Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

Article

In vitro anti-microbial Activity of Essential oils and other Extracts from *salvia officinalis* against Some Bacteria

Amna Yousif Mohamed[1] and Ahmed Ali Mustafa[2*]

[1]Biotechnology Department, Faculty of Science and Technology, Omdurman Islamic University, P.O.Box#382, Omdurman Sudan.

[2]Botany Department, Faculty of Science and Technology, Omdurman Islamic University, P.O.Box#382, Omdurman Sudan.

*Correspondence: E.Mail: ahmad.ali11526@gmail.com tel: 00249911511696

Abstract: This study aimed to screen the antibacterial activity of essential oils from different parts (leave and stem) of *Salvia officinalis* against some Gram positive and Gram negative bacteria using agar disc diffusion test, then the extracts were prepared by hydro distillation to extract the essential oils. Maceration and hexane extraction by Soxhlet were used to obtain crude extracts from the leave and stem. Essential oils from the leaves and the ethyl acetate extract of the leaves showed higher antimicrobial activity, while hexane extract of leaves and stems showed moderate antibacterial activity. In contrast the essential oil from the stems showed very low antibacterial activity. It was observed that the results gram positive bacteria (*staphylococcus aureus*) was more sensitive than Gram negative (*Echerichia coli*).

Keywords: Antimicrobial activity, Essential oils, *Salvia officinalis*, Sudan.

1. Introduction

Nature has provided a complete storehouse of remedies to cure ailments of mankind. About 80% of the world's population depends wholly or partially on traditional medicines for its primary health care needs [1]. Herbal medicines as the major remedy in traditional medical system have been used in medical practice for thousands of years and have made a great contribution to maintain human health [2]. Herbal treatments are becoming increasing by popular as the herbal preparations have no or less side effects [3]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of order to validate their use in folk medicine. Systematic screening of them may result into the discovery of novel active compounds [4]. The family Lamiaceae is widely distributed over the world. It comprises over 5,000 medicinal and aromatic plant species whose essential oils have multiple applications [5, 6]. The genus *Salvia* commonly called Sage, is the largest member of lamiaceae or mint family containing over 900 species throughout the world [7]. Sage the dialect name of the genus *Salvia* is attributed to different species that are widely used in the food, drug and fragrance industry. The high diversity in secondary metabolites (essential oils and the phenolic derivatives) isolated from sage plants, possess excellent antimicrobial activity as well as antioxidant capacity and some are used as anticancer agents or have hypoglycemic effect [8-10].

Sage tea has been traditionally used for the treatment of digestive and circulation disturbances bronchitis, cough, asthma, angina, mouth and throat information, depression, excessive sweating skin diseases, and many other diseases. Salvia essential oils have been used in the treatment of a wide range of diseases like those of the

© 2019 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

nervous system, heart and blood circulation respiratory system digestive system, and metabolic and endocrine disease [11]. In addition, they possess a number of biological activities including antiseptic, antimicrobial [12, 13] antioxidant [14] astringent, anti-inflammatory [15-17] antiviral [18,19] antitumoral [20] cytotoxic [21, 22] spasmolitic, anticonvulsant [23]., antimycobacterial [24], and carminative activities [25]. Also *salvia officinals* has long history of medicinal, culinary and many different uses [26].

The present work aimed to study the antibacterial activity of essential oils from different parts (leave and stem) of *salvia offcinalis* against some Gram positive and Gram negative bacteria in Sudan.

2. Materials and Methods

2.1 Plant material

Salvia officinalis (Leave and stem) was collected in February 2018, at Khartoum State, Sudan, based on the available market samples brought from Syria. The identification of the plant material has been carried out at the Department of Botany, Faculty of Science and Technology, Omdurman Islamic University.

2.2 Extraction procedure

2.2.1 Extraction by maceration

The plant material has been air dried and ground to produce a fine powder, 50g of the plant material was macerated in 100 ml ethyl acetate (organic solvent) at room temperature, After 24 hours the solute was filtered using what man filter paper No.1. The procedure was repeated three times to ensure complete extraction of the plant material. The extracts were concentrated and the evaporator at 40 ^{o}C. The extracts were further dried by freeze-drying and kept in a refrigerator at 4 ^{o}C, until used.

2.2.2 Soxhlet Extraction

Sage (*Salvia offcinalis*) areal parts was packed in thimble the thimble was covered with cotton wool to prevent the packed material from floating out. The packed thimble was placed into Soxhlet glass that was connected ed to an extraction flask. The excess solvent was poured through the soxhlet glass. Then a condenser was attached and he extract was carried out continuously till the extract is exhausted from the crushed sage.

2.2.3 Hydro distillation extraction

1kg of crushed sage was put in a Clevenger distiller apparatus. Then the sample was covered by distilled water. The temperature was adjusted at 66 ^{o}C and the condenser was attached. The extraction was carried out for 3hrs. The mixture obtained was separated and the resulted oil was collected then it was treated with anhydrous sodium sulphate to eliminate all the water, and then stored in a refrigerator at approximately 4c until used.

2.3Antimicrobial susceptibility investigation

2.3.1 Preparation of the tested organisms

2.3.1.1 Preparation of standard bacterial suspension

Mueller Hinton agar powder 2.8 g was dissolved into1000 ml distilled water and allowed to soak for 10 minutes. Then each 20 ml of prepared solution was put in 5 bottles. These were sterilized by autoclaving at121 C/ 15minutes, after which they were cooled at room temperature. The bacteria were incubated at 37 C in broth media (Oxoid Ltd England) the essential oil and other extraction was dissolved in normal saline (N.S) in serial dilutions(1/1,1/2,1/4,1/8) and applied in different concentrations. N.S was used as negative control and the antibiotic Ciprofloxin was used as positive control. *Staphylococcus aureuse* and *Etherichia coli* reference isolates, which were kindly provided by the authorities of the Department of Bacteriology, were utilized throughout the antimicrobial susceptibility testing.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

2.3.1.2 Disk diffusion Method

The disk diffusion assay was used to determine the antibacterial activity of the essential oil and other extracts of sage according to Hindler (1995) [27]. Overnight bacterial cultures were spread or swabbed onto the surface if Mueller Hinton agar. Sage extracts were applied to 10 mm disks (What man filter paper No.1), then placed onto the inoculated dishes and after 24 hours of incubation at 37 °C, the antibacterial activity was assessed by measuring the diameter of growth inhibition zones.

3. Results

3.1 Antimicrobial results

The antimicrobial activity of essential oil and other different extract has leaves and stem of *S.officinalis* was screened against gram positive bacteria (*Staphylococcus aureus*) Gram negative (*E.coli*) using disc diffusion methods.

3.1.1 Esential Oil of leaves by hydro distillation extract

Essential oil of leaves exhibited high antibacterial activity against both against, Gram positive bacteria *staphylococcus aureus* and Gram negative (*E.coli*), gram positive bacteria *Staphylococcus aureus* was more sensitive than gram negative *E .coli* (figure1)

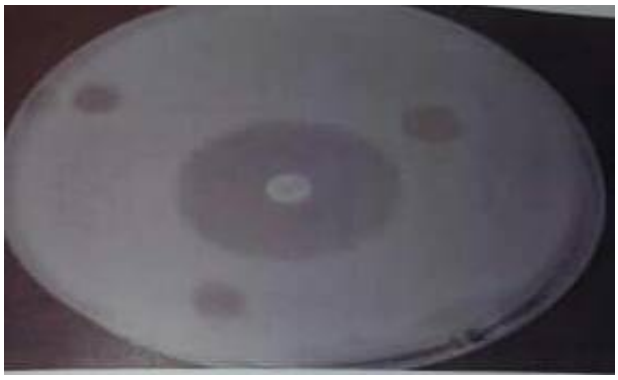

Figure1: Inhibition zone of essential oil (E.O) of leave against *Staphylococcus aureus*.

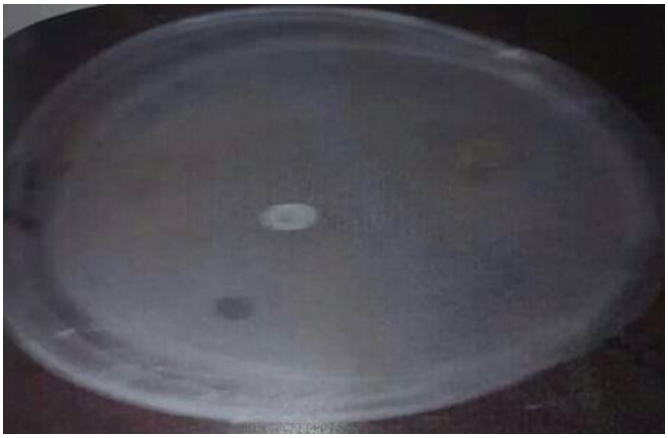

Figure 2: Inhibition zones of E.O leave against *E. coli*.

3.1.2 Esential Oil of steam by hydro distillation extract

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

Essential oil of steam has antimicrobial activity against gram positive bacteria, *staphylococcus aureus* but very low activity or no against *E.coli*. (Figure 4,5)

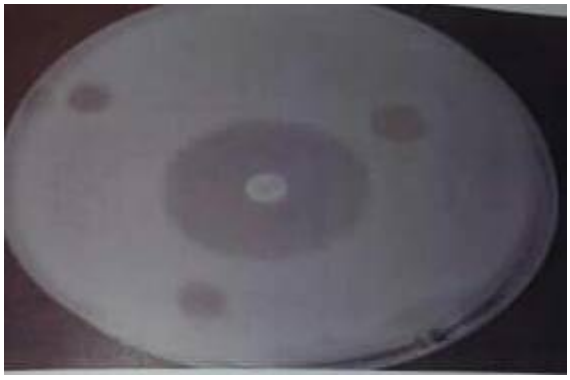

Figure3: Inhibition zones of E.O leave against *E.coli*.

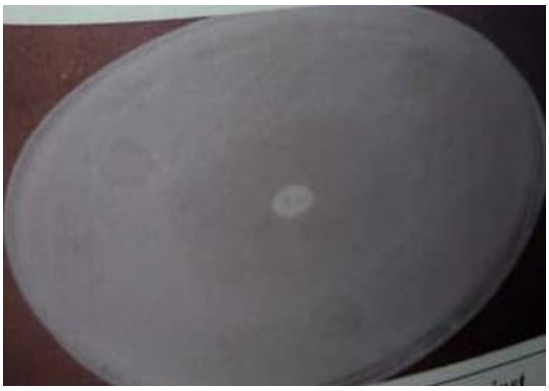

Figure4: Inhibition zones of E.O stems against *Staphylococcus aureuss*.

3.1.3 Ethyl acetate extract from leaves by maceration

Ethyl acetate showed high antibacterial activity against both test bacteria but gram positive *staphylococcus aureus* was more susceptible than the gram negative *E.coli*.(figure 6,7)

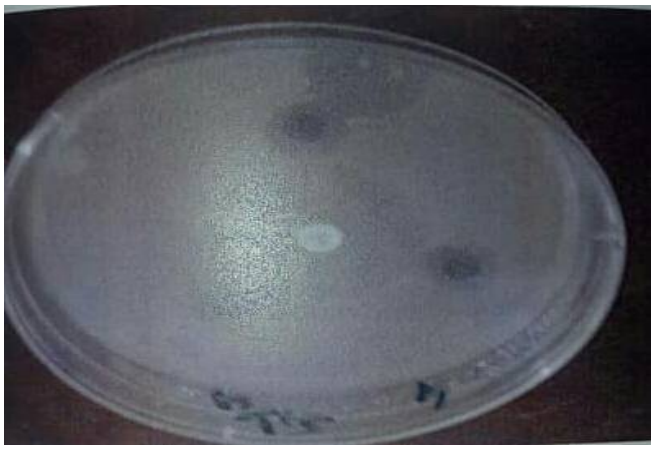

Figure 5: Inhibition zone of ethyl acetate extract of leave against *Staphylococcus aureuss*.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

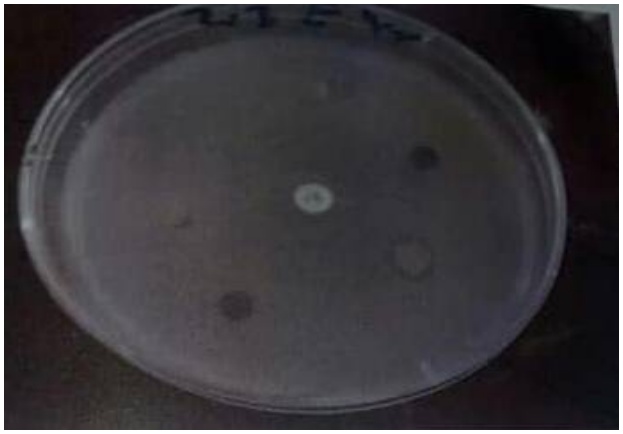

Figure6: Inhibition zone of ethyl acetate extract of leave against *E.coli*

3.1.3 Hexane produced from steams by soxhlet:

Hexane extract of steams showed high antibacterial activity against gram positive and gram negative bacteria (figure 8, 9, 10)

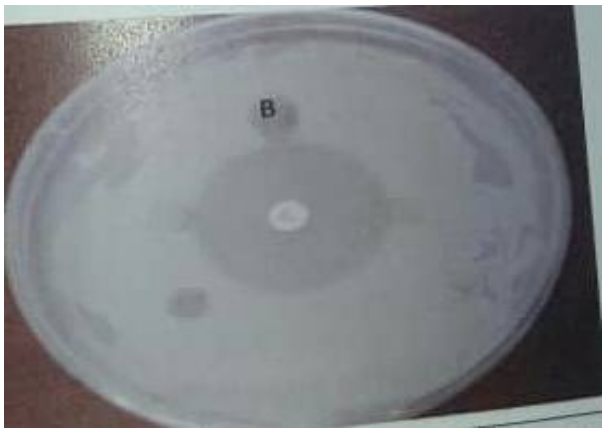

Figure7: Inhibition zone of hexane extract from stem against *E.coli*

Figure 8: Inhibition zones of hexane extract of stems against *Staphylococcus aureus*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

Figure 9: Inhibition zones of hexane extract of stems against *Staphylococcus aureus*

3.1.4 Hexane extraction obtained from leaves by Soxhlet:

It showed remarkable antimicrobial activity against *staphylococcus aureus* as well as in *E. coli* that showed much more sensitivity than that of the *S.aureus*. (figure 11,12,13)

Figure10: Inhibition zones of hexane extract of leaves against *E.coli*

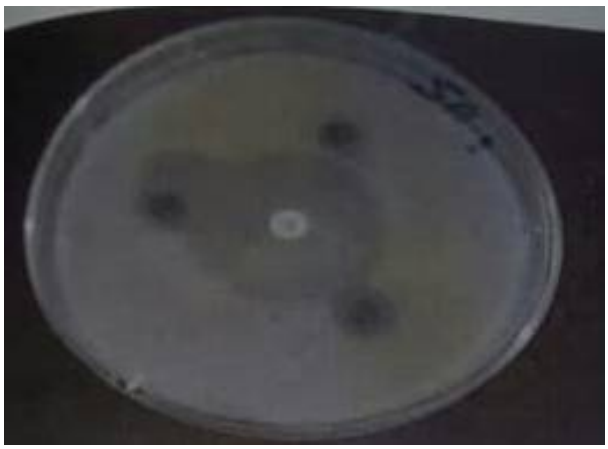

Figure11: Inhibition zones of hexane extract of leaves against *Staphylococcus aureus*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

Figure12: Inhibition zones of hexane extract of leaves against *Staphylococcus aureus*

4. Discussion

The study aimed to show the antibacterial activity of volatiles and crude extracts from leaves and stems of Sage. It has been found that, hexane extract obtained from the leaves by Soxhlet had higher antibacterial activity compared with stem extract by the same method. Among the leave extracts (essential oil and ethyl acetate) both possessed remarkable antibacterial activity. However, volatiles extract from leaves showed higher activity than the extract from stem.

Moreover, Gram positive bacteria, Staphylococcus aureus was more sensitive than Gram negative E.coli . Conforming results already reported by [28-30]. Several studies had demonstrated the antibacterial activity of essential oil of leaves and aqueous extract of sage against Gram +ve and Gram- ve bacteria, But there have no studies to test the activity of stems extract of sage. Also this work showed that, Gram n- ve bacteria E.coli was resistant to the essential oil obtained from stem.

5. Conclusion

The antimicrobial activity results obtained confirmed that the essential oil of leave and the crude extract produced from leaves and stem of *Salvia officinalis* possesses an antibacterial activity. Therefore, it is beneficial to human health. In contrast essential oil of the stem showed very low antibacterial activity. This antimicrobial activity was more obvious against Gram positive than Gram negative.

6. References

1-Kunwar, R.M. and N. Adhikari., 2005., Ethnomedicine of dolpa : The plants of their vernacular names and uses., Lyonia, PP 43-49.

2- Rahman M.S., M.M.H. Khan and M.A.H.M Jamal., A. Parvin and M.K.Alam., 2011 Antimicrobial activity of Argemone Mexicana L., .against waterborne Microbes., Res. J. Med. Plant, 5: PP 621-26.

3- Rajasekaran. S., Sivagananam K., Narayanan V., Subramaniam S., 2001 Publications of Indian Association of Biomedical Sciences, PP41-45.

4-Tomoko N., Takashi A., Hiromu T., Yuka I., Hiroko M., 2002 ‘Antibacterial activity of Extracts prepared from tropical and subtropical plants on methicillin – resistant Staphylococcus Aureus, J. Health Science, P 273-276.

5-Sakkas H, Papadopoulou C 2017. Antimicrobial activity of basil, oregano and thyme essential oil., Journal of Microbiology and Biotechnology 27(3):429-438.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

6-Piras A, Goncalves MJ, Alves J, Falconieri D, Porcedda S, Maxia A, Salgueiro L 2018. *Ocimum tenuiflorum* L. and *Ocimum basilicum* L., two spices of Lamiaceae family with bioactive essential oils. Industral Crops and Products 113:89-97.

7-Hamidpour, Hamidpour, S., Hamidpour,M., and Shahlari,M., 2013. Sage: the functional novel medicine for preventing and curing chronic illnesses. International journal of case reportec and images vol.4, pp671-677.

8-Kintzios, S.E., 2000. Sage: the genus *Salvia*, Harwood academic publisher, Australia, Canada.

9-Miladinovic, D., Miladinovic, Lj., 2000. Antimicrobial activity of essential oil of sage from Serbia. Facta Universitatis, Series: Physics, Chemistry and Technology 2 (2), 97-100.

10- Khalil, R. and Zheng-Guo, L., 2011. Antimicrobial activity of essential oil of *Salvia officinalis* L. collected in Syria. African Journal of Biotechnology 10 (42), 8397-8402.

11-Hamidpour, Hamidpour, R., Hamidpour, S., and Shahlari, M., 2014. Chemistry pharmacology, and Medicinal property of Sage (Salvia) to prevent and cure lllnesses such as obesity, Diabetes, Depression, Dementia, Lupus, Autism, Heart Disease, and cancer. J tradit complement med., Vol 4, pp 82-88.

12-Yang, Z., Kitano, Y., Chiba, K., Shibata, N., Kurokawa, H., and Doi, Y., 2001. "Synthesis of variously oxidized abietanediterpenes and their antibacterial activities against MRSA and VRE." Bioorg Med Chem., vol. 9, pp. 347-356.

13- Abd-Elmageed, M. A. M. and Hussein, B. A., 2008. "Cytotoxicity and antimicrobial activity of Salvia officinalis L. flowers." Sudan JMS., vol. 3, pp. 127-130.

14-Lima, C. F., Andrade, P. B., Seabra, R. M., Fernandes-Ferreira, M., and Pereira-Wilson, C., 2005. "The drinking of a Salvia officinalis infusion improves liver antioxidant status in mice and rats." J Ethnopharmacol, vol. 97, pp. 383–389.

15-Baricevic, D., Sosa, S., Della, L. R., Tubaro, A., Simonovska, B., Krasna, A., and Zupancic, A., 2001. "Topical antiinflammatory activity of Salvia officinalis L. leaves: The relevance of ursolic acid." J Ethnopharmacol, vol. 75, pp. 125-132.

16-Akkol, E. K., Goger, F., Kosar, M., and Baser, K. H. C., 2008. "Phenolic composition and biological activities of Salvia halophila and Salvia virgata from Turkey." Food Chem, vol. 108, pp. 942-949.

17-Çadirci, E., Süleyman, H., Gürbüz, P., Kuruüzüm, U. A., Güvenalp, Z., and Demirezer, L. Ö., 2012. "Anti-inflammatory effects of different extracts from three Salvia species." Turk. J. Biol, vol. 36, pp. 59-64.

18-Tada, M., Okuna, K., Chiba, K., Ohnishia, E., and Yoshiia, T., 1994. "Antiviral diterpenes from Salvia officinalis." Phytochemistry, vol. 35, pp. 539-541.

19-Smidling, D., Mitic-Culafic, D., Vukovic-Gacic, B., Simic, D., and Knezevic-Vukcevic, J., 2008. "Evaluation of antiviral activity of fractionated extracts of Sage Salvia officinalis L (Lamiaceae)." Arch BiolSci Belgrade, vol. 60, pp. 421–429.

20-Fiore, G., Nencini, C., Cavallo, F., Capasso, A., Bader, A., Giorgi, G., and Micheli, L., 2006. "In vitro antiproliferative effect of six Salvia species on human tumor cell lines." Phytother Res, vol. 20, pp. 701-703.

21-Ryu, S. Y., Lee, C. O., and Choi, S. U., 1997. "In vitro cytotoxicity of tanshinones from Salvia miltiorrhiza." Planta Med, vol. 63, pp. 339-342.

22-ZareShahneh, F., Valiyari, S., Baradaran, B., Abdolalizadeh, J., Bandehagh, A., Azadmehr, A., and Hajiaghaee, R., 2013. "Inhibitory and cytotoxic activities of salvia officinalis L. Extract on human lymphoma and leukemia cells by induction of apoptosis." Adv Pharm Bull, vol. 3, pp. 51-55.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 April 2019 doi:10.20944/preprints201904.0012.v1

23-Coelho, d. e., Souza, G. P., and Elisabetsky, E. I., 1998. "Ethnobotany and anticonvulsant properties of Lamiaceae from Rio Grande de Soul (Brasil). In: Harley R, Payton A, Harvey T, eds. Lamiales Newsletter Royal Botanic Gardens, Kew " p. 10.

24- Aşkun, T., Başer, K. H. C., Tümen, G., and Kürkçüoğlu, M., 2010. "Characterization of essential oils of some Salvia species and their antimycobacterial activities." Turk J Biol, vol. 34, pp. 89-95.

25-Lawrence, B. M., 2005. The Antimicrobial/Biological Activity of Essential Oils, Allured Publishing Corp. USA: Carol Stream, IL.

26-Mayekisco B, Magwa M L, Coopoosamy R M 2008. The chemical composition and antimicrobial activity of leaf extract of S. reports Burch Exbenth, journal of medicinal plants Research 2(7) pp159-162.

27-Hindler J (1995). Special antimicrobial susceptibility test : In text book of diagnostic Microbiology, ED Connie C Mahon, George Manuselis,89-96.

28-Ebd-Elmageed .M.A and Hussein. B.A (2008). Cytotoxicity and antibacterial activity of *Salvia officinalis* flowers. Sudan. J.M.S.(3):127-130

29-Bonsic T, Softic. D and Vasic. JK (2006).Antibacterial activity of some essential oil and major constituents of medicinal 35(1) 19-21

30-Culafic D. M,gacic B,Vukcevic JK, Stankovic.S and Simic S (2005).Comparative study on antibacterial activity of volatiles from Sage (*Salvia officinals*). Arch.bio. sci., Belegrade 57(3) 173-178.