https://doi.org/10.33472/AFJBS.6.13.2024.4007-4038

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

A STUDY TO COMPARE THE EFFECT OF NEW SYNTHESIZED EUGENOL DERIVATIVE AND SILVER NANOPARTICLES OF CLOVE OIL AGAINST ENDOCARDITIS CAUSING BACTERIA

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Article Info

Volume 6, Issue 13, July 2024

Received: 04 June 2024

Accepted: 05 July 2024

Published: 31 July 2024

doi: 10.33472/AFJBS.6.13.2024.4007-4038

ABSTRACT:

Infection of heart valve through blood stream results in endocarditis that is commonly treated using antibacterial agents. Drug resistance due to extensive use of antibacterial agents motivates the researchers to explore some new antibacterial agents synthetically and naturally from plants sources. This study was intended to compare the antibacterial response of a new eugenol derivative and biosynthesized silver nanoparticle against *S. aureus* (the endocarditis causing bacteria). The study involved synthesis of new imino derivative of eugenol (IDE) and silver nanoparticles of eugenol rich clove oil. The study involved synthesis of IDE by esterification of eugenol followed by hydrazination and Schiff reaction. The study also involved biosynthesis of silver nanoparticles of clove oil (SNPCO) using $AgNO_3$. The synthesized IDE and biosynthesized SNPCO were characterized using IR, NMR & MASS spectrometry, and UV-vis spectrometry respectively. The synthesized IDE and SNPCO were further evaluated for their antibacterial potential against *S. aureus* using disk diffusion method. Study revealed high antibacterial potential of IDE when compared to SNPCO. Although the current study reports the new IDE as promising antibacterial agents for infectious endocarditis, however the new IDE must be further evaluated for its preclinical and clinical significance.

Key words: Synthesis; eugenol derivative; Silver nanoparticles; Endocarditis; Characterization

1. INTRODUCTION

Evidence highlights that human microbiota exhibits bacterial and human cells in the ratio of 1:1 [1-4]. Minor alteration in this ratio of may lead to various infections and diseases [5-8]. A minor change in this ratio invites countless infections in the human body. Infectious endocarditis (infection of heart valve) is generally caused by transfer of *S. aureus* bacteria through blood stream [9]. For endocarditis treatment use of strong antibacterial agents is the first choice [10-12]. Extensive use of antibacterial commonly leads to resistance development, which stimulates the researchers to explore some new antibacterial moieties both from synthetic as well as natural sources [13-32]. The phenolics both from natural [33-62] and synthetic source [63-72] are known for their strong antibacterial potential. Numerous inventions suggest synthesis of various antibacterial agents. Many studies highlighted synthesis of various derivatives by esterification, hydrazination and imination [73-78]. Plants are the richest source for modern medicine in various indications, such as cancer [79-99], cardiac disorders [100-104], ageing [105], arthritis [106,107], nephrotoxicity [108], infections [109-116], anti-inflammatory [117-123], parkinsonism [124], dengue [125-129], hyperlipidemia [130,131], diabetes [132-140], depression [141], hepatic disorders [142-151], Alzheimer disease [152,153], diabetes [154], neurodegeneration [155-164] and many other disorders [165-181]. Due to high phenolic content, they are reported to possess high antioxidant activity [182-195] and thereby maintain the health [196]. Studies highlight importance of computer aided drug designing [197-213], nanotechnology [214-248], and biomaterials [249-267] in drug development. Biosynthesis of nano formulations, using biomaterials especially from plants source is a boon for the investigators [268-286]. Wide application of silver nanoparticles (SNP) for improvement antibacterial and other biological activities, among metallic nanocomposites always draws high attention of researchers. Today synthesis of SNP using plants source is common approach attributed to their environmental biosafety and cost effectiveness [287-300]. The economy of the treatment is also one of the important factors that is generally considered while selecting the modality for the treatment [301-303]. The phytomolecules may synergistically act with SNP and are applicable to various food, medical and pharmaceutical industries [304-313]. Phytochemicals act as reducing agent for green synthesis of SNP [314-317]. Therefore, based on the involvement of *S. aureus* in endocarditis, and importance of nanotechnology, and antibacterial agents both from natural and synthetic sources current study was aimed to perform to compare the effect of new imino derivative of eugenol (IDE) derivative and silver nanoparticles of clove oil (SNPCO) against endocarditis causing bacteria.

2. MATERIAL AND METHODS

Chemicals, biologicals and reagents for the present study were procured from various companies such as: Sigma-Aldrich Co. (USA), Merck KGaA (Germany), and Hi-Media. Melting points of new compound was determined using Stuart SMP11 melting point apparatus and are uncorrected. The proton magnetic resonance (1H-NMR) spectrum was recorded on a Bruker 400 MHz instrument using tetramethylsilane (TMS) as internal standard. Infrared (IR) spectrum was recorded using KBr on Shimadzu FT-IR 8300 instrument between 400 to 4000 cm-1. Mass spectrum was recorded on JEOL DX 303 HF spectrometer with MASPEC SYSTEM (msw/9629) at 70 eV. Compounds purity was monitored by TLC.

Synthesis of 2-(4-allyl-2-methoxyphenoxy)-N'-(1-(4-aminophenyl)ethylidene)acetohydrazide (2)

The compound 2 (IDE) was synthesized based on standard literature with minor modification [4,5]. Briefly, hydrazide (1) was subjected to Schiff reaction by refluxing with 4-aminoacetophenone in equimolar concentration (0.001 M) using 1 drop of acetic acid for 8 hours. Obtained crude was recrystallized to offer pure compound 2. The reaction monitoring was done by TLC. White crystals (yield: 82%, m.p.: 205 ºC); IR spectrum (cm^{-1}): 3264 (N-H str.), 3069 (=C-H), 2929 (C-H str.), 1684 (C=O), and 1586 (C=N)); ^{1}H-NMR spectrum (DMSO, ppm) δ: 1.1 (s, 3H, CH_3), 3.22 (d, 2H, CH_2-Ph), 3.73 (s, 3H, CH_3), 4.5 (s, 2H, NH_2), 4.83 (s, 2H, CH2-C=O), 5.12-5.14 (m, 2H, =CH_2), 6.31 (m, 1H, =CH), 6.42-7.48 (m, 7H, Ar-H), 9.12 (s, 1H, CONH); Mass spectrum (m/z): 353 (M+).

Preliminary Phytochemical screening of clove oil

The clove oil was subjected to qualitative testing as per the standard procedure given in the literature [318-335].

Biosynthesis of SNPCO

Biosynthesis was done based on the standard literature with minor modifications [216, 220, 221, 233]. Briefly, the clove oil was diluted in acetone at a ratio of 1:150 and 1 mM of AgNO3 solution was prepared and stirred on magnetic stirrer. 2 mL of the dilute clove oil was added drop by drop into AgNO3 solution with stirring until color of solution changed from colorless to brown. The mixture was next incubated at room temperature in dark overnight, followed by centrifugation for 15 min at 10,000 rpm to separate SNPCO and addition of few drops of distilled water to resultant SNPCO pellet. Finally, SNPCO pellet was scraped, dried and stored at room temperature.

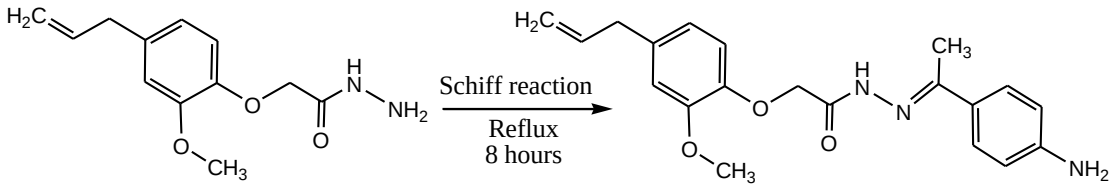
Biological Evaluation

The newly synthesized IDE and SNPCO were further tested for their antibacterial activity against S. aureus by modified disk diffusion method based on standard literature [336-352]. Experiment involved 90 mm circular Mueller-Hinton plates, and dissolution of IDE and SNPCO in 1 mL of acetone. For study, bacteria were grown to log phase overnight at 37 °C, followed by spreading of S. aureus cultures onto the MH plates agar media, placement of discs of 6 mm diameters impregnated with 100 µg/mL solution of IDE and SNPCO, 50 µg/mL of gentamicin and acetone over MH plates agar media. Next, the plates were incubated for 24 h at 37 °C in triplicate, and finally the zones of inhibition of were measured on mm scale.

3. RESULTS AND DISCUSSION

Synthesis

For the synthesis of compound 2 firstly esterification of eugenol was done based on standard literature with minor modification [63-78]. Briefly, eugenol was refluxed with ethylchloroacteate in equimolar concentration for 17 hours. The synthesized ester was further hydrazinated using hydrazine hydrate in equimolar concentration. The obtained hydrazide was subjected to Schiff reaction on refluxing with 4-aminoacetophenone in equimolar concentration (0.001 M) following standard procedures with minor modification. Synthesized compound was further subjected to IR, 1H-NMR, and Mass spectral analysis. The scheme to synthesize compounds 2 is presented in figure 1.



(1) **(2)**

Figure 1: Scheme to synthesize compound 2 (IDE)

The synthesis and characterizations study result compound 2 (IDE) were also correlated with other studies [63-78]. This study successfully synthesized and elucidated structure of compound 2 (IDE), which correlates synthesis of IDE with its physical and chemical structures of present study [63-65 & 353,354].

Preliminary Phytochemical screening of clove oil

The clove oil was subjected to qualitative testing as per the procedure given in standard references [318-335] and group of compounds identified are presented in table 1.

Table.1: Preliminary data for phytochemical screening of clove oil

S. No.	Tests	Phytoconstituents
1	Carbohydrates	+
2	Proteins	-
3	Terpenoids	+
4	Alkaloids	+
5	Flavonoids	-
6	Glycosides	+
7	Steroids and sterols	+
8	Tannins and Phenols	+

Biosynthesis of SNCOP

The visual inspection and UV–Visible spectrometric analysis revealed successful synthesis of SNPCO. The visual color changes from yellow to brown after 60 min indicated the formation of SNPCO. The SNPCO synthesis was also confirmed by UV-Visible spectrometry, which offered an absorption spectrum containing curves 1, 2, & 3 (Figure 1). In figure 1, curve 1 represents AgNO3, curve 2 represents SNPCO, and curve 3 represents Clove oil. Appearance of SPR peak at 406 nm in curve 2 confirmed synthesis of SNPCO. In UV-Vis spectrum, curve 3 of the clove oil did not shown any signal near 406 nm.

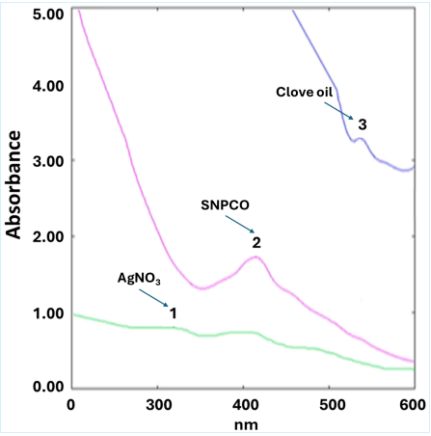


Figure 1. UV–Vis spectrum of $AgNO_3$, SNPCO, and Clove oil

Biological Evaluation

The antibacterial activity of IDE, SNPCO and clove oil was determined using standard protocols as mentioned in the experimental section. The inhibitory potential of IDE, SNPCO and clove oil was determined against S. aureus (the endocarditis triggering pathogen). Tables 2 presents the zones of inhibition (ZOI) exhibited by SNPCO and clove oil.

Table 2: Antibacterial activity (Zone of inhibition)

Compound	**Zone of inhibition in mm**
2 (IDE)	24
SNPCO	22
Clove oil	20
DMSO (10%)	-
Amoxycillin	25

After carrying out the antibacterial activity, it was found that compound 2 (IDE), SNPCO and dilute clove oil possess good antibacterial activity against *S. aureus* when compared with amoxycillin (+ve control). Many studies highlighted the various mechanisms of actions of phytomolecules [355-399]. Also, the new compound 2 (IDE) exhibits higher inhibitory activity when compared with SNPCO. The results of the present study are also compared with other studies where different biomaterials were used [398, 399]. Hence based on the antibacterial activity biosynthesized IDE and SNPCO can be indicated for endocarditis treatment, however prior to that preclinical and clinical investigations are must.

4. CONCLUSION

Current study reveals that biosynthesis of SNPCO is eco-friendly, and cost-effective technique. In this study, the compound 2 (IDE) and SNPCO exhibited good inhibitory potential against *S. aureus*. Present study establishes clove oil as an efficient biomaterial for the biosynthesis of silver nanoparticles. This study confirms that compared to SNPCO, and clove oil, the compound 2 (IDE) exhibits the highest antibacterial potential against *S. aureus*. Hence, current study establishes that synthesized IDE and SNPCO can be indicated for endocarditis treatment, however prior to that preclinical and clinical investigations are must.

ACKNOWLEDGEMENTS

All the authors of this manuscript are thankful to Lovely Professional University and SGT University for their support in successful completion of this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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