Synthesis of Silver Nanoparticles from *Ocimum sanctum* (Tulsi) for Enhanced Healthcare System

[1]Prashant Sharma, [2]Harshita Joshi, [3]Ritu Singh, [4]Gayatri Devi, [5]Shakira Malik

[1,3]M.Pharm Student, [2]B.Pharm Student, [4,5]Assistant Professor
Department of Pharmacy
[1,3]Dr. K.N Modi Institute of Pharmacy and Research. Modinagar, Uttar Pradesh (India)
[2,4,5]Dr. Ram Manohar Lohia College of Pharmacy, Modinagar (Uttar Pradesh)
CORRESPONDING AUTHOR: PRASHANT SHARMA

Abstract*- Nano-sized drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines. The synthesis of nanoparticles is the most emerging method of synthesis. We report the synthesis of antibacterial silver nanoparticles by using aqueous leave extract of *Ocimum sanctum*. The silver nanoparticles formation was characterized by a technique UV-visible absorption spectroscopy in the range from 350 – 650 nm further confirmed by their change of colour to dark brown. The quantitative phytochemical screening showing of plant extract was carried out for detection of the bio-active marker like alkaloids, flavonoids, saponins, tannins and polyphenols. The antibacterial activity of synthesized AgNPs against dissimilar pathogenic bacteria was determined via well diffusion method. The silver nanoparticles were found to exhibit effective activity against pathogenic bacteria *E. coli, S. aureus.

***Keywords:* Nanoparticles, Antibacterial, AgNPs, E. coli, S. aureus.**

INTRODUCTION

Nanotechnology is an important field of modern research dealing with the development of nanomaterials and nanoparticles (NPs)[1] for their utilization in diverse Fields such as catalysis, electrochemistry, biomedicines, pharmaceuticals, sensors, food technology, cosmetics, biomedical, food and feed, drug-gene delivery, environment, health, mechanics, optics, chemical industries, electronics, space industries, energy science, light emitters, single electron transistors, nonlinear optical devices and photo-electrochemical applications. etc.[2] Nanoparticles (NPs) are nanometer-sized (<100 nm) atomic or molecular scale solid particles having some excellent physical properties compared to the bulk molecules depending on their size and morphology. This size range all the properties (chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk.[3]

Among all types of NPs, metal and metal oxide nanoparticles have been thoroughly examined using science and technology due to their excellent properties such as high surface to volume ratio, high dispersion in solution, *etc*.[4]

Biological sample -

Ocimum sanctum Linn known as Holy Basil is an aromatic plant native to the tropics of Asia and Africa being medicinally important plant in the family Lamiaceae.[5] The family Lamiaceae is one of the most exploited medicinal plant family worldwide not only as a source of medicinal plants but also with its valuable essential oils being used as spices and flavours for various food products.[6] Morphologically O. sanctum is an erect about 75 cm tall, much branched with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed.[7]

Scientific classification
Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Lamiales
Family : Lamiaceae
Genus : Ocimum
Species : O.Tenuiflorum
Binomial name : *Ocimum sanctum*.[8]


Fig:1-*Ocimum sanctum*

Chemical composition

The chemical composition of Tulsi is highly complex, containing many nutrients and other biological active compounds. Fresh leaves and stem of Ocimum sanctum International Journal of Academic Research and Development extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugeno.[9]

The leaves of *Ocimum sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene. Two flavonoids orientin and and vicenin from aqueous leaf extract of *Ocimum sanctum* have been isolated.[10]

MEDICINAL USES-

In the Ayurveda, the Charaka Samhita, the tusli is use in the treatment of the headaches, rhinitis, stomach disorders,inflammation, heart diseases, various forms of poisoning and malaria. Each part of this plant has its own important in the world of pharmacy and it is proved.[11] The aqueous extract from the leaves have various pharmacological activities are as follows.

- Antimicrobial activity
- Anti-inflammatory activity
- Antidiabetic activity
- Anticancer activity
- Antiulcer activity
- Antifungal/Anticandidal Activity
- Thyroid activity[12]

Experimental Method

Plant collection and identification

Ocimum Sanctum, is an aromatic much branched erect herb with 4 angled stems, bearded nodes and lanceolate or ovate lanceolate leaves. It is a common weed of open waste lands.[13] For the present study fresh plants were collected from GREEN CARE NURSERY MURADNAGAR.

Preparation of leaf extract

For the preparation of leaf extract, fresh leaves were collected in a beaker and washed several times with water to remove the dust and finally with double distilled water. 10 g washed leaves were cut into fine pieces and crushed with the help of mortar and pestle in 100 ml double distilled water. After grinding the aqueous extract was taken in 250 ml beaker and boiled for 10 min at 80 °C temperature.[14] The plant extract was allowed to cool at room temperature and then filtered with whatman filter paper. The filtrate was centrifuged for 20–25 min at 10000 rpm, the supernatant was collected and stored at 4 °C. This filtrate was used as a stabilizing and reducing agents.[15]

Preparation of 1 mM silver nitrate ($AgNO_3$) solution

The concentration of 1 mM Silver Nitrate was prepared by dissolving 0.169 g $AgNO_3$ in 1 L De-Ionised water and stored in amber coloured bottle to prevent the self-oxidation of silver nitrate solution[16].

Green synthesis of silver nanoparticles

The preparation of silver nanoparticles (AgNPs) is a single step synthesis 10 ml of tulsi leaf extract, prepared was added to 90 ml silver nitrate solution and the mixture was heated up at 80 °C for 15 min . The colour of the solution turned from light yellow to brown indicating the formation of silver nanoparticles (AgNPs). The Ag NPs synthesized were separated by the process of centrifugation from the reaction mixture[17].

Purification of silver nanoparticles

In order to purify the nanoparticles prepared as described in section above, The synthesized AgNPs were kept in 12 N hydrochloric acid solution for 24 h. The Ag NPs were isolated by the process of centrifugation from the mixture. The mixture was washed with distilled water till hydrochloric acid was completely removed.[18]

Qualitative Phytochemical Analysis of Tulsi (*Ocimum sanctum*) leaves extract-

Phytochemical evaluation Phytochemical analysis of methanol and aqueous extracts of O. sanctum, A. indica and P. emblica demonstrated the presence of phytoconstituents like tannin, saponins, flavonoids, glycosides, reducing sugar, steroids and alkaloids. The medicinal value of the plant lies in bioactive phytochemical action on the human body. Some of the most important bioactive phytochemical constituents were alkaloids, flavonoids, tannins, saponins etc compounds reported earlier.[19] Antibacterial properties of several plant extracts have been attributed to some of these secondary metabolites. The phytochemical screening of aqueous leaf extract of O. sanctum, revealed the presence and absence of alkaloids, steroids, and tannin compound.[20]

Test for Tannins

1ml of sample was added with 20µl of 0.1% ferric chloride and the appearance of brownish green or black colour indicates the presence of tannins.

Test for Saponins

1ml of sample was added with 2ml of distilled water, shaken vigorously and observed for foam appearance which indicates the presence of saponins.

Test of Flavonoids

1ml of sample was added to 200 µl of concentrated hydrochloric acid and 2 pellets of magnesium chloride, change of colour from pink to red was observed.

Test for Alkaloids

1 ml of sample was added to 20µl of Dragendroff's reagent (gram's iodine). Formation of orange colour indicates the presence of alkaloids.[21]

Test for Proteins

1ml of sample was added to 100µl of Bradford reagent. Appearance of blue colour indicates the presence of protein.

Test for Steroids

1ml of sample was added to 200µl of 10% concentrated sulphuric acid and the appearance of green colour indicates the presence of steroids.

Test for Quinones

1ml of sample was added to 200 µl of aqueous sodium hydroxide. Appearance of yellow colour in aqueous layer indicates the presence of quinones.

Test for Terpenoids

1ml of sample was added to 400 µl of chloroform and 200 µl of concentrated sulphuric acid. The development of reddish brown colour indicates the presence of terpenoids.

Test for Cardio Glycosides

To 1ml of sample, 0.4ml of glacial acetic acid, 200µl of ferric chloride and 200 µl of concentrated sulphuric acid were added. The appearance of brown ring indicates the presence of cardio glycosides.[22]

Characterization of silver nanoparticles AgNPs

1- By observing color change: The plants extract added to silver nitrate solution, the color of the sample solution changed from pale yellow to reddish-brown after 5hr because of the process of reduction of Ag^+ to Ag^O nanoparticles colour change were possible because of ag ions is reduced due to the effects of heat and produces Ag+ complex. This complex was responsible for changing color from pale yellow to reddish brown. This colour change indicate the formation of Ag nanoparticles[23].

Antibacterial Activity Analysis

Antibacterial activity of synthesized AgNPs was observed against two bacteria viz.; Staphylococcus aureus and Escherichia coli bacteria. For this purpose, a disc diffusion method was used. This method was used to identify two important things, namely the sensitivity of bacterial strains and zone of inhibition towards the antibiotics. All the data of the present study are given in Table 1, which showed that the developed AgNPs exposed good inhibition against both of the bacterial strains (Staphylococcus aureus and Escherichia coli).

Table 1: Antibacterial activity of synthesized AgNPs using *Ocimum Sanctum* leaf extract

Name of
Micro-
organism
Zone of Inhibition in mm
Doxycycline
(10 µg/disc)
Concentration
of Silver
Nanoparticles
(µL)
Silver
Nano-
particles
Staphylococcus
Aureus
20 15 1.8
20 30 2.2
20 45 2.5
Escherichia
Coli
20 15 1.5
20 30 1.9
20 45 2.0

Conclusions

The plant-mediated nanoparticles can be produced at large scale, and they can have compatibility for medical and pharmaceutical applications. From the study, it can be concluded that the plant-mediated synthesis of nanoparticles is a simple, nontoxic, ef□cient, and ecofriendly method. In the present investigation leaves extract of *Ocimum sanctum* and a known concentration of silver nitrate solution was used to synthesize the AgNPs. Different types of Microscopic and Spectroscopic techniques were used to characterize the developed nanoparticles. The antimicrobial activity of the developed AgNPs was studied against Staphylococcus aureus and Escherichia coli bacteria, and it was found that the synthesized nanoparticles show good stability and good antimicrobial activity against both of the bacterial strain

RESULTS

1. PREPARATION OF PLANT EXTRACT

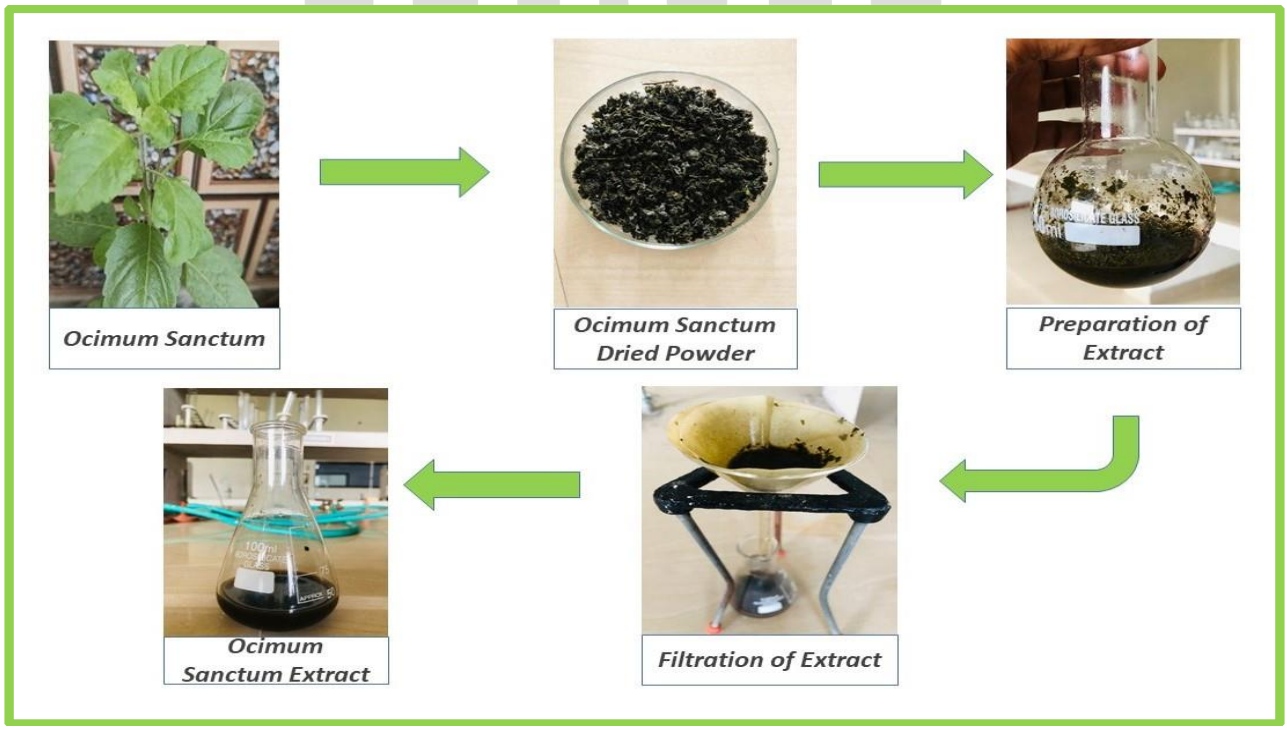

Fig:2-Preparation of Plant Extract (*Ocimum Sanctum*)

2. SYNTHESIS OF SILVER NANOPARTICLE

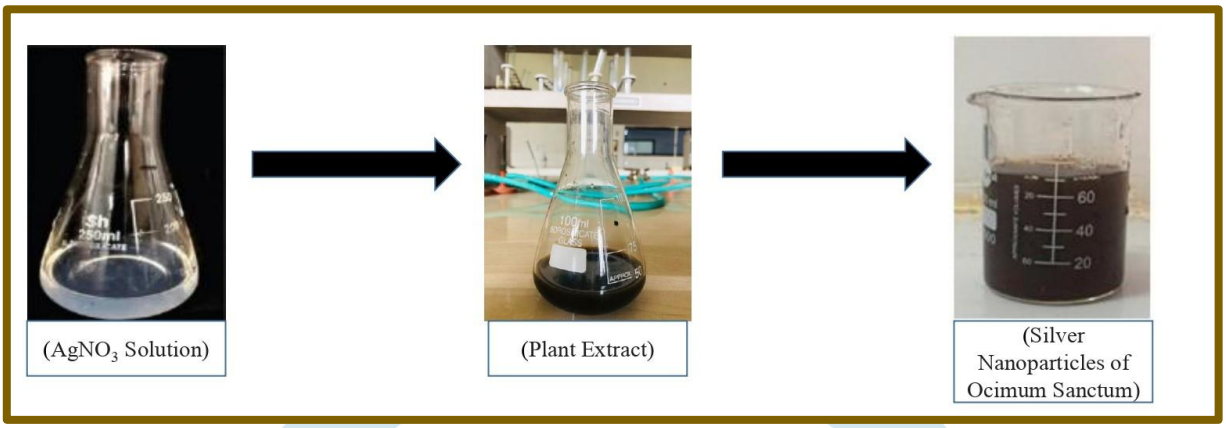

Fig:3-Synthesis of Silver Nanoparticle of *Ocimum Sanctum* Plant Extract

3. Phytochemical Screening of Synthesized Silver Nanoparticle *(Ocimum Sanctum)*

Table.1 Qualitative determination of phytochemical groups of aqueous leaf extract of O. sanctum Active chemical constituents in plant Extract.

S. No	Phytochemical	Present (+)	Absent (-)
1.	Alkaloids	+	
2.	Flavanoids	+	
3.	Glycosides		-
4.	Tannin	+	
5.	Saponin	+	
6.	Oil		-
7.	Carbohydrate		-
8.	Steroids		-
9.	Test for Protein		-

4. Antibacterial Activity

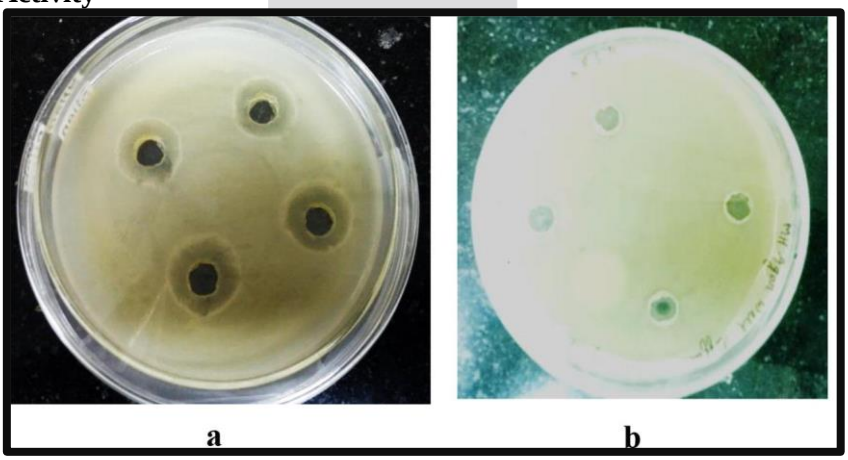

Fig:4- Antibacterial Activity of Synthesized Ag Nanoparticles of *Ocimum sanctum* Plant Extract Against Staphylococcus aureus(a) And E. coli(b).

Table:2- Zone of Inhibition Diameter of Test Sample against Staphylococcus aureus and E. coli

S.No	Name	Zone of Inhibition against S. aureus	Zone of Inhibition against E.coli
1.	Plant Extract (*O. Sanctum*) Sample	18mm	11mm
2.	$AgNo_3$ Solution	30mm	28mm
3.	O. Sanctum NP_s	32mm	27mm
4.	Ciprofloxacin (Standard Positive Control)	40mm	32mm

Conclusions

The plant-mediated nanoparticles can be produced at large scale, and they can have compatibility for medical and pharmaceutical applications. Leaves of *Ocimum Sanctum* Plant are rich in Alkaloids, Tannins, Saponins and flavonoids which are popular Phytochemical constituents. From the study, it can be concluded that the plant-mediated synthesis of nanoparticles is a simple, nontoxic, efficient, and ecofriendly method. In the present investigation leaves extract of *Ocimum sanctum* and a known concentration of silver nitrate solution was used to synthesize the AgNPs. Different types of Microscopic and Spectroscopic techniques were used to characterize the developed nanoparticles. The antimicrobial activity of the developed AgNPs was studied against Staphylococcus aureus and Escherichia coli bacteria, and it was found that the synthesized nanoparticles show good stability and good antimicrobial activity against both of the bacterial strain.

REFERENCES:

1. Ambedkar A.K Gautam D, *Ocimum Sanctum* leaf Extract-Assisted Green Synthesis og Pd-Doped CuO Nanoparticle for highly sensitive and Selective NO_2 Gas Sensors, ACS Publication, 2023;8(32).29663-29673
2. Tailor PG, Yadav BL *et.al*, Green synthesis of silver nanoparticles using *Ocimum sanctum* and their anti-bacterial activity Biochemistry and Biophysics Reports 2020 Vol;24, 100848
3. Saranya T, Noorjahan C.M. *et.al* PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF TULSI Plant . Int. Res. J. Pharm. 2019, 10 (6), 2230 – 8407.
4. Desai D, Pillai T.G *et.al* Nanoparticle from Tulsi (*Ocimum Sanctum*) in Immune Enhancement against Covid 19 Situation Determination in Nanomedicine and Nanotechnology 2021 Vol-2(3) 2832-4439
5. Cohen MM *et.al* Tulsi- Ocimum Sanctum ; a herb for all Reasons J-Ayurveda Integr Med. 20145(4) 251-259.
6. Sethi J, Sood S, *et.al*. Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum*. *Indian J Clin Biochem.* 2004;19:152–5.
7. Cohen M.M, Study of Tulsi – *Ocimum sanctum*: A herb for all reasons. Journal of Ayurveda and Integrative Medicine 2014; 5(4): 251–259. 0975-9476
8. Gupta SK, Prakash J, *et.al* Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. *Indian J Exp Biol.* 2002;40:765–73.
9. Verma Sunita, Study of Chemical constituents and pharmacological action of *Ocimum sanctum* (Indian holy basil-Tulsi. The Journal of Phytopharmacology 2016; 5(5): 205-207.
10. Tanwar, R., Pahare, A. *et.al*, *Ocimum sanctum* (tulsi), excellent source of phytochemicals. Indo American Journal of Pharmaceutical Research, 2015, 12(5): 2231-6876.
11. Singh D, Chaudhuri P, A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). Industrial Crops and Products 2018 Vol-118, Pages 367-382.
12. Gulhane N.S, Ghode CD *et.al*, Study of Medicinal uses of *Ocimum Sanctum* (Tulsi) Journal of Pharmacognosy and Phytochemistry,2021;10 (2) 1427-1431

13. Ahmed S, Ahmad M *et.al*, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise J Adv Res. 2016;7(1): 17–28
14. Verma OP, Singh P *et.al* Phytochemical Screening of *Ocimum sanctum* (Tulsi), Azadirachta indica (Neem) and Phyllanthus emblica (Amla) International Journal of Current Microbiology and Applied Sciences 2019 Vol;8 (04), 2319-7706.
15. Joshi Manoj , Yadav B.L, *et.al* Green synthesis of silver nanoparticles using *Ocimum sanctum* and their anti-bacterial activity. Biochemistry and Biophysics Reports 2020, 24: 100848.
16. Rao Y.S, Kotakadi V.S, *et.al* Green synthesis and spectral characterization of silver nanoparticles from Lakshmi. Journal of Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy Vol 103, 2013,156-159.
17. Rout Yogeswari, Behera Sikha, *et.al*. Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) and study of their antibacterial and antifungal activities" *J. Microbial Antimicrobe.* 2012;4(6):103–109.
18. L. Harsha, Kumar R *et.al*.Synthesis ,Characterization and Antimicrobial Activity of Silver Nanoparticle derived from Methax Piperita X, Ocimum Teniflorum. An in-vitro Study, J Adv. PharmTechnol Res,2022 13;5272-5276.
19. Goyal S, A Study on Synthesis of Silver Nanoparticles Using *Ocimum Sanctum* L (Tulsi) Leaf Extract & their Antimicrobial Activity: A Green Chemistry Approach 2020 vol;8, 2582-0397.
20. Saranya T, Noorjahan C.M. *et.al* PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF TULSI Plant . Int. Res. J. Pharm. 2019, 10 (6), 2230 – 8407.
21. Rajesh, H., Rao, S.N., *et.al*, Phytochemical analysis of aqueous extract of *Ocimum sanctum* Linn ,International journal of universal pharmacy and bio sciences,2013 2(2):462-468.
22. Verma OP, Singh P *et.al* Phytochemical Screening of *Ocimum sanctum* (Tulsi), Azadirachta indica (Neem) and Phyllanthus emblica (Amla) International Journal of Current Microbiology and Applied Sciences 2019 Vol;8 (04), 2319-7706.
23. A. Monica Senthilkumar. S.R *et.al* Study of Green synthesis and characterization of Silver nanoparticles from Ocimum basilicum and their Antimicrobial Antioxidant and Anticancer activity. Research Journal of Pharmacy and Technology 2020;0947360X.
24. Subramanian, G., Tiwari, B.B., *et.al*, Studies of Antimicrobial Properties of Different Leaf Extract of Tulsi (Ocimum tenuiflorum) against Human Pathogens. American International Journal of Contemporary Research, 2014 4(8): 149-157.
25. Lopez-Romero J. C., González-Ríos H., *et.al* Antibacterial Effects and Mode of Action of Selected Essential Oils Components against *Escherichia coli* and Staphylococcus aureus. 2015 *Evid. Based Compl. Alternat. Med.* 2015:e795435. 10.1155/2015/795435.