaene	36.796	0,14
β -caryophyllene	39.061	5,04
α -caryophyllene	40.742	2,76
Total:		100,00

Figure 1. Qualitative-guantitative characteristics of the using sage essential oil

Table 1.

Bacterial counts [cfu.g^{-1} of faeces] in piglets from the experimental and control group in order to evaluate sage essential oil application

microorganisms	anaerobes		*Escherichia coli*	
days	EG mean ±SD	CG mean ±SD	EG mean ±SD	CG mean ±SD
21	$4.46x10^{7a}\pm3.91$	$5,67x10^{6}\pm1.25$	$5.87x10^{6a}\pm3.81$	$1.67x10^{5}\pm0.94$
35	$4.14x10^{6b}\pm1.96$	$5.67x10^{6}\pm1.25$	$4.60x10^{4b}\pm3.74$	$1.67x10^{5}\pm0.94$
42	$5.50x10^{6b}\pm3.20$	$1.03x10^{6}\pm0.83$	$4.14x10^{4}\pm3.27$	$4.20x10^{5}\pm4.14$

Footnotes: EG – experimental group (n=7), CG –control group (n=3); SD – standard deviation; [a, b] values in one column with different superscripts differ significantly (p<0.05)

Table 2.

Sage essential oils and its influence on bacterial counts [cfu.g^{-1} of faeces] in piglets

microorganisms	enterobacteria		enterococci	
days	EG mean ±SD	CG mean ±SD	EG mean ±SD	CG mean ±SD
21	$3.84x10^{7a}\pm3.32$	$1.73x10^{6}\pm1.41$	$2.40x10^{6a}\pm1.89$	$3.43x10^{5}\pm3.23$
35	$6.57x10^{5b}\pm5.26$	$1.73x10^{6}\pm1.41$	$4.89x10^{5b}\pm4.05$	$3.43x10^{5}\pm3.23$
42	$6.07x10^{5}\pm4.43$	$7.67x10^{6}\pm5.91$	$1.24x10^{3}\pm1.01$	$1.87x10^{4}\pm1.47$

Footnotes: EG –experimental group (n=7); CG – control group (n=3); SD – standard deviation; a, b – values in one column with different superscripts differ significantly ($p<0.05$)

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [8]. The antimicrobial activity of plant essential oils and extracts has been recognized for many years. There appears to be a revival in the use of traditional approaches to protect livestock and food from diseases, pests and spoilage in industrial countries. This is especially true in regard to plant essential oils and their antimicrobial evaluation [9-11]. Essential oils are potential sources of novel antimicrobial compounds [12], especially against bacterial pathogens and they can be used in the treatment of infectious diseases caused by resistant microbes [4, 13]. Several studies have been conducted on the antimicrobial properties of the essential oil from sage plants. [14]. The antimicrobial activity of sage essential oil is in general agreement with previously reported studies [10, 13, 15].

CONCLUSION

Due to negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural and in particular, plant extracts, including their essential oils and essences. The popular press and empirical evidence have suggested that plant extracts may offer benefits in terms of boosting the immune system and preventing disease.

Microbial induced diarrhea remains one of the crucial problems, especially in large-scale farm animal production. The antimicrobial properties of the essential oils from sage were evaluated against selected bacteria in a model experiment in crossbred piglets (Slovak White x Pietrain) weaned at 28 days of age. As alternatives to in-feed antibiotics sage essential oils show significant antimicrobial effects. Further investigations would be required to determine the inhibitory concentrations of this essential oil. According to the preliminary results obtained in our model experiment we can conclude that plant feed additives may be used as an appropriate alternatives to antibiotics.

ACKNOWLEDGEMENTS

The study was supported by the Ministry of Education of the Slovak Republic, Government Programme for R & D, No. 2004 SP 260280C05.

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OLEJEK ETERYCZNY Z *SALVIA OFFICINALIS* L. I JEGO WPŁYW NA LICZBĘ BAKTERII U PROSIĄT W DOŚWIADCZENIU MODELOWYM

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Streszczenie

Roślinne olejki eteryczne i ich składniki aktywne działają przeciwbakteryjnie, przeciwutleniająco, stymulująco na przemianę materii, przeciwzapalnie, pobudzają apetyt, poprawiają nastrój. Właściwości przeciwbakteryjne olejku eterycznego z szałwi (*Salvia officinalis* L., rodzina Lamiaceae) badano w odniesieniu do wybranych bakterii w doświadczeniu modelowym przeprowadzonym na prosiętach-mieszańcach (Słowacki Biały i Pietrain), karmionych mlekiem matki do 10. dnia życia. Olejek eteryczny podawano codziennie w dawce 0,05% jako dodatek do paszy ČOS 1 i ČOS 2 przez 3 tygodnie, poczynając od 21. dnia życia. Porównywano stężenia bakterii patogennych u 7 prosiąt z grupy badanej i 3 z grupy kontrolnej. Analizowano próbki kału prosiąt z 21., 35., i 42. dnia życia. Zbadano liczbę beztlenowców, *Escherichia coli*, enterobakterii i enterokoków. Różnica w liczbie wybranych bakterii w grupie kontrolnej w czasie doświadczenia nie była istotna statystycznie, natomiast liczby w grupie badanej różniły się istotnie statystycznie ($p<0,05$) w przypadku wszystkich badanych bakterii w próbce 1 i 2. Liczba bakterii beztlenowych różniła się znacznie pomiędzy 21. a 42. dniem życia. Liczba bakterii *Escherichia coli* w grupie badanej 35. dnia była zdecydowanie niższa niż w grupie kontrolnej. Wszystkie różnice istotne statystycznie wskazywały na spadek liczby wybranych bakterii. Podczas stosowania olejku z szałwi nie zaobserwowano działań niepożądanych. Dlatego można polecać stosowanie tego olejku jako dodatku pochodzenia roślinnego do standardowych pasz o działaniu przeciwbakteryjnym.

Słowa kluczowe: *zdrowie zwierząt, olejek eteryczny, mikroflora, prosięta, szałwia*