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Original Research Article

ANTIBACTERIAL AND ANTIBIOFILM POTENTIAL OF BIOSYNTHESIZED PALLADIUM NANOPARTICLES USING *Senna occidentalis* LEAF EXTRACT ON *Salmonella typhi*

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ABSTRACT

Owing to various side effects of synthetic drugs, medicinal plants have gained much popularity in the treatment of a number of infections. The use of medicinal plants has long been established due to their potentials in mediating the synthesis of nanomaterials. The present research aimed at determining antibacterial and antibiofilm potential of biosynthesized Palladium Nanoparticles (PdNPs) using S. occidentalis leaf extract on *S. typhi*. The test bacteria were obtained from Dutse General Hospital, and confirmed using standard phenotypic and genotypic techniques. The phytochemicals screening, antibacterial, antibiofilm, MIC and MBC of *S. occidentalis* leaf extracts and biosynthesized PdNPs on S. typhi were determined using standard techniques. The phytochemicals screening revealed the presence of alkaloids, tannin, anthraquinones, saponin, flavonoid, and phenols in the extracts. The statistical analysis using independent t-test showed no significant difference ($P > 0.05$) between 100, 80 and 60 mg/ml concentrations of extracts and its biosynthesized PdNPs treatments on *S. typhi*. Moreover, statistical results showed significant difference ($P < 0.05$) between extracts and its biosynthesized PdNPs treatments on *S. typhi* at 40 mg/ml concentration. The statistical results using one-way ANOVA indicated no significant difference ($P > 0.05$) between all concentrations of ethanolic and methanolic extracts and its biosynthesized PdNPs treatments on *S. typhi*. Moreover, statistical results indicated significant difference ($P < 0.05$) between all concentrations of ethyl acetate extracts and its biologically synthesized PdNPs treatments on *S. typhi.* The for MIC and MBC of extracts and its biosynthesized PdNps on *S. typhi* showed that the isolate had MIC and MBC ranged from 8.0 to 10.0 mg/ml. The *in vitro* antibiofilm assay of S. occidentalis leaf extracts and its biosynthesized PdNPs on *S. typhi* revealed that biosynthesized PdNps exhibit significant inhibitory effect on biofilm formation with OD value of 0.545 compared to extract with OD of 0.9. The present findings showed that *S. occidentalis* leaf extracts and its biosynthesized PdNPs had potentiality in inhibiting S. typhi and can be considered in antimicrobial treatment.

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INTRODUCTION

Medicinal plants have been used for long in prevention and treatment of a number of diseases. *Senna occidentalis* also known as *Coffee senna* in English, *Majamfari/ Rai dore* in Hausa language, is found in open dumping sites, water canals and by road sides, its leaves are being used in the treatment of typhoid fever, hepatitis and malaria in Northern Nigeria [1]. According to [2] local healers in Jos

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and Niger State, Nigeria, have reported that *S. occidentals* leaf infusion is effective for typhoid fever treatment, while decoction or steam bath of its fresh leaf are used for fever treatment. The Green synthesis method for Nanoparticles (NPs) is preferred method of NPs synthesis because it is eco-friendly and has low cost [3]. More so, the right method has to be employ in producing particles capable of penetrating the biofilm formed by bacteria. Natural compounds in plant have excellent antimicrobial and antibiofilm potential, but factors such as solubility and stability restrict their applications. The use of NPs is an interesting approach in improving delivery process to these compounds [4].

Scientists in the field of nanotechnology are currently researching the idea of forming metallic NPs biologically using reducing agents present in plant extracts, microbes and their biomass [3]. NPs used for antibiofilm therapy have gained a tremendous impetus because of their unique features of having broad spectrum of activity, by easily penetrating into the biofilm matrix to act, unlike a number of antibiotics [4]. Palladium (Pd) is generally used in biomedical fields due to its biocompatible nature and industrial applications. It is also used as catalyst because of its unique optical, thermal, morphological and chemical characteristics [5]. Palladium nanoparticles (PdNPs) has been widely used as a catalyst to manufacture pharmaceuticals, degrade pollutants, sensor for various analytes detection, drug carriers, antibiofilm and antimicrobial agents [5].

Bacterial infections among the populace of mankind are the principal cause of diseases and death in this cosmos, due to their resistance to antibacterial drugs which posed serious threat to the health sector. Consequently, bacterial multidrug-resistant and biofilm-associated diseases present a great challenge that strengthen the need for new antimicrobial drugs development, thus, the work of scientists to discover an additional pharmaceutical drugs are very vital to human health. [4].

Biofilm are complex microbial communities surrounded in extracellular matrix made up of protein, polysaccharide, and lipids, form by microorganism which initiate or facilitate microbial adhesion to biomaterials. It plays an important role in bacterial infections and antimicrobial-resistance, by offering protection to surrounded microorganisms to drugs as well as immune response, leading to high tolerate of up to 1000 times antibiotic concentration by bacterial cells than planktonic one [4]. The biofilm mediated infections treatment present challenges in healthcare setting that require potent and sensitive antibiofilm drugs for their inhibition [4]. National Institutes of Health (NIH) recent report revealed that, about 80% of human soft and hard tissue diseases are caused by pathogenic biofilms forming microbes. Many researchers have embarked in testing and evaluating plants and animals' products from a number of samples to come up with antimicrobial solution for biofilms associated infections [6].

Typhoid fever is cause by *salmonella enterica serovar typhi.* Typhoid and paratyphoid fever are collectively known as enteric fever [7]. The disease is one of the lead cause of morbidity and mortality in the tropical and subtropical countries within all age groups worldwide. It is transmitted by taking of contaminated food and/ or water [7, 8]. Cultures diagnostic approach is the best diagnostic method in suspected typhoid fever cases [7].

The present study aimed to determine the antimicrobial and antbiofilm potential of biosynthesized PdNPs using *S. occidentalis* leaf extract on *S. typhi*.

MATERIALS AND METHODS

Plant Sample Collection and Preparation

Plant sample collection and preparation was as described by [9]. The leaf of *S. occidentalis* was handpicked in Jahun Local Government, Jigawa State, Nigeria, and transported to the Department of Botany, Federal University Dutse, Jigawa State, Nigeria, where it was identified. The leaf was clean thoroughly under running tap water, and shade dried under the shed for 14 days at room temperature. It was then grinded using motor and pestle into fine powder. The powdered sample was kept in an air tight containers at room temperature for further use.

Extraction of Plant Sample

Percolation method as reference to the method of [9] was used for extraction of plant leaf. About 125 g of the powdered *S. occidentalis* leaf was soaked into 1250 ml of ethanol, methanol, and ethyl acetate respectively in a conical flask. The mixture was kept with vigorous shaking in a magnetic shaker for 48 h, and filtered using sieve wire gauge, then Whatman No.1 filter paper. Water bath at 60^{o}C was used to concentrated the filtrates by complete evaporation of

the solvents. The dried extracts were stored at 4°C before use.

Extract Percentage Yield

Extract percentage yield was calculated using the formula below as adopted from [1]:

Extraction yield (%) = $\frac{X_1}{X_0} \times 100$

Where:

X_1 = extract weight after evaporation

X_0 = weight of dry powder before extraction

Phytochemicals Screening

The phytochemicals screening of the extracts were carried out as described by [10] for the detection of alkaloids, tannins, anthraquinones, saponins, phenols, glycosides, resins, cardiac glycosides, and flavonoids.

Biogenic Synthesis of Palladium Nanoparticles

The methods of [11, 12] were used in the synthesis of PdNPs. About 30 ml of the extracts were dispensed into 15 ml of 5 mM Palladium chloride ($PdCl_2$) solution. The mixtures were kept at 60°C for 2 h on magnetic stirrer. Formation of brown colloidal solution indicated PdNPs biosynthesis. The PdNPs solution obtained was centrifuged at 10,000 rpm for 18 min. Supernatant was discarded, where the pellet was dried in hot air oven at 60°C, and stored at 4°C before used.

Preparation of Different Concentrations of the Samples

The preparation of different concentrations of the sample was as described by [10]. About 1 g of the dried extract and biosynthesized PdNPs were weighed and dissolved in 1 ml of DMSO to obtain a concentration of 1000 mg/ml as stock solution. The concentrations of 100, 80, 60, 40 mg/ ml were prepared from stock solution using the formula below:

$Cc \times Vc = C_D \times V_D$

Where:

Cc = concentration of initial stock solution

Vc = required volume (from stock solution)

C_D = final concentration (taken from stock solution)

V_D = volume required concentration

$Vc = C_D \times V_D / Cc$

Test Bacteria

Clinically isolated *S. typhi* were collected from Dutse General Hospital, Jigawa State, Nigeria, and subculture on nutrient agar and salmonella shigella agar plates.

Phenotypic and Molecular Characterization of the *S. typhi*

The gram reaction and biochemical tests comprise of indole, catalase, urease, citrate, motility, and oxidase tests were conducted on bacterial isolate as described by [13]. In addition, molecular characterization of the bacterial isolate was carried out to confirmed the identity of the bacterial isolate using DNA extraction, PCR amplification and the 1.5% agarose gel electrophoresis of 16S rRNA genes of *S. typhi* using (27F): (5'-AGAGTTTGATCCTGGCTCAG-3') and (1492R): (5'-GGTTACCTTGTTACGACTT-3') (Invitrogen™) as forward and reverse primers as described by [14].

Antibacterial Activity of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs on *S. typhi*

The agar well diffusion method was used for studying the antibacterial activity of *S. occidentalis* leaf extracts and biosynthesized PdNPs on *S. typhi* as described by [15-17].

Minimum Inhibitory Concentration (MIC) Determination

The MIC was determined using broth dilution method as described by [17-18]. The concentration ranged from 10.0 to 4.0 mg/ml were used. The test tubes that had lowest concentration that showed no visible growth were recorded as MIC.

Minimum Bactericidal Concentration (MBC) Determination

MBC refers to lowest concentration of antimicrobials capable of killing 99.9% of the organism over a fixed period of time. MBC was determined as adopted from [16]. The concentrations showed no visible growth on agar plates were recorded as MBC.

Detection of Biofilm Formation of *S. typhi*

The biofilm formation *S. typhi* was detected using Congo red method according to the method of [15, 19]. About 1% Congo red solution was dispensed to NA medium and poured onto a Petri dish, and allowed to solidified. The bacterial samples

standardized to 0.5 McFarland standard was inoculated by quadratic streaking on agar plates, and incubated at 37°C for 24 to 48 h. The presence of black colonies on the agar surface indicate biofilm formation.

Antibiofilm Assay of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs on *S. typhi*

Antibiofilm activity of *S. occidentalis* leaf extracts and biosynthesized PdNps were determined using crystal violet assay in 96 well plates as reference to the method of [5, 20, 21]. Overnight bacterial culture of *S. typhi* was grown in 1 ml Mueller Hinton broth (MHB) for 24 h to reach an optical density (OD 570 nm) of 1:0 (10^6 CFU/ ml). The bacterial suspension was inoculated (1:100 dilution) into 1 ml MHB and incubated at 37°C for 24 h. About 0.1 ml of extract and biosynthesized PdNps (100 mg/ml to 40 mg/ml) were dispensed into 96-well plate containing 0.1 ml MHB supplemented with 0.5% (w/v) sucrose and Congo red respectively. About 0.01 ml of *S. typhi* suspension (10^6 CFU/ ml) was dispensed into 96-well plates and incubated at 37 °C for 24 h. After incubation, non-adherence cell were removed by washing three times with distilled water from each well. Whereas adherent cells were stained with 1% crystal violet for 3 min, and rinsed twice with distilled water. The plates were then de-stained with 95% ethanol for 45 min. The color intensity was determined using spectrophotometer at OD of 570 nm using micro titer plate ELISA reader to define the optical density of biofilm formation. The optical OD of biofilm was compared with $PdCl_2$ control and the isolates were group as follows:

A. OD ≤ ODc = non-biofilm former
B. OD_C < OD ≤ 2 × ODc = weak biofilm former
C. 2 × ODc < OD ≤ 4 × ODc = moderate biofilm former
D. 4 × ODc < OD = strong biofilm former

Key: OD_C = optical density control, OD = optical density.

Statistical Analysis

The data generated were analyzed using one-way ANOVA and independent t-test using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The results were presented in figures, charts and tables.

RESULTS

Percentage Yield of *S. occidentalis* Leaf Extracts

The percentage yield of *S. occidentalis* leaf extracts (Table 1) showed that the percentage yield obtained for the three solvent ranged from 9.04 to 14.24%. The highest percentage yield of 14.24% was observed in ethanolic extract, followed by methanolic extract with 10.32%, while ethyl acetate extract had the least percentage of 9.04 %.

Table 1: Extraction Yield Percentage of *S. occidentalis* Leaf Extracts

Plant	Part used	Extract	Weighed of powdered(g)	Weight of extract(g)	Percentage yield %
S. occidentalis	Leave	Ethanol	125	17.8	14.24
		Methanol	125	12.9	10.32
		Ethyl acetate	125	11.3	9.04

Phytochemicals Screening

The phytochemicals screenings of *S. occidentals* leaf extracts (Table 2) revealed the presence of alkaloids and anthraquinones in all the extracts. Tannins were present in ethenolic and methanolic extracts, while resins were present in methanolic and ethyl acetate extracts. The findings showed that there was absence of glycoside and cardiac glycoside in all the extracts.

Table 2: Phytochemicals Screening of *S. occidentalis* Leaf Extracts

Plant used	Phytochemical compound	Type of extract		
S. *occidentalis* Leaves		**Ethanol**	**Methanol**	**Ethyl acetate**
	Tannins	+	+	-
	Anthraquinones	+	+	+
	Saponins	-	+	-
	Flavonoids	+	-	-
	Alkaloids	+	+	+
	Phenols	-	+	-
	Glycosides	-	-	-
	Resins	-	+	+
	Cardiac glycoside	-	-	-

Key: + = Present, - = Absent

Phenotypic and Molecular Characterization of the *S. typhi*

The phenotypic characterization of the *S. typhi* (Table 3) showed that the isolate was Gram –ve short rod that produced smooth, colorless with black centered colonies on SSA media. The isolate was tested +ve for catalase and motility and was –ve for indole, urease, citrate and oxidase tests, and was identified as *Salmonella* sp. The molecular characteristics of *S. typhi* showed the gel electrophoresis of 16S rRNA genes (Figure 1). The results showed bands at 750 bp of 16S rRNA genes of *S. typhi* of the 100 bp plus DNA marker.

Table 3: Phenotypic Characterization of *S. typhi*

Test	Results						Probable Organism
Morphological Characteristics	Colonial morphology on SS agar			Smooth, colorless with black centered colonies			***Salmonella typhi***
	Cellular/microscopic observation			Short rod singly appeared			
	Gram reaction			Gram negative			
Biochemical Test	Indole	Catalase	Urease	Citrate	Motility	Oxidase	
	-	+	-	-	+	-	

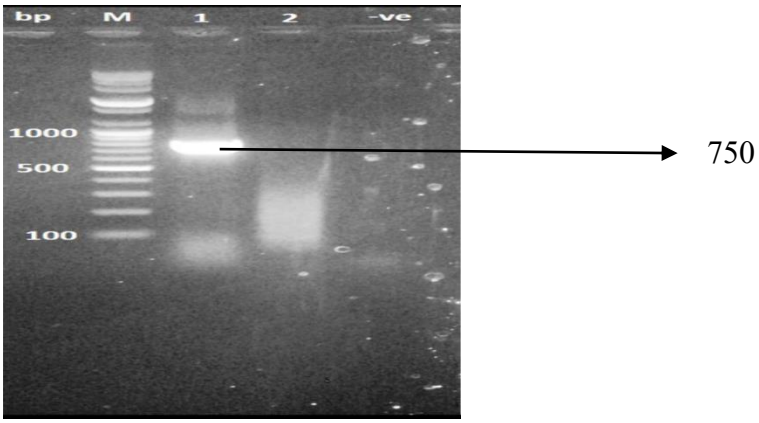


Figure 1: Gel Electrophoresis of 16S rRNA Gene of *Salmonella typhi*
Key: M = 100 bp Marker, 1 = *S. typhi*, 2 = *S. typhi*, bp = base pair, -ve = Negative control

Antimicrobial Activity of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs against *S. typhi*

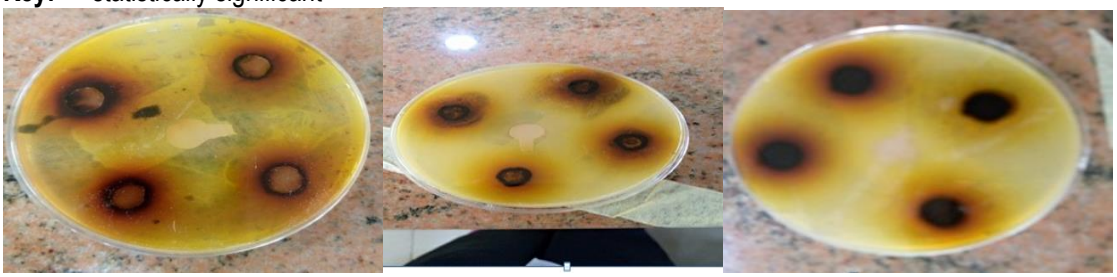
The antimicrobial activity of *S. occidentalis* leaf extracts and biosynthesized PdNPs against *S. typhi* (Table 4) showed that the degree of inhibition zones varied with concentrations. The statistical results using independent t-test indicate no significant difference (P > 0.05) between 100, 80 and 60 mg/ml concentrations of the ethanolic, methanolic, and ethyl acetate extracts, and biologically synthesized PdNPs treatments on *S. typhi*. Moreover, statistical results using independent t-test showed significant difference (P < 0.05) between ethanolic, methanolic and ethyl acetate extracts and biologically synthesized PdNPs treatments on *S. typhi* at 40 mg/ml. The Figure 2 showed antimicrobial activity of ethanolic, methanolic and ethyl acetate extracts and biosynthesized PdNPs on *S. typhi*.

Table 4: Antimicrobial Activity of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs on *S. typhi*

Solvents type	Diameter zones of growth inhibition (mm)		P-value (one tail)	P-Value (two tail)
	Extract	PdNPs		
	100 mg/ml			
Ethanol	14	17		
Methanol	12	16	0.145	0.289
E. acetate	10	11		
	80 mg/ml			
Ethanol	12	15		
Methanol	10	14	0.057	0.114
E. acetate	9	11		
	60 mg/ml			
Ethanol	11	13		
Methanol	10	14	0.078	0.155
E. acetate	9	10		
	40 mg/ml			
Ethanol	8	10		

Methanol	8	10	0.001*	0.002*
E. acetate	7	10		

Key: *= statistically significant



A **B** **C**

Figure 2: Antimicrobial Activity of Biosynthesized PdNPs on *S. typhi*: A= Ethanolic extract PdNPs, B= Methanolic extract PdNPs, C= Ethyl Acetate extract PdNPs

Comparative Antimicrobial Activity of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs on *S. typhi*

The comparative antimicrobial activity of *S. occidentalis* leaf extracts and biosynthesized PdNPs on *S. typhi* (Table 5) showed that the degree of inhibition zones varied with concentrations. The statistical results using one-way ANOVA revealed no significant difference (P > 0.05) between 100, 80, 60 and 40 mg/ml concentrations of the ethanolic and methanolic extracts and biosynthesized PdNPs treatments on *S. typhi*. Moreover, Statistical results revealed significant difference (P < 0.05) between 100, 80, 60 and 40 mg/ml concentrations of ethyl acetate extracts and biosynthesized PdNPs treatments on *S. typhi*. The Figure 3 showed comparison chart between extracts and biosynthesized PdNps.

Table 5: Comparative Antimicrobial Activity of *S. occidentalis* Leaf Extract and Biosynthesized PdNPs on *S. typhi*

Conc. (mg/ml)	Diameter of zones of growth inhibition (mm)		f-value	p-value
	Extract	PdNPs		
Ethanolic extract				
100	14	17		
80	12	15		
60	11	13	1.65	0.25
40	8	10		
Methanolic extract				
100	12	16		
80	10	14		
60	9	14	6.08	0.05
40	8	10		
Ethyl acetate extract				
100	10_a	11_a		
80	9_a	11_a	6.39	0.04*

60	9_b	10_b
40	7_b	10_b

Key: *= Statistically significant, $_a$= significant difference in mean of the 2 groups, $_b$= no significant difference in the means of 2 groups

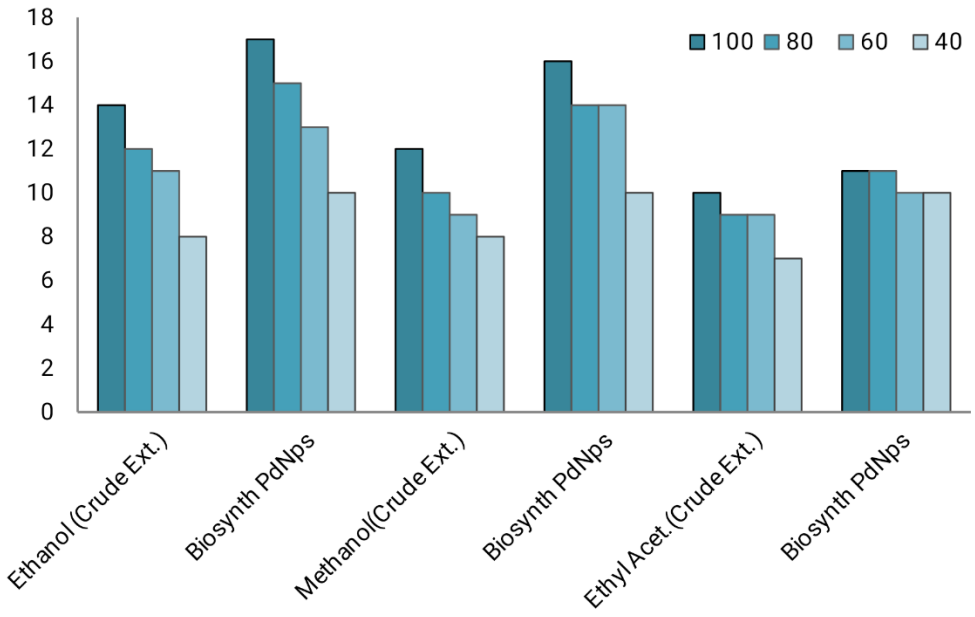


Figure 3: Comparison Chart Between Extracts and Biosynthesized PdNps

MIC and MBC of *S. occidentalis* Extracts and Biosynthesized PdNps on *S. typhi*

The MIC of the extracts and biosynthesized PdNps on *S. typhi* (Table 6) showed that *S. typhi* had MIC ranged from 8.0 to 10.0 mg/ml. The ethanolic and methanolic extracts and biosynthesized PdNPs had MIC of 8.0 mg/ml. However, the ethyl acetate extracts and biosynthesized PdNPs MICs were not detected. Moreover, the MBC of the extracts and biosynthesized PdNps on *S. typhi* (Table 7) showed that *S. typhi* had MBC ranged from 8.0 to 10.0 mg/ml. The ethanolic and methanolic extracts and biosynthesized PdNPs had MBC of 8.0 mg/ml. Whereas the ethyl acetate extracts and biosynthesized PdNPs had the MBCs were not detected.

Table 6: MIC of the Extracts and Biosynthesized PdNps on *S. typhi*

Conc. mg/ml	Ethanol extract	Biosynthesized PdNps	Methanol extract	Biosynthesized PdNps	Ethyl acetate	Biosynthesized PdNps
100	-	-	-	-	+	+
80	-	-	-	-	+	+
60	+	+	+	+	+	+
40	+	+	+	+	+	+

Key: + = Presence of turbidity, - = Absence of turbidity

Table 7: MBC of Extracts and Biosynthesized PdNps on *S. typhi*

Conc. mg/ml	Ethanol		Methanol		Ethyl acetate	
	Crude	PdNps	Crude	PdNps	Crude	PdNps
100	-	-	-	-	+	+
80	-	-	-	+	+	+
60	+	+	+	+	+	+
40	+	+	+	+	+	+

Antibiofilm Assay of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs on *S. typhi*

The Biofilm formation of *S. typhi* was measured photometrically at optical density of 570 nm (Table 8). The results revealed that *S. typhi* was capable of biofilm formation. It produced an intermediate biofilm with high OD value of 1.65. The antibiofilm assay of *S. occidentalis* leaf extracts biosynthesized PdNPs on *S. typhi* (Table 8) revealed that biosynthesized PdNps exhibit a significant inhibitory activity on the biofilm formation with OD value of 0.545 which was lower compared to the extract OD of 0.9.

Table 8: Antibiofilm Assay of *S. occidentalis* Leaf Extracts and Biosynthesized PdNPs on *S. typhi*

Test sample	Mean	Biofilm	Formula
Control (A)	0.548	Non-biofilm former	$OD \leq OD_{(c)}$
S.typhi (B)	1.65	Moderate biofilm former	$2 \times OD_{(c)} < OD \leq 4 \times OD_{(c)}$
Biosyn. PdNps(C)	0.545	Non-biofilm former	$OD \leq OD_{(c)}$
Extract (D)	0.9	Weak biofilm former	$OD_{(c)} < OD \leq 2\times OD_{(c)}$

Key: OD = Optical Density, OD_C = Optical density control (Palladium chloride)

DISCUSSION

The extraction percentage yield of *S. occidentalis* leaf extracts showed that the percentage yield obtained for the three solvent range from 9.04 to 14.24%. This is in consistent with findings of [22] that reported percentage yield of 11.3% for methanolic extracts of *S. occidentalis*. Abdullahi *et al* [1] also reported the percentage yield of 12.29% for methanolic extract of *S. occidentalis*. Khadka *et al* [23] described climatic conditions, extraction methods, extraction time, temperature and parts of the plant used as vital parameters effecting percentage yields and contributing immensely in the isolation of bioactive compounds.

The phytochemicals screenings of *S. occidentals* leaf extracts revealed the presence of alkaloids and anthraquinones in all the extracts. Tannins were present in ethenolic and methanolic extracts, while resins were present in methanolic and ethyl acetate extracts. The findings showed that there were absence of glycoside and cardiac glycoside in all the extracts. Justina and Solomon [24] attributed the difference in phytochemical constituents of plant extracts to the planting location, environmental, and seasonal variations. Alexanda [25] stated that the plant metabolites have varied pharmacological actions in human and animals and its presence suggest great potentials of the plants as a source of useful phytomedicines. Cheng *et al* [26] suggested that the phytochemicals are naturally occurring non nutritive chemicals in plants that serve as medicines for the treatment and prevention of diseases.

The phenotypic characterization of the *S. typhi* are in line with phenotypic characteristics of the *Salmonella* spp reported by [27]. The molecular characterization of *S. typhi* showed the gel electrophoresis of 16S rRNA genes of the *S. typhi* showed bands at 750 bp of 16S rRNA genes of *S. typhi* of the 100 bp plus DNA marker. This is in line with findings of [28] who reported the bands at 789 bp of 16S rRNA genes of the bacterial isolates.

The synthesis of PdNPs was observed visually by formation of brown colloidal solution after 2 hours at 60°C on magnetic stirrer. This is due to interaction between Pd^+ and *S. occidentalis* leaf extracts which served as the stabilizing and reducing agents as a result of excitation of the surface plasma vibrations [29]. The antimicrobial activity of *S. occidentalis* leaf

extracts and biosynthesized PdNPs on *S. typhi* showed that the degree of inhibition zones varied with concentrations. The statistical results using independent t-test revealed no significant difference ($P > 0.05$) between 100, 80 and 60 mg/ml concentrations of the ethanolic, methanolic and ethyl acetate extracts and biosynthesized PdNPs treatments on *S. typhi*. Moreover, Statistical analysis using independent t-test showed significant difference ($P < 0.05$) between ethanolic, methanolic and ethyl acetate crude extracts and its biosynthesized PdNPs treatments on *S. typhi* at 40 mg/ml concentration.

In addition, comparative antimicrobial activity of *S. occidentalis* leaf extracts and biosynthesized PdNPs on *S. typhi* using one-way ANOVA revealed no significant difference ($P > 0.05$) between 100, 80, 60 and 40 mg/ml concentrations of the ethanolic and methanolic extracts and biosynthesized PdNPs treatments on *S. typhi*. Moreover, statistical results showed significant difference ($P < 0.05$) between 100, 80, 60 and 40 mg/ml concentrations of ethyl acetate extracts and biosynthesized PdNPs treatments on *S. typhi*. Thus, the study upholds the claims of traditional healers on the usefulness of *S. occidentalis* leaf extracts in the cure of typhoid fever. This is in conformity with [16] findings who reported the effectiveness of the leaf extract at 100 mg/ml. Concurrently the results of this research work empirically proved the evidence of the previous findings that reported antimicrobial activity to have direct relation to increasing the extract concentration [30]. Nel *et al* and Marambio-Jones and Hoek [31, 32] reported that NPs are positively charged and can attach bacterial cell membranes with ease, since its negatively charged to alter membranes physicochemical characteristics by interfering with permeability, osmoregulation and respiratory functions. Nel *et al* and Marambio-Jones and Hoek [31, 32] also stated that NPs interaction with DNA, proteins, nitrogen, sulphur and phosphorus containing molecules in bacterial cell, leading to altered function. They attributed NPs free radicals' formation to be the cause of bacterial membrane damage, that lead to the leakage of cell constituents.

The MICs and MBC of the extracts and biosynthesized PdNps on *S. typhi* showed that *S. typhi* had MIC and MBC ranged from 8.0 to 10.0 mg/ml. The ethanolic and methanolic extracts and biosynthesized PdNPs had MIC and MBC of 8.0 mg/ml. Whereas ethyl acetate extracts and biosynthesized PdNPs MIC and MBC of were not detected. This is in agreement with [17] findings that reported MIC and MBC of 6.0 and 4.0 mg/ml for ethanolic extracts of PdNPs. Gonelimali *et al* and Al-Hashimi [30, 33] reported lower MIC for ethanol extract compared to aqueous extracts.

The antibiofilm activity of *S. occidentalis* extract and biosynthesized PdNPs on *S. typhi* revealed that *S. typhi* was capable of biofilm formation that exhibit intermediate biofilm formation with high OD value of 1.65. This is in conformity with the findings of [21] who reported the OD of 1.74 for *V. chlorae*. It was revealed from present study that biosynthesized PdNps exhibit significant inhibitory activity on biofilm formation with OD value 0.545 compared to the extract with OD of 0.9. This enhance potential might be because of NPs ability to penetrate into the cell [5]. Prasannaraj and Venkatachalam [15] attributed antibacterial and antibiofilm potential to the presence of bioactive compounds, and the interaction between Pd^{2+}, and phosphate and carboxylate groups of lipo polysaccharide present in the cell wall leading to alteration of cell membrane permeability. Prasannaraj and Venkatachalam [15] also associated antibacterial potency of NPs to the size and dose, as smaller size NPs would be transported into the bacterial cell very faster than the larger counterparts.

Based on present findings, the antibacterial and antibiofilm potential of *S. occidentalis* leaf extracts and biosynthesized PdNPs in inhibiting *S. typhi* were revealed and may be considered as an alternative antimicrobial treatment for *S. typhi*.

CONCLUSION

The findings revealed the extracts percentage yield, and phytochemicals constituents of *S. occidentalis* leaf extracts. The phenotypic and genotypic characterizations confirmed the identities of the organism. The statistical results using independent t-test showed no significant difference ($P > 0.05$) between 100, 80 and 60 mg/ml concentrations of the extracts and biosynthesized PdNPs treatments on *S. typhi*. Moreover, statistical results revealed significant difference ($P < 0.05$) between extracts and biosynthesized PdNPs treatments on *S. typhi* at 40 mg/ml concentration. The statistical results using one-way ANOVA showed no significant difference ($P > 0.05$) between all concentrations of the ethanolic and methanolic extracts and biosynthesized PdNPs

treatments on *S. typhi*. Moreover, statistical results revealed significant difference ($P < 0.05$) between all concentrations of ethyl acetate extracts and biosynthesized PdNPs treatments on *S. typhi*.

The MIC and MBC of extracts and biosynthesized PdNps on *S. typhi* ranged from 8.0 to 10.0 mg/ml. The results revealed that *S. typhi* isolate was capable of biofilm formation, with intermediate biofilm of OD 1.65. The antibiofilm assay of *S. occidentalis* leaf extracts and biosynthesized PdNPs on *S. typhi* revealed that biosynthesized PdNps exhibit significant inhibitory effect on biofilm formation with OD of 0.545 compared to the extracts with OD of 0.9. Based on the present findings the antibacterial and antibiofilm potential of *S. occidentalis* leaf extracts and biosynthesized PdNPs in inhibiting *S. typhi* were revealed and may be considered as an alternative antimicrobial treatment for *S. typhi*.

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AUTHORS' CONTRIBUTION

RAU: Data curation, methodology, software, formal analysis, writing original draft, proof read and approved the manuscript. **LD:** Conceptualization, methodology, supervision, data curation, project administration, validation, formal analysis, resources, visualization, writing original draft, proof read and approved the manuscript. **NMS**: Conceptualization, supervision, resources, visualization, validation, writing original draft, proof read and approved the manuscript. **MI**: Data curation, methodology, writing original draft, proof read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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