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INHIBITORY ACTIVITY OF NEW SESAMOL DERIVATIVE AND SILVER NANOPARTICLES OF SESAME SEED OIL AGAINST OSTEOMYELITIS CAUSING BACTERIA

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ABSTRACT:

Staphylococcus aureus is the common cause for osteomyelitis in adults and children, that can be treated by antibacterial agents. Resistance development on continuous exposure to antibacterial agents, is the motivation to find some new antibacterial agents from synthetic and natural source. This research was aimed to determine the antibacterial activity of new sesamol derivative and biosynthesized silver nanoparticles against *S. aureus* (the osteomyelitis causing bacteria). Study involved synthesis of new imino derivative of sesamol (IDS) and silver nanoparticles of sesamol rich Sesame seed oil. Current investigation involved synthesis of IDS by esterification of sesamol followed by hydrazination and Schiff's reaction. This study also involved biosynthesis of silver nanoparticles of Sesame seed oil (SNPSSO) using $AgNO_3$. The synthesized IDS and biosynthesized SNPSSO was characterized using IR, NMR & MASS spectrometry, and UV-Vis. Spectrometry respectively. The synthesized IDS and SNPSSO were further evaluated for their antibacterial potential against *S. aureus* using disk diffusion method. Study revealed high antibacterial potential of IDS when compared to SNPSSO. Although, current study reports the new IDS as promising antibacterial agents for infectious osteomyelitis, however the new IDS should be further evaluated for its preclinical and clinical significance.

Key words: Synthesis; sesamol derivative; Silver nanoparticles; Osteomyelitis; Characterization

1. INTRODUCTION

Facts suggests human microbiota to accommodate bacterial and human cells in the ratio of 1:1 [1-4]. A very small change in this ratio of might lead to different infectious diseases [5-8]. A minor change in this ratio invites countless infections in the human body. Infectious osteomyelitis is generally caused by *S. aureus* [9]. For osteomyelitis treatment use of strong antibacterial agents is the first choice [10-12]. High use of antibacterial generally offers resistance development, which forces investigators to explore some new antibacterial moieties from synthetic and 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. A large number of 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 maintains the health [196]. Studies highlights 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]. Long range of application of silver nanoparticles (SNP) with improved antibacterial and other biological activities, among metallic nanocomposites always draws high researchers' attention. Synthesis of SNP using plants source is common approach attributed to their environmental biosafety and cost effectiveness. Economy of treatment is major factor that is considered while selection of modality for any treatment [287-300]. The phytomolecules may synergistically act with SNP and are applicable various food, medical and pharmaceutical industries [301-317]. Phytochemicals acts as reducing agent for green synthesis of SNP. Therefore, based on the involvement of *S. aureus* in osteomyelitis, and importance of nanotechnology, and antibacterial agents both from natural and synthetic sources present study was intended to compare the effect of new imino analogue of sesamol and silver nanoparticles of Sesame seed oil (SNPSSO) against osteomyelitis causing bacteria.

2. MATERIAL AND METHODS

Reagents and chemicals for current 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 N-(1-(4-aminophenyl)ethylidene)-2-(benzo[d][1,3]dioxol-5-yloxy)acetohydrazide (2)

Synthesis of compound 2 (IDS – imino derivative of sesamol) was done 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: 86%, m.p.: 198 ˚ C); IR spectrum (cm^{-1}): 3249 (N-H str.), 3055 (=C-H), 2924 (C-H str.), 1696 (C=O), and 1579 (C=N)); ^{1}H-NMR spectrum (DMSO, ppm) δ: 1.1 (s, 3H, CH_3), 4.6 (s, 2H, NH_2), 4.83 (s, 2H, CH_2-C=O), 5.6 (s, 1H, CH_2), 6.45-7.49 (m, 7H, Ar-H), 9.06 (s, 1H, CONH); Mass spectrum (m/z): 327 (M+)

Preliminary Phytochemical screening of Sesame seed oil

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

Biosynthesis of SNPSSO

Biosynthesis was done based on the standard literature with minor modifications [220-221]. Briefly, the Sesame seed oil was diluted in acetone at a ratio of 1:150 and 1 mM of $AgNO_3$ solution was prepared and stirred on magnetic stirrer. 2 mL of the dilute Sesame seed 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 SNPSSO and addition of few drops of distilled water to resultant SNPSSO pellet. Finally, SNPSSO pellet was scraped, dried and stored at room temperature.

Biological Evaluation

The newly synthesized IDS and SNPSSO 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 IDS and SNPSSO 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 IDS and SNPSSO, 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 sesamol was done based on standard literature with minor modification [72-76]. Briefly, sesamol 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.

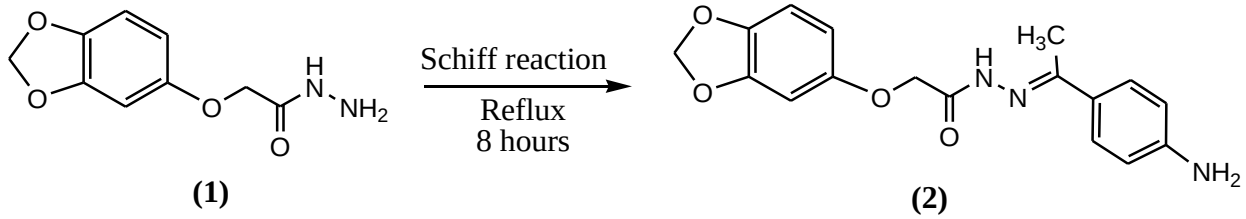

Figure 1: Scheme to synthesize compound 2 (IDS)

The synthesis and characterizations study result compound 2 (IDS) were also correlated with other studies [19-22]. This study successfully synthesized and elucidated structure of compound 2 (IDS), which correlates synthesis of IDS with its physical and chemical structures of present study [353,354].

Preliminary Phytochemical screening of Sesame seed oil

The Sesame seed oil was subjected to qualitative testing as per the procedure given in standard references [322-328] and group of compounds identified are presented in table 1.

Table.1: Preliminary data for phytochemical screening of Sesame seed oil

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

Biosynthesis of SNPSSO

The visual inspection and UV–Visible spectrometric analysis revealed successful synthesis of SNPSSO. The visual color changes from yellow to brown after 60 min indicated the formation of SNPSSO. The SNPSSO 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 SNPSSO, and curve 3 represents Sesame seed oil. Appearance of SPR peak at 398 nm in curve 2 confirmed synthesis of SNPSSO. In UV-Vis spectrum, curve 3 of the Sesame seed oil did not shown any signal near 406 nm.

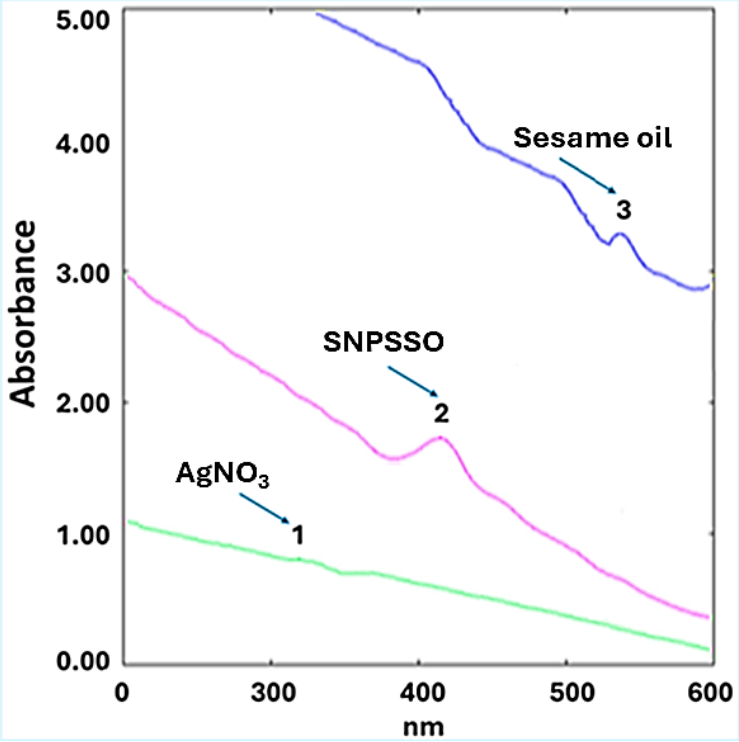

Figure 1. UV–Vis spectrum of $AgNO_3$, SNPSSO, and Sesame seed oil

Biological evaluation

The antibacterial activity of IDS, SNPSSO and Sesame seed oil was determined using standard protocols as mentioned in the experimental section. The inhibitory potential of IDS, SNPSSO and Sesame seed oil was determined against *S. aureus* (the osteomyelitis triggering pathogen). Tables 2 presents the zones of inhibition (ZOI) exhibited by SNPSSO and Sesame seed oil.

Table 2: Antibacterial activity (Zone of inhibition)

Compound	Zone of inhibition in mm
2 (IDS)	24
SNPSSO	23
Sesame seed oil	22
DMSO (10%)	-
Amoxycillin	25

After carrying out the antibacterial activity, it was found that compound 2 (IDS), SNPSSO and dilute Sesame seed oil possess good antibacterial activity against *S. aureus* when compared with amoxycillin (+ve control). Many studies highlighted the various mechanism of actions of phytomolecules [355-399]. Also, the new compound 2 (IDS) exhibits higher inhibitory activity when compared with SNPSSO. The results of present study are also compared with other studies. Hence based on the antibacterial activity it is clear that synthesized IDS and SNPSSO can be indicated for osteomyelitis treatment, however prior to that preclinical and clinical investigations are must.

4. CONCLUSION

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

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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