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## RESEARCH ARTICLE

# GENETIC EVALUATION OF *TABEBUIA AVELLANEDAE* AGAINST *STAPHYLOCOCCUS AUREUS*

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**ABSTRACT**

Tabebuiaavellanedae *"tabebuiaavellanedae"* containing two antimicrobial active elements. This study aimed to cover one of the antimicrobial effect of Tabebuiaavellanedae against one of food pathogenic microorganism *Staphylococcus aureus.* About 50 gm dried Tabebuiaavellanedae coarse powder extracts were collected and stock solution of concentration of 10 mg/ml in (acetone and methanol). It was tested against one gram positive bacteria (*Staphylococcusaureus*) (ATCC 25923) then measuring the zone of inhibition. *S. aureus* enterotoxins genes multiplex PCR detected also. Results viewed the different inhibition zones on sensitivity test against *Staphylococcus aureus* using different concentrations of Tabebuiaavellanedae EO as following: the complete absence of inhibition effect in control samples while the inhibition effect were gradual grow up with higher Tabebuiaavellanedae concentrations as following; 1cm, 2cm, 6cm diameter around the disc immersed by 2.5%, 5% &10% concentrations respectively. Tabebuiaavellanedae had inhibition effect against; *Sea, Sec, Sed, See* virulence genes while not effect on *Seb* virulence genes.Further studies are recommend to increase researches study the genetic effect of Tabebuiaavellanedae*Staphylococcus aureus and other* bacterial virulence's genes.



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## INTRODUCTION

Tabebuiaavellanedae"*tabebuiaavellanedae"*is a tree that grows in tropical rainforest and use their wood medically. Tabebuiaavellanedae containing two antimicrobial active elements known as "naphthoquinones"; lapachol and beta-lapachone which is a toxic to nearly all types of harmful organisms and have strong killing effect against bacteria, viruses, parasites and fungi with strong anti-inflammatory activity. Tabebuiaavellanedae are performed in capsule, extract and tea forms which enhances the immune system, radiation protection, It has many applications in feed and food additives and drug industries, science and cosmetic as a food in human, aquaculture, vet and poultry and food industries including beverages, bakery products, candy, gel desserts in different countries. (Byeon, *et. al.,* 2008; Maddaly*et. al.,* 2010; Hosseini, *et. al.* 2013; Mosy*et. al.,* 2014; Ghaeni, &Roomiani, 2016 and Pyne, *et. al..,* 2017). Consuming of cup of Tabebuiaavellanedae inner bark tea daily orally and externally are very helpful in treatment of prostate inflammation, treat arthritis, pain, fever, dysentery and ulcers.

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High doses of this compound can lead to dangerous side effects, like reproductive toxicity. Tabebuiaavellanedae. This study aimed to cover one of the antimicrobial effect of Tabebuiaavellanedae against one of food pathogenic microorganism *Staphylococcus aureus.*

## MATERIAL AND METHODS

**TabebuiaavellanedaeOil Extraction and Microbiological Quality (APHA, 1992 and AOAC, 2005):** About 50 gm dried Tabebuiaavellanedae coarse powder was soaked in 300 ml of acetone and methanol then the flasks were covered with aluminum foil and stand for 7days. Then filtered by Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and stock solution of concentration of 10 mg/ml in (acetone and methanol). It was tested against one gram positive bacteria (*Staphylococcusaureus*) (ATCC 25923) were cultured on Muller Hinton then impress Tabebuiaavellanedae discs with different concentrations and incubated at 37°C/24 hours. Then measuring the zone of inhibition (Jonathanand Fasidi, 2003; Hemashenpagam and Selvaraj, 2010 and Balakumar *et al*., 2011).

**Identification of Isolated Organisms (Biochemical behaviors):** Biochemical tests were applied as recommended

by APHA (2002). The pure cultures of suspected colonies were subjected to the following tests for confirmation and identification as follows: PCR Detection: genomic DNA was extracted from (Qiagen, Germany) isolates *S. aureus*, using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit. Primers used were supplied from (Biobasic, Canada) as listed in table (1). Then PCR amplification performed for *S. aureus* enterotoxins genes multiplex PCR, primers were utilized in a 50 µl reaction containing 25 µl of 2X DreamTaq Green mastermix kit, 1 µl of each primer of 20 pmol concentration, 7 µl of water, and 8 µl of DNA template. The reactions were performed in applied biosystem 2720 thermal cycler.

**Analysis of the PCR Products:** The thermal cycler pattern was Primary denaturation the products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl and 30 µl of the uniplex and multiplex PCR products respectively were loaded in each gel slot. Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**Statistical Analysis (SPSS, 2007):** The statistical program, SPSS version 16 for window, was used for determination of means, standard error and analysis of variance (ANOVA) using the one way (mean at significance level of ($P<0.05$). Statistical significance was tested at the 5% level of significance in this study.

## RESULTS AND DISCUSSION

Effect of Different Tabebuiaavellanedae EOs Concentration against *Staphylococcus aureus*; Fig. (1) which declared the different inhibition zones on sensitivity test against *Staphylococcus aureus* using different concentrations of Tabebuiaavellanedae EO as following: the complete absence of inhibition effect in control samples while the inhibition effect were gradual grow up with higher Tabebuia avellanedae concentrations as following;1cm, 2cm, 6cm diameter around the disc immersed by 2.5%, 5% &10% concentrations respectively. The prevalence of *S. aureus* enterotoxins*:* presented in fig. (2) That Tabebuiaavellanedae had inhibition effect against; *Sea, Sec, Sed, See* virulence genes while not effect on *Seb* virulence genes. There is no any research performed to test the effect of Tabebuiaavellanedae effect against *Staphylococcus aureus* virulence genes but the antimicrobial effect and inhibition effect against *Staphylococcus aureus* by many researchers such as; (Machado, *et. al.,* 2003; Pereira, *et. al.,* 2006; Kung, *et. al.,* 2008 and Coelho, *et. al.,* 2010) whom studied the Tabebuiaavellanedae effect against methicillin-resistant *Staphylococcus aureus.*

**Table 1. Primers sequences, target genes, amplicon sizes and annealing temperatures of PCR reactions**

| Target agent | Target gene | Primers sequences | Amplified segment (bp) | Annealing | Reference |
|---|---|---|---|---|---|
| *S. aureus* | *16rRNA* | 5-GTAGGTGGCAAGCGTTATCC-3<br>5-CGC ACATCAGCGTCAG-3 | 228 | 60 | Monday and Bohach, (1999) |
| | *Sea* | GGTTATCAATGTGCGGGTGG<br>CGGCACTTTTTTCTCTTCGG | 102 | 50˚C | Mehrotra*et al*., (2000) |
| | *Seb* | GTATGGTGGTGTAACTGAGC<br>CCAAATAGTGACGAGTTAGG | 164 | | |
| | *Sec* | AGATGAAGTAGTTGATGTGTATGG<br>CACACTTTTAGAATCAACCG | 451 | | |
| | *Sed* | CCAATAATAGGAGAAAATAAAAG<br>ATTGGTATTTTTTTTCGTTC | 278 | | |
| | *See* | AGGTTTTTTCACAGGTCATCC<br>CTTTTTTTTCTTCGGTCAATC | 209 | | |

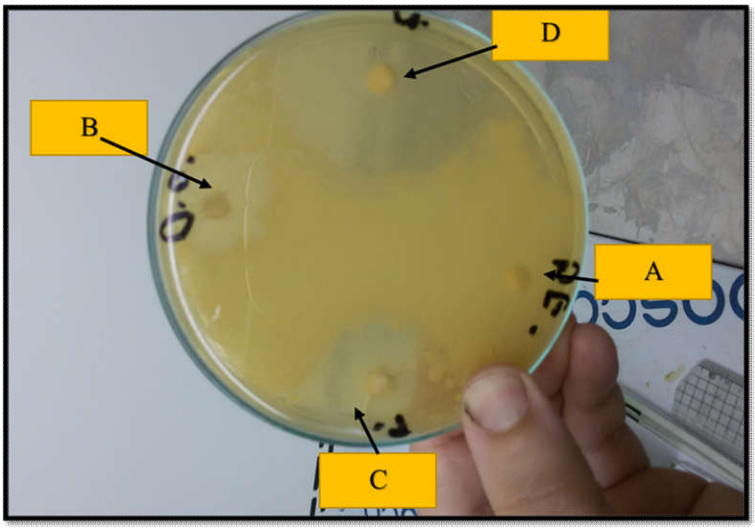


**Figure 1. Effect of Different Tabebuiaavellanedae Concentration against *Staphylococcusaureus***

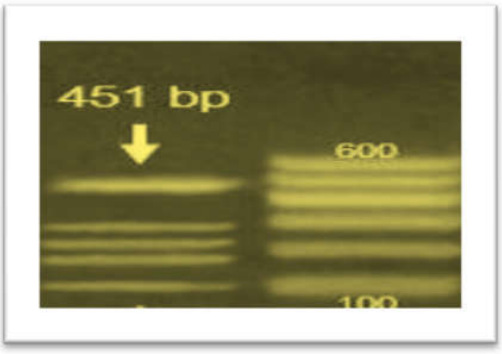


**Figure 2. Agarose gel electrophoresis of specific dose-dependent amplification of *Staph. aureus*pathogenic gene *(Seb)***

Park *et. al.,* (2006) and Park *et. al.,* (2005) showed that Tabebuiaavellanedae had strong antibacterial activity against gram-negativeandgram-positive bacteria. In conclusion, this survey revealed that Tabebuiaavellanedae has strong inhibition effect which increased with higher concentrations. In addition to its strong inhibition effect against many virulence genes of *Staphylococcus aureus "Sea, Sec, Sed, See"* while not effect on *Seb* virulence genes. All these findings suggest that the consumption of Tabebuiaavellanedae have strong antibacterial effect and the study recommend to increase researches study the genetic effect of Tabebuiaavellanedae*Staphylococcus aureus and other* bacterial virulence's genes.

***Staphylococcusaureus***

- No inhibition zone around the control disc.
- About 1 cm inhibition zone around the disc containing 2.5% Tabebuiaavellanedae EO.
- About 2 cm inhibition zone around the disc containing 5% Tabebuiaavellanedae EO.
- About 6 cm inhibition zone around the disc containing 10% Tabebuiaavellanedae EO.

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