Evaluation of Antibacterial Activity of Salvia Officinalis L. Sage Oil on Veterinary Clinical Isolates of Bacteria

Bhoj Raj Singh

Indian Veterinary Research Institute Izatnagar Bareilly Uttar Pradesh INDIA

Abstract

In the study except a strain of Dermatophilus congolensis isolated from a dog blood suffering from pyrexia, all bacterial isolates from diseased animals were resistant to discs containing 2 microL sage oil. The only sensitive strain of D. congolensis also showed narrow zone (8 mm) of growth inhibition while the sensitive reference strain of E. coli-382 had 10-12 mm zone of growth inhibition around SEO discs. The MIC for the reference E. coli strain was 0.64 microL/ ml while for D. congolensis strain it was 1.28 l/ ml with all three methods (agar dilution, broth dilution and agar well methods) of MIC determination. Sage oil MIC for four Streptococcus species strains was 2.56 l to 5.12 microL/ ml while for three strains of Pasteurella canis and four strains of Plesiomonas shigelloides was 5.12microL / ml. For screening purpose, disc diffusion assay for antimicrobial activity appeared useful tool. The study revealed that MIC of sage oil could be determined using any of the three (broth dilution, agar dilution or agar well) methods without any significant variation among results.

Keywords: Sage oil, MIC, Salmonella, Klebsiella pneumoniae, Pasteurella canis, Plesiomonas shigeeloides, Dermatophilus congolensis, E. coli, Moraxella osloensis, Raoultella terrigena

Introduction: Oils are synthesized in plants either as food reserves or as by-product of several biochemical synthesis pathways and stored in or excreted from plant tissues. Most of the plant oils are chemically complex having several dozen compounds to hundreds of compounds as constituents. Essential oils mainly contain terpenes consisting of variety of hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, sulphur compounds, acetylene derivative, tropolons, coumarins and organic acids [1, 2, 3]. More than 300 essential oils (EO) are produced on industrial scale and posses many pharmaceutical uses. The strong antimicrobial activity has been reported in Salvia officinalis (sage) essential oil [4]. Biologically active ketones and alcohols in sage EO (SEO) responsible for its antimicrobial activity include thujene (2.5-30%), camphor (2-46%) and 1-8-cyneol (2-18%) (5, 6). Studies on antimicrobial properties of SEO revealed its activity against Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Yersinia enterocolitica, Shigella flexneri, Listeria monocytogenes, Staphylococcus aureus, Staph. epidermidis, Enterococcus faecalis, Salmonella spp., Bacillus subtilis and Aspergillus niger [7, 8]. Variation in composition of the sage EO of different origin is well documented and might be responsible for variation in the activity of SEO of different origin [8, 9, 14]. Further most of the studies on antimicrobial activity of SEO have been conducted on reference strains [8-14]. Therefore, this study was planned to evaluate commercially available SEO of Indian origin for its antimicrobial activity on bacteria isolated either from samples from animals either suffering or died with different ailments.

Materials and Methods: A total of 60 isolates of aerobically growing bacteria belonging to 29 species of 20 genera and isolated from clinical samples or post-mortem samples of animals of seven species (Table. 1) available in Epidemiology Section of Centre for Animal Disease Research and Diagnosis of the Institute were revived from glycerol stocks. All the cultures were

 ISSN 1941-2681. http://www.notoare.com/15341289

rechecked for purity and identity [15, 16] and maintained on nutrient agar (Hi-Media, Mumbai) slants throughout the study. All the strains were tested for their sensitivity to sage EO using disc diffusion method (each disc contained 2 mg of Sage EO) as described earlier [17]. Besides, minimum inhibitory concentration (MIC) of SEO for different bacteria was determined using agar dilution, broth dilution and agar well methods. For agar dilution method Mueller Hinton agar (MHA, Hi-Media) plates having 5.12, 2.56, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04 and 0.02 microL SEO / ml of MHA were used [17] and test strains were spot inoculated from overnight broth culture with a sterile loop and incubated for 24 h at 37oC. The growth of bacteria was determined by formation of bacterial colony on the site of inoculation and on the SEO dilution plate at which the inoculated bacteria did not grew was taken as MIC of SEO for the test strain. For broth dilution method, SEO was first diluted (51.2 microL/ ml) in dimethyl sulphoxide (DMSO, Merck India Ltd.) and then 200 microL of it was dispensed in to the first tube having 1.9 ml of Mueller Hinton broth (MHB, Hi-Media) to have final dilution of 5.12 microL of SEO/ ml. From the first tube one ml was transferred to the second tube having one ml of sterile MHB, and such two-fold serial dilutions were made up to 9th tube to have 5.12, 2.56, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04 and 0.02 microL SEO/ ml of MHB in first to 9th tube, respectively. To each tube 10 microL of overnight grown test culture was added and incubated at 37oC for 24 hr and observed for growth of bacteria. The highest dilution where growth was not observed (no turbidity) was recorded as MIC of SEO for the test strain.

For agar well method eight two-fold serial dilutions of SEO (102.4 to 0. 8 microL/ ml) were made in DMSO while the 9th tube contained only DMSO. Six mm diameter eight wells were cut in MHA plate in a circle (1 cm inside to the periphery of the plate) and 9th well was cut in the centre of the plates pretested for sterility through 36 h incubation at 37oC. Bases of wells were sealed using 10 l of sterile molten MHA. Thereafter, MHA plates were inoculated using swabs dipped in overnight test culture of bacteria grown in MHB. After inoculation plates were allowed to dry for 15 min at ambient temperature in laminar flow before dispensing 50 l of diluted SEO from well number one to eight and only DMSO in central well. The well with highest dilution showing clear zone of growth inhibition was considered as the MIC well and MIC was calculated by dividing the SEO concentration dispensed in the MIC well by 20, viz., if the MIC well was the 3rd well (Fig. 1) then MIC was equal to 1.28 microL/ml. All tests were conducted in triplicate. A reference strain of E. coli (E382), received from National Salmonella Centre, IVRI, Izatnagar, Bareilly, India, sensitive to all antimicrobial drugs, was used as control to determine the MIC of SEO.

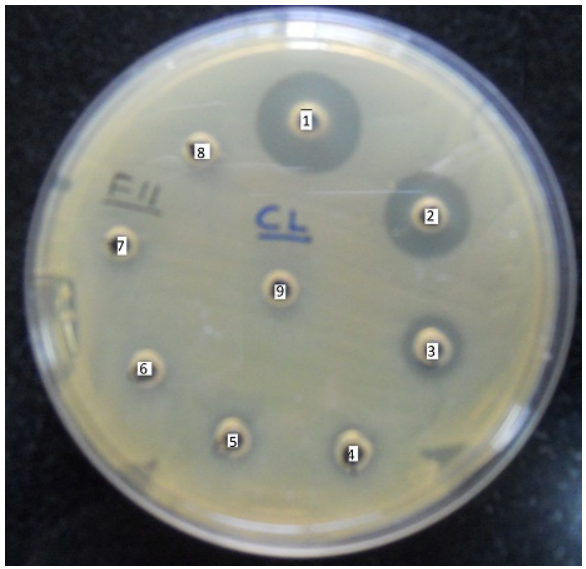

Fig. 1:

Results: In disc diffusion test for antimicrobial action of SEO, except a strain of Dermatophilus congolensis isolated from a dog blood suffering from pyrexia, none of the remaining 59 bacterial isolates from different disease conditions of animals was detected sensitive to SEO (Fig. 2). The only sensitive strain of D. congolensis also showed narrow zone (8 mm) of growth inhibition while the sensitive reference strain of E. coli-382 had 10-12 mm zone of growth inhibition around SEO discs. The MIC studies also corroborated with disc diffusion test results. There was no difference in MIC calculated for different strains with three different methods. The reference E. coli strain had MIC equal to 0.64 microL/ ml while D. con-

golensis strain had MIC 1.28 microL/ ml with all three methods of MIC determination. Sage oil MIC for all four Streptococcus species strains was determined to be 2.56 microL to 5.12 microL/ ml while for three strains of Pasteurella canis and four strains of Plesiomonas shigelloides was 5.12 microL/ ml.

Table 1. MIC of bacterial isolates from clinical/ post-mortem cases of animals for sage oil

Bacteria	Number of isolates	Disease condition/ source of isolates	MIC in µl/ ml
Actinobacillus equeli	1	Urinary tract infection (UTI) in horse	>5.12
Aeromonas salmonicida ssp. *salmonicida*	1	Vaginal swab of aborted goat	>5.12
Alcaligenes faecalis	3	Spleen and lungs of dead pig (2), UTI in Dog (1)	>5.12
Burkholderia mallei	3	Nasal swabs of horses suspected for glanders	>5.12
Brucella abortus	1	Vaginal swab of an aborted cow	>5.12
Dermatophilus congolensis	1	Blood of dog with pyrexia	1.28
Enterobacter agglomerans	2	Inflicted wounds of horses	>5.12
Enterococcus solitarius	2	UTI in dog	>5.12
Escherichia coli	15	Wound of buffalo (1), spleen of aborted foetus of cattle (1), vaginal swab of aborted cows (3), UTI in dogs (3), vaginal swabs of aborted goats (2), wound of horse (1), spleen of a dead pig (1), liver dead tigers (3)	>5.12
Klebsiella pneumoniae	3	Spleen of a dead pig (1), kidney and liver of a dead tiger (2)	>5.12
Klebsiella oxytoca	1	Spleen of an aborted foetus of cattle	>5.12
Moraxella osloensis	1	Stomach fluid of an aborted cattle foetus	>5.12
Pasteurella canis	3	Spleen of an aborted foetus of cattle (1), vaginal swabs of aborted cows (2)	5.12
Plesiomonas shigeeloides	4	Spleen and stomach contents of aborted foetus of cattle (3), vaginal swabs of aborted cows (1)	5.12
Proteus mirabilis	3	Spleen of dead tiger (1), wound of horse (1), UTI in dog (1)	>5.12
Proteus penneri	1	UTI in dog	>5.12
Pseudomonas pseudoalcaligenes	1	Spleen of an aborted foetus of cattle	>5.12
Raoultella terrigena	1	UTI in dog	>5.12
Salmonella enterica spp. *enterica* serovar Kentucky	1	Diarrhoea in tiger	>5.12
Staphylococcus auricularis	1	Otorrhoea in dog	>5.12
Staph. capitis ssp. *urealyticus*	1	Prostatitis in dog	>5.12
Stap. chromogenes	1	Spleen of dead tiger	>5.12
Staph. hyicus	1	UTI in dog	>5.12
Staph. lentus	2	Wound and otorrhoea of dogs	>5.12
Staph. epidermidis	1	Spleen of dead tiger	>5.12
Streptococcus adjacens	1	UTI in dog	2.56
Streptococcus defactivus	2	Vaginal swab of aborted goats	5.12
Streptococcus equi ssp. *equisimilis*	1	UTI in horse	5.12
Vibrio mimicus	1	Stomach fluid of an aborted cattle foetus	>5.12
Escherichia coli 382	1	Reference strain	0.640

Fig. 2:

Discussion: In the present study most of the bacterial strains isolated from sick or dead animals were resistant to SEO and had MIC more than 5.12 mg/ ml (Fig. 2). The observation at first sight appeared to be contradicting the earlier observations on SEO reporting it as a good antimicrobial substance [8-14]. However, the observations in the present study are not much different from the earlier observations on reference strains. In an earlier study on antimicrobial activity of sage, rosemary, eucalyptus, melissa, lavender and thyme essential oils, sage was found to have weak activity against Staphylococcus aureus Bacillus subtilis E. coli and Pseudomonas aeruginosa [10-12). In another study [13] SEO had significant antibacterial effect against Staph. aureus and B. subtilis, MIC ranging from 1.25 to 2.5 microL/ ml for Staph. aureus and 0.15 to 2.5 microL ml for B. subtilis. However, Mitic-Culafic and co-workers [13] reported SEO ineffective against E. coli and Salmonella enterica ssp. enterica serovar Typhimurium even when 30 microL of oil was put in each disc. Bernotien and co-workers [14] reported that most of the bacteria including Pseudomonas aeruginosa, E. coli, Klebsiella pneumoniae, Staph. aureus, Staph. epidermidis, Enterococcus faecalis and Salmonella bruneii were efficiently inhibited by sage oil to give good zone around discs containing 20 microL of the oil, the concentration 10 times of that used in our study. No variation in MIC of three strains of Pasteurella canis (isolated from an aborted foetus and its dam) and four strains of Plesiomonas (isolated from an aborted foetus and its dam) shigelloides might be due to the possibility of their being from single source. The study concluded that bacteria isolated from diseased animals are mostly resistant to sage oil and have MIC more than 5.12 microL/ ml except a few strains of Dermatophilus congolesis, Pasteurella canis, Plesiomonas shigelloides and Streptococcus spp. For screening purpose disc diffusion assay for antimicrobial activity is a useful tool but researchers have suggested that instead of disc diffusion assay MIC determination is better method to evaluate antimicrobial activity of herbal oils [13]. This study also support this view because quantification of antimicrobial activity is possible only with MIC determination and without quantifying the antimicrobial activity of herbal oils and other antimicrobials their utility cannot be explored. The present study also revealed that MIC can be determined using any of the three (broth dilution, agar dilution or agar well) method at least for aerobic strains of bacteria without any significant variation among results obtained using three methods.

Acknowledgments

Author is thankful to Shubh Flavour and Frances Pvt. Ltd. New Delhi for providing free samples of Sage oil, Mr. HC Joshi and Mr. Laik for constant help in laboratory procedures, Joint Director CADRAD and Director IVRI, Izatnagar for permission to work, funds and facilities for conducting the study.

References

[1] Sienkiewicz M., Denys P., Kowalczyk E., *"Antibacterial and immunostimulatory effect of essential oils."*, Internation Review of Allegology and Clinical Immunology, Vol. 17, No. 1-2, 40-44, 2011.

[2] Cal K., *"Skin penetration of terpenes."*, Pharmaceutical Technology Europe, Vol. 19, No. 2, 42-48, 2007.

[3] Lamer-Zarawska E., Kowal-Gierczak B., Niedworok J., *""*, Phytotherapy and Plant Medicines. PZWL Medical Publisher, Warsaw, Vol. , No. , , 2007.

[4] Price A., Price L., *""*, Aromatherapy for health professionals. 3rd Edition, Churchill Livingstone, London ., Vol. , No. , , 1999.

[5] Kelen M., Tepe B., *"Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora."*, Bioresource Technology., Vol. 99, No. 10, 4096-4104, 2008.

[6] Jirovetz L., Wlcek K., Buchbauer K., Gochev V., Girova T., Stoyanova A., Schmidt E., Geissler M., *"Antifungal activities of essential oils of Salvia lavandulifolia, Salvia officinalis and Salvia sclarea against various pathogenic Candida species."*, Journal of essential Oil Bearing Plants, Vol. 5, No. 10, 430-439, 2007.

[7] Rota C., Carraminana J.J., Burillo J., Herrera A., *"In Vitro Antimicrobial Activity of Essential Oils from Aromatic Plants against Selected Foodborne Pathogens"*, Journal of Food Protection, Vol. 67, No. 6, 1252-1256, 2004.

[8] Miladinovi D., Miladinovi LJ., *"Antimicrobial activity of essential oil of sage from Serbia"*, Facta Universitatis. Series: Physics, Chemistry and Technology, Vol. 2, No. 2, 97-100, 2000.

[9] Jorge A. P., Mirna E., Victor F., *"Essential Oil of Sage (Salvia officinalis L.) Grown in Cuba"*, Journal of Essential Oil Research, Vol. 9, No. 2, 221-222, 1997.

[10] Bosni T., Softi D., Gruji-Vasi J., *"Antimicrobial Activity of Some Essential Oils and Major Constituents of Essential Oils"*, Acta Medica Botanica, Vol. 35, No. 1, 9-14, 2006.

[11] Skovic M., Tzakaou O., Pitarokili D., Couladis M. . (2002) 46(5) 317-320., *"Antifungal activities of selected aromatic plantsgrowing wild in Greece, Nahrung"*, Food, Vol. 46, No. 5, 317-320, 2002.

[12] Baratta M.T., Dorman H.J.D., Deans S.G., *"Chemical Composition, Antimicrobial and Antioxidative Activity of Laurel, Sage, Rosemary, Oregano and Coriander Essential Oils"*, Journal of Essential Oil Research, Vol. 10, No. November-December, 618-627, 1998.

[13] Miti-ulafi D., Vukovi-Gai B., Kneevi-Vukevi J., Stankovi S., Simi D., *"Comparative study on the antibacterial activity of volatiles from sage (Salvia officinalis L.)"*, Archives of Biological Sciences, Belgrade, Vol. 57, No. 3, 173-178, 2005.

[14] Bernotien G., Nivinskien O., Butkien R., Mockut D., *"Essential oil composition variability in sage (Salvia officinalis L.)"*, Chemija, Vol. 18, No. 4, 38-43, 2007.

[15] Holt J.G., Sneath P.H.A., Mair M.S., Sharpee M.E. (1986)., *""*, Bergey,s Manual of Systematic Bacteriology. Vol. 2. Williams &Wilkins, 428 east Preston Street, Baltemore, MD 211202, USA, Vol. , No. , , 1986.

[16] Singh B.R., *""*, Labtop for Microbiology Laboratory, ISBN 978-3-8383-1574-40, Lambert Academic Publishing, Germany, Vol. , No. , , 2009.

[17] Singh B.R., Singh V., Singh R.K., Toppo S., Haque N., Ebibeni N., *"Antimicrobial effect of Artemisia Vulgaris essential oil"*, Natural Products: An Indian Journal, Vol. 7, No. 1, 5-12., 2011.