

Research Article



The *In-vitro* Antibacterial Effect of the Methanolic Extract of *Boswellia serrata* in Combination with Dextrin and Glycerin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

¹Abdulkareem H Abd, ²Bahaa F Hussein, ³Hayder B Sahib*, ⁴Abbas M Khalil

¹Al-Nahrain University, College of Medicine, Pharmacology Department, Iraq.

²Baghdad University, Veterinary Medicine, Department of Anatomy and Histology, Iraq.

³Al-Nahrain University, College of Pharmacy, Pharmacology Department, Iraq.

*Corresponding author's E-mail: haider_bahaa@yahoo.com

Accepted on: 28-07-2016; Finalized on: 30-09-2016.

ABSTRACT

The objective of the study is to evaluate the anti-microbial effect of the methanolic extract of *Boswellia serrata*-dextrin-glycerin combination on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The dried powder of the oleo gum resin was extracted by cold method (maceration) using methanol as solvent and then the dried extract was mixed with dextrin and glycerin. Antimicrobial susceptibility testing for different concentration of methanol extract of *Boswellia serrata*-dextrin-glycerin combination was done by well diffusion method against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and then minimum inhibitory concentrations were calculated using agar dilution method. Both of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were sensitive to the methanolic extract of *Boswellia serrata*-dextrin-glycerin combination and the susceptibility of bacteria was proportional to the concentration of the compound used. Methanolic extract of *Boswellia serrata*-dextrin-glycerin combination had a concentration dependent antimicrobial activity against both of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Keywords: *Boswellia serrata*, methanol extract, dextrin, antimicrobial activity, combination.

INTRODUCTION

One of the most common problems worldwide is burns which are the leading cause of ugly skin scarring and massive handicapping. The effect of burns extends to the entire body besides the skin¹. The skin functions as a protection to the internal body organs from any hostile external environment of different pollution, temperature, humidity and radiation. Also, skin has important function in preserving water and heat regulation². A burn is a kind of skin injury that is caused by heat, electricity, chemicals, light radiation, extreme cold or friction³. The world health organization reports that nearly more than 90% of burn injuries occur in the developing countries or underdeveloped ones where the death from large burns (more than 40% of total body surface area) reach 100%⁴.

Gram-positive bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis* are the clinically significant pathogens and the antibiotic resistance in these pathogens has become one of the main worldwide health problems⁵. Biofilms are communities of surface-associated micro-organisms which is embedded in a self-produced extracellular polymeric matrix that are difficult to eradicate and are the source of many resistant infections⁶⁻¹⁰. Staphylococci are well known to form biofilms on an implanted medical device or damaged/dead tissues and these biofilms are difficult to disrupt¹¹.

Frankincense, the gum resin of the *Boswellia serrata* tree, was well known to ancient civilizations and is still used for ritual purposes in the Catholic Church and traditional

ceremonies in Northern Africa¹². High performance liquid chromatography analysis of Indian and African samples of *B. serrata* gum resin give rise to 12 different pentacyclic triterpenic acids the most important is alpha boswellic acid and acetyl boswellic acid.

This method provides differentiation and standardization of the resin of different origin and gum resin phytopharmaceuticals¹³.

Dextrin is mainly produced by an enzyme called amylase in human that is usually present in saliva mixes with the food in the mouth, and then acts on the starch in a slightly alkaline medium to convert it to dextrin¹⁴.

Glycerin (CAS No. 56-81-5) is a polyhydric alcohol which its molecular formula is C₃H₈O₃.

Glycerin (also referred to as glycerol) is a simple polyol compound that has three hydroxyl (OH) groups¹⁵.

The objective of the present study is to evaluate the antimicrobial activity of the methanolic extract of *Boswellia serrata*-dextrin-glycerin combination on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Extraction Process

Dried oleo gum resin of *Boswellia serrata* was purchased from the local market the gum was crushed into a fine powder with an electric grinder in the department of pharmacology in the college of medicine, Al Nahrain university.

The extraction process was first done on the Oleo gum resin using methanol by maceration (cold method), then



the methanolic extract was dried using rotary evaporator to get rid of the solvent, then the dried extract mixed with dextrin and glycerin with continuous heating and stirring using magnetic stirrer and electric heater, the final combination was left to cool down¹⁶⁻¹⁷.

Mueller Hinton media was prepared according to the manufacturer company.

Well Diffusion Method

Antimicrobial susceptibility testing was done by well diffusion method to detect anti-bacterial effect of the product (ME of *B. serrata*, dextrin and glycerin).

The bacteria were immersed into the culture broth and compressed around the tubes side walls to remove excess inoculum and apply it evenly on the Muller Hinton Agar plates.

The well diffusion method was done according to Perez¹⁸. Four wells were made then 15 µl of the (ME of *B. serrata*, dextrin and glycerin) combination that diluted with

normal saline was added to three well in each plate in different concentrations (100mg/ml(3), 50mg/ml(2), 25mg/ml(1)) respectively as shown in Figure 1, and the last well was the control filled with 15 µl normal saline.

Agar Dilution Method

Agar dilution is a quantitative susceptibility testing method because MIC values can be obtained using the method. For agar dilution, two-fold serial dilutions of the compound were made in molten tempered (45°C) Mueller-Hinton agar (MHA) medium (pH 5.9) to obtain the desired final concentrations of 1%, 0.5%, 0.25%, 0.125% and 0.06% by mixing the agar and the compound solutions thoroughly¹⁹.

RESULTS

Antimicrobial Susceptibility Test (Well Diffusion Method)

The results of the antimicrobial test by the well diffusion method is described in Table 1.

Table 1: Antimicrobial Susceptibility Test of ME of *Boswellia serrata*

Tested Compound	Concentration of Tested Compound mg/ml	Mean Inhibition Zone of <i>P. aeruginosa</i> (cm)	Mean inhibition zone of <i>S. aureus</i> (cm)
BDG	1000	2.2	2.7
BDG	500	2.0	2.2
BDG	250	1.8	2.0
C: N/S	0.9 %	-	-

BDG= Methanolic extract of *Boswellia serrata*-dextrin-glycerin combination, N/S= normal saline

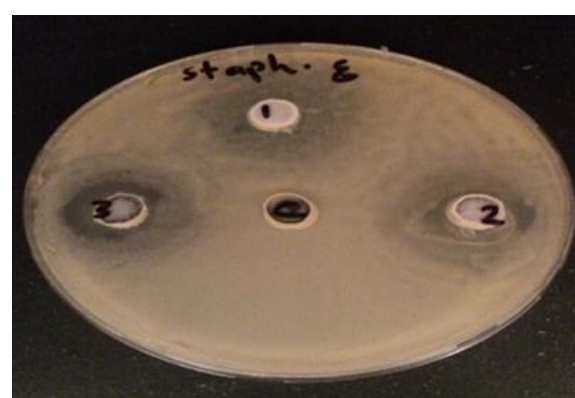
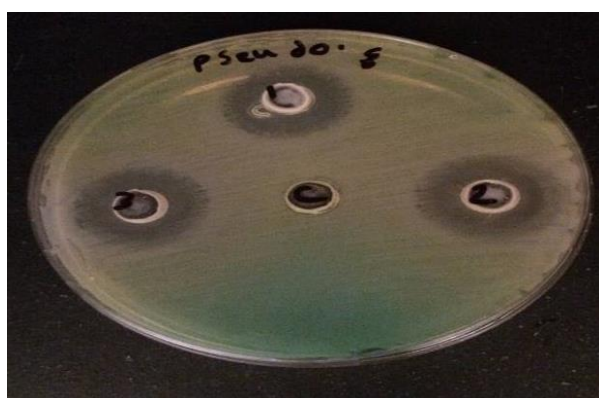


Figure 1: Well Diffusion Method for *S. aureus* and *P. aeruginosa*

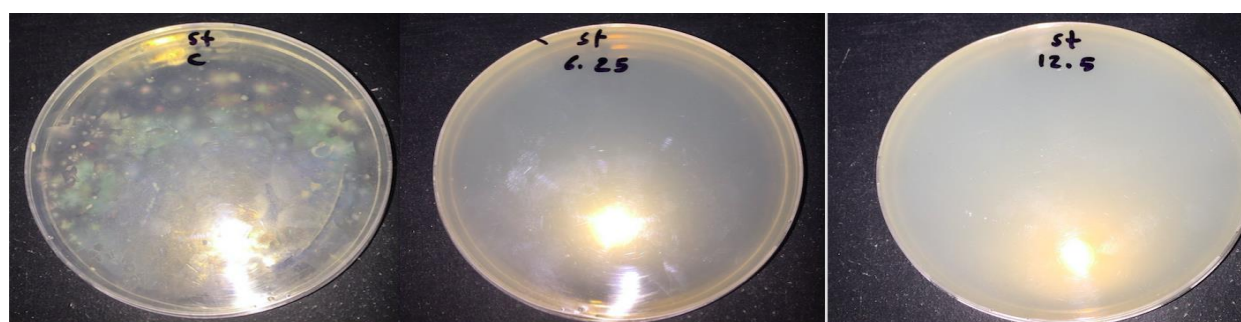


Figure 2: minimum inhibitory concentration for *S. aureus* where c= control, 6.25 mg/ml showed minor growth, 12.5 mg/ml showed complete inhibition

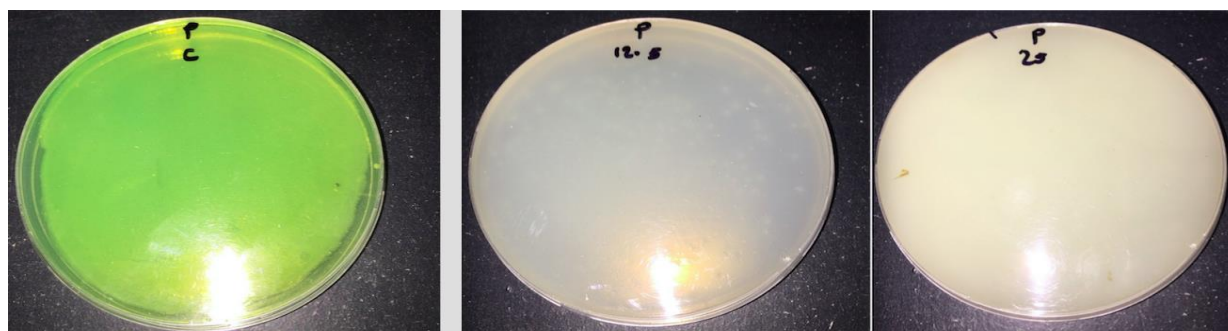


Figure 3: minimum inhibitory concentration for *P. aeruginosa* where c= control, 12.5 mg/ml showed minor growth, 25 mg/ml showed complete inhibition

Table 2: Minimum Inhibitory Concentration of ME of *Boswellia serrata*

Bacteria	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	control
<i>S. aureus</i>	++	NG	NG	NG	NG	+++
<i>P. aeruginosa</i>	++	+	NG	NG	NG	+++

+++ = high bacterial growth, ++ = moderate bacterial growth, + = mild bacterial growth, NG = no bacterial growth

Minimum Inhibitory Concentration (MIC)

For *S. aureus*, 6.25 mg/ml concentration showed moderate inhibition of growth, while 12.5 mg/ml showed complete inhibition Figure 2.

For *P. aeruginosa*, 12.5 mg/ml showed high inhibition of bacterial growth, while 25 mg/ml showed complete inhibition Figure 3.

And Table 2 summarize the results of the agar dilution method.

DISCUSSION

The gum exudate obtained from the bark of *Boswellia serrata* has been widely used by the practitioners of medicine for various medical conditions such as arthritis, asthma, ulcers, and skin diseases²⁰. For the purpose of screening, antimicrobial test is required to identify the sensitivity of specific bacteria to the plant extract. Accordingly, disk diffusion method is useful to do so. The test is fast and simple. However, it's characterized by low accuracy as zone of inhibition widely affected by medium composition and interference of some ions with spread of antibiotics throughout the medium²¹. For research purposes, MIC is a useful measure tool to specify minimum concentration of antibiotic showing inhibition of growth, such concentration might be a helpful parameter used to estimate antibacterial activity of antibiotic and medicinal agents²².

Accordingly, for testing medicinal agent and antibiotic sensitivity, the two methods were used wherever it is needed. The present study showed that both of *P. aeruginosa* and *S. aureus* strains were sensitive to the ME of *Boswellia serrata*, dextrin and glycerin combination with the three different concentrations. This result agrees with Raja who stated that AKBA (acetyl keto boswellic acid) has significant antibacterial compound against

Gram-positive pathogens, he showed that boswellic acid exerted bacteriostatic antibacterial activity against *S. aureus* and exhibited a significant post antibacterial efficacy (PAE).

The proposed mechanism of action is inhibition of formation of biofilms generated by *S. aureus* as well as reducing the performance of bacterial biofilms. Increasing the uptake of propidium iodide and leakage of 260 and 280 nm absorbing material by the treated cells of *S. aureus* indicating that the antibacterial mode of action of the combination probably occurred via disruption of microbial membrane structure²⁰.

However, boswellic acids had no significant antimicrobial action against gram negative bacteria (*p. aeruginosa*) where this study didn't agree with it since the combination showed significant activity against the mentioned bacteria and this may be attributed the change of the physical properties of the gum extract of *Boswellia serrata* when mixed with glycerin and dextrin. On the other hand, Abdaallah²³ was recorded that extracts of two species belonging to the genus *Boswellia* had antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus flavus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida maltosa*.

CONCLUSION

Methanolic extract of *Boswellia serrata*-dextrin-glycerin combination had a concentration dependent antimicrobial activity against both of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Recommendation

Further studies are needed to investigate the effect of different concentrations of methanolic extract of *Boswellia serrata*-dextrin-glycerin combination on the parameters used in current study to determine the most

effective concentration that confirm the potent burn healing effect on infected burns with *S.aureus* and *P. aeruginosa*.

REFERENCES

1. Abdel Hamid AAM and Soliman MFM. Effect of topical aloe vera on the process of healing of full-thickness skin burn: a histological and immunohistochemical study. *J Histol Histopathol.* 2, 2015, 3.
2. Gawkrödger. *Illustrated color Text of Dermatology*, 3rd ed. Churchill Livingstone, 2002, 2-10.
3. Herndon D. *Total Burn Care*, 3rd Edition, Saunders. 2007, 880.
4. Potokar TS, Ali S, Chamania S, Prowse S and Whitaker IS. A global overview of burns research highlights the need for forming networks with the developing world. *Burns*, 34, 2008, 3-5.
5. Millar BC, Prendergast BD, Moore JE: Community-associated MRSA (CA- MRSA): an emerging pathogen in infective endocarditis. *J Antimicrob Chemother*, 61, 2008, 1-7.
6. Brown MR, Allison DG, Gilbert P: Resistance of bacterial biofilms to antibiotics: a growth-rate related effect? *J Antimicrob Chemother*, 22, 1998, 777-780.
7. Chambers HF: The changing epidemiology of *Staphylococcus aureus*. *Emerg. Infect Dis*, 17, 2001, 178-182.
8. Stewart PS: Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbio*, 292, 2002, 107-113.
9. Shirtliff ME, Mader JT, Camper AK: Molecular interactions in biofilms. *Chem Biol*, 9, 2002, 859-865.
10. Adam B, Baillie GS, Douglas LJ: Mixed species biofilms of *Candida albicans* and *Staphylococcus epidermidis*. *J Med Microbiol*, 51, 2002, 344-349.
11. Wu JA, Kusuma C, Mond JJ, Kokai-Kun JF: Lysostaphin Disrupts *Staphylococcus aureus* and *Staphylococcus epidermidis* Biofilms on Artificial Surfaces. *Antimicrob Agents Chemother*, 47, 2003, 3407-3414.
12. PoECKel D and O Werz. Boswellic acids: biological actions and molecular targets. *Curr Med Chem*, 13, 2006, 3359.
13. A Sharma, S Chhikara, S N Ghodekar, S Bhatia, M D Kharya, V Gajbhiye, A S Mann, AG Namdeo and K R Mahadik. Phytochemical and Pharmacological investigations on *Boswellia serrata*. *Phcog Rev*, 3(5), 2009, 206-215.
14. Monsivais P, Carter BE, Christiansen M, Perrigue MM and Drewnowski A. (2010). Soluble fiber 527 dextrin enhances the satiating power of beverages. In: *Appetite*. Elsevier Ltd.
15. Soap and Detergent Association, 1990.
16. Patel U, Kulkarni M, Undale V and Bhosale A. Evaluation of Diuretic Activity of Aqueous and Methanol extracts of *Lepidium sativum* Garden Cress (Cruciferae) in Rats. *Trop. J. Pharm. Res.* 8, 2010, 215-219.
17. Sahib H.B., A.F. Aisha, Yam Mun Fe., Z. Ismail., Z. Asmawi., S. M. Salhimi., A.M.S. Abdul Majid. Anti-angiogenic and anti-oxidant properties of *Orthosiphon stamineus* Benth. methanolic leaves extract. *International journal of pharmacology*, 5(2), 2009, 162-167.
18. Perez C, Pauli M and Bazerque P. An antibacterial assay by agar well diffusion method. *Acta Bio Et Med Exp*, 15, 1990, 113-115.
19. L Jiang, F Wang, F Han, W Prinyawiwatkul, HK No and B Ge. Evaluation of diffusion and dilution methods to determine the antimicrobial activity of water-soluble chitosan derivatives. *Journal of Applied Microbiology*, 114, 2009, 956-963.
20. Alsaba F Raja, Furqan Ali, Inshad A Khan, Abdul S Shawl, Daljit S Arora, Bhahwal A Shah and Subhash C Taneja. Anti-staphylococcal and biofilm inhibitory activities of acetyl-11-keto-b-boswellic acid from *Boswellia serrata*. *BMC Microbiology*, 11, 2011, 54.
21. Baron EJ, Peterson LR and Finegold SM. *Bailey and Scott'S Diagnostic Microbiology* 1994.9th ed., The C.V. Mosby, Co., USA.
22. Junaid S A, Olabode A O, Onwuliri F C, Okori A E J and Agina S E. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *African Journal of Biotechnology*, 5(22), 2006, 2315-2321.
23. E M Abdaallah, A S Khalid and N Ibrahim. *Sci. Res. Essays*, 4, 2009, 351.

Source of Support: Nil, Conflict of Interest: None.

