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EFFECT OF SAMPLE PREPARATION ON PHYTOCHEMICAL CONTENT, SILVER NANOPARTICLES SYNTHESIS, ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *ZINGIBER OFFICINALE* AND *CURCUMA LONGA* SYNERGISTIC COMBINATION

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Keywords:

Hot aqueous extraction, Blending, Silver nanoparticles synthesis, Antioxidant activity, Antibacterial activity

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ABSTRACT: This work compared the effect of hot aqueous extraction and blending on phytochemical content, AgNPs synthesis, antibacterial and antioxidant properties of ginger and turmeric. Qualitative and quantitative phytochemical screening was conducted on a hot aqueous extract of ginger and turmeric combination (CHAE) and blended ginger & turmeric combination (CBLE). An attempt was then made to synthesize silver nanoparticles by reducing 1mM $AgNO_3$ solution with CHAE & CBLE, respectively. The biosynthesized AgNPs were characterized using UV-Visible spectroscopy, FTIR, and SEM. Using existing protocols, the antioxidant and antibacterial properties of CHAE, CBLE, and biosynthesized AgNPs were evaluated. The result of the phytochemical screening revealed the presence of phytochemicals required for synthesizing silver nanoparticles, such as flavonoids and phenols in CBLE but absented in CHAE. The UV-Visible spectrum confirmed the synthesis of AgNPs using CBLE (AgNPs-CBLE), with the highest absorbance peak observed at 412 nm. The FTIR analysis revealed the involvement of phenolic compounds in the bioreduction of Ag^+ as well as the capping/stabilization of the biosynthesized nanoparticles. AgNPs-CBLE were irregularly granulated and highly aggregated, as revealed by the SEM micrograph. The result of the antioxidant activity revealed that the test samples (CHAE, CBLE, and AgNPs-CBLE) possessed a concentration-dependent antioxidant power. Also, antibacterial result revealed that CHAE had no zone of inhibition against *Salmonella typhi* and *Bacillus substilis*. At the same time, CBLE exhibited antibacterial activity against *Salmonella typhi* with an inhibition zone diameter of 20 mm and none against *Bacillus subtilis*. However, AgNP-CBLE exhibited a greater antibacterial activity against Bacillus subtilis and a significant antibacterial activity against Salmonella typhi compared to Levofloxacin. These results indicate that the sample preparation method influenced the phytochemical levels, synthesis of silver nanoparticles, antibacterial and antioxidant capacities of the ginger and turmeric combination.

INTRODUCTION: The World Health Organization (WHO) claims that 65% of the world's popular prefers therapeutic plants; most antioxidant and antibacterial agents are derived from these plants [1-3]. Therefore, these plants' pharmacokinetics and performance can be significantly enhanced if utilized for nanoparticle synthesis [4, 5].

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Nanoparticles are materials that are nanometers, most times 100 nm in size [6]. Nanoparticles are usually used mainly due to their compact size, higher surface area-to-volume ratio, and ability to be used *in-vivo* for drug delivery [7].

Metal nanoparticles are metal precursors, including Pl, Pd, Ag, and Au. Recent investigations on nanoparticle synthesis, characterization, and applications have utilized metal nanoparticles [8-10]. Because of their distinct physical and chemical properties, AgNPs stand out among various metal nanoparticles. Plants' extracted phytochemicals, including flavonoids, polysaccharides, terpenes, alkaloids, phenolics, saponins, and tannins, have

been reported to reduce silver ions 11-13 rapidly. In phytochemicals, hydroxyl, aldehyde, ketone, carboxyl, and amino groups can reduce Ag^+ ions [14]. However, plant source, a combination of two or more plant materials, and preparation of plant material (extract preparation) are some factors determining the quantity and mixtures of phytochemicals present in a particular plant extractive [15].

This implies that the properties of biosynthesized AgNPs can be selected or altered by controlling extract composition [16]. AgNPs have been synthesized using aqueous extract of ginger and turmeric separately/ singly [17, 18] however in this study, an attempt was made to optimize the synthesis and properties of silver nanoparticles by making use of differently prepared (blended and hot aqueous extracts) ginger and turmeric combination.

MATERIALS AND METHOD: The materials (ginger and turmeric) used for this study were acquired from the market and authenticated by a Botanist at Covenant University. Analytical grade reagents and chemicals were used.

Sample Preparation: Both materials were cleaned under running tap water; the skins of the samples were peeled separately using a sterile table knife, chopped into smaller fractions, and washed again with distilled water.

Preparation of Extract: Adesipe and Iweala 19, Ogori *et al.,* 20 were employed for extract preparation with little modification. In brief, 25g of chopped turmeric and 25g of chopped ginger rhizomes were weighed and completely blended with 100 mL of distilled water, sieved, and labeled sample CBLE (Combined Blended extract). The hot water extract of ginger and turmeric combination (CHAE) was obtained by heating 25 g each of chopped ginger and turmeric rhizomes in 100ml of distilled water at 60 ^oC in a water bath for 15 minutes.

Phytochemical Analysis: Phytochemical analysis was performed on CBLE and CHAE for secondary metabolites identification and quantification using the phytochemical methods which have been previously described [21, 22].

Synthesis of Silver Nanoparticles: In two flasks containing 90ml of aqueous 1 mM $AgNO_3$ each, 10 ml of CBLE and CHAE were introduced separately [23, 24].

Characterization of Biosynthesized AgNPs: UV-Vis spectrophotometer was used to validate the bioreduction of Ag^+ into AgNPs between 200 and 600 nm. The biosynthesized AgNPs' FTIR spectra were obtained between 350 and 4400 cm-1 using a Nicolet IS5 model from Thermos Scientific with a resolution of 0.4cm-1. The size of the AgNPs was determined using a scanning electron microscope.

Determination of Antioxidant Property: The antioxidant activities of the test samples (CHAE, CBLE, biosynthesized AgNPs, and Ascorbic acid) were assessed by analyzing their 1,1-diphenyl-2-picrylhydrazyl (DDPH), reducing power and Nitric oxide radical scavenging activities.

Test Samples' Scavenging Capacity of DPPH Radical: The test sample's capacity to scavenge DPPH was assessed by mixing various doses (25-100 µg/ml) of either CHAE, CBLE, AgNPs-CBLE, or Ascorbic acid (standard) to newly prepared 200 M DPPH methanolic solution in the dark. After 30 minutes, absorption at 517 nm was measured for each reaction combination 25, 26.

The following formula was used to compute radical scavenging activity:

$$\% \text{ Scavenging activity of DPPH} = (ABS_{control} - ABS_{sample}) / (ABS_{control}) \times 100$$

$ABS_{control}$ equals DPPH + methanol's absorbance, while ABS_{sample} equals DPPH + sample's (CHAE/CBLE, / AgNPs-CBLE / standard), respectively.

Reducing Power Determination of Test Samples: To evaluate the reducing power of the test samples (CHAE, CBLE, and biosynthesized AgNP), different concentrations of the sample (25 100 µg/ml) were combined with phosphate buffer (0.2 mol/L, pH 6.6, 2.5 ml), $K_3Fe(CN)_6$; potassium ferricyanide (1%, 2.5 ml) and at 50oC incubated for 20 min. The mixture was centrifuged for 10 minutes after adding trichloroacetic acid (10%, 2.5 mL). At 700 nm, absorbance was read after adding distilled water to the supernatant (2 ml each) and

0.5 ml of 0.1% $FeCl_3$. The reaction mix's enhanced absorbance suggested greater reducing power [27].

Test Sample's Scavenging Activity of Nitric Oxide: The method of Garret [28] as described by Venkatachalam and Muthukrishnan [29] was followed for this determination. In brief, 5mM Sodium nitroprusside; $Na_2[Fe(CN)_5NO]$ in phosphate-buffered saline, pH 7.3 and varied doses of the test samples were combined to make a reaction mix of 5 ml and incubated at 25°C for 3 hours. The radical generated (nitric oxide) coupled with oxygen to form nitrite ion was quantified by adding an incubation mixture (1 ml) with the same quantity of Griess reagent at 30-minute intervals. Following nitrite ions diazotization with sulfanilamide and naphthylethylenediamine dihydrochloride coupling, the chromophore (purple azo dye) was measured at 546 nm.

Antibacterial Activity Determination:

Preparation of Samples for Antimicrobial Assay: The samples; Hot aqueous extract (CHAE), bended extract (CBLE), and the biosynthesized Silver nanoparticles (AgNPs-CBLE) were kept in the oven at 60 ^oC until they were dried completely [4]. The resultant dried extracts were dissolved in sterile distilled water to make solutions with concentrations ranging between 190-310 mg/mL. The test organism (*Salmonella typhi* and *Bacillus typhi*) used for this study were clinical isolates collected from the University of Lagos Microbiology Laboratory.

Antibacterial Analysis of Test Samples: Agar well diffusion method was used to analyze the test samples' antibacterial efficacy following Adesipe and Adebayo [23], Abhay and Rupa [30] with appropriate modifications.

Before pour plating, 1ml of calibrated test organisms were seeded separately into warm agar and mixed thoroughly using the roll-palm method. Wells of 10 mm diameter were drilled using a cork borer after the nutrient agar plates had solidified. About 300mg/ml of 100% PBE and UPBE, as well as 6.25 g/ml of Levofloxacin, were placed into the wells and left to stand for several hours to allow proper diffusion. The plates were checked for zones of inhibition after a 24-hour incubation period at 37°C.

RESULT AND DISCUSSION:

Phytochemical Screening: Because plants contain active biological components, they provide basic raw materials for some of the most important drugs [31, 32, 33]; however, methods of plant processing have been reported to affect their phytochemical content, which subsequently could affect their efficiency for drug development or therapeutic action [34]. The result of the phytochemical content of the hot aqueous extract of ginger: turmeric combination (CHAE) and blended extract of ginger: turmeric combination (CBLE) are presented in **Tables 1** and **2.** The phytochemical screening of CHAE and CBLE revealed the presence of steroids, saponin, cardiac glycoside, and phlorotannin in both extracts. However, additional phytochemicals such as reducing sugar, tannin, flavonoid, phlorotannin, cardiac glycoside, and phenol were confirmed to be present in CBLE. The disparity in the phytochemical contents of CHAE and CBLE could be due to the sample sizes and the low temperature that was used to prepare CHAE. The efficiency of extraction, that is, solubility/diffusion of bioactive components, is usually improved by tiny particle size and relatively high temperatures [35].

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF CHAE AND CBLE

Test	CHAE	CBLE
Steroid	+	+
Terpenoid	-	-
Reducing sugar	-	+
Tannin	-	+
Anthraquinone	-	-
Saponin	+	+
Flavonoid	-	+
Cardiac glycoside	+	+
Phenol	-	+
Phlobatanin	+	+

Key: + = present, - = Not present.

The phytochemical result obtained for a blended extract of ginger and turmeric combination in this study is superior to the single phytochemical contents reported for ginger and turmeric extract. For example, Arawande *et al.*, [36] reported the absence of phenol, tannin, saponin, and glycosides in the aqueous extract of ginger and also reported the absence of phenol, tannin, reducing sugar, phlorotannin in aqueous extract of turmeric, however, they were present in the combination of ginger and turmeric in this study.

TABLE 2: QUANTITATIVE PHYTOCHEMICAL SCREENING OF CHAE AND CBLE

Test	CHAE	CBLE
Steroid	23.890 ± 0.113	27.43 ± 0.16
Terpenoid	-	-
Reducing sugar	-	29.46± 0.23
Tannin	-	23.54 ± 0.2
Saponin	29.760 ± 0.467	40.475 ± 0.525
Flavonoid	-	40.835 ± 0.135
Cardiac glycoside	28.555 ± 0.177	29.535± 0.21
Phenol	-	32.825 ± 0.275

Note: Values are represented as mean ± SEM of duplicate.

Silver Nanoparticles Biosynthesis: Silver nanoparticles were successfully synthesized using a blended ginger and turmeric combination by a cost-effective and environmentally friendly pathway. The synthesis was visually confirmed by the change of colour of the reaction mixture of $AgNO_3$+CBLE from grey to dark brown **Fig. 1D, E.** Several studies have reported this colour change for silver nanoparticles as AgNPs usually look brownish in the aqueous medium due to surface Plasmon vibrations [37-41]. However, no colour change was observed for $AgNO_3$ + CHAE **Fig. 1a, B,** indicating that CHAE could not reduce Ag^+ to Ag^0. This could be due to the absence of bioactive compounds such as phenol and flavonoid that have been reported to rapidly reduce silver ions in CHAE [11-13].

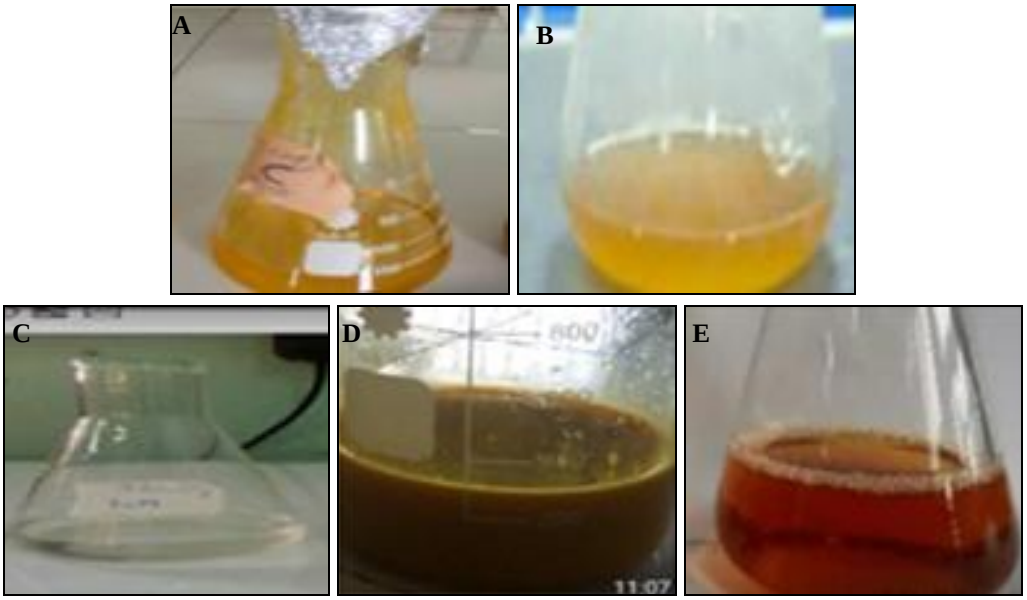

FIG. 1: (A) CHAE (B) $AGNO_3$+CHAE (C) $AGNO_3$ SOLUTION (D) CBLE (E) $AGNO_3$+CBLE

Characterization of Biosynthesized Silver Nanoparticles:

UV-Visible spectrophotometry: Since UV-Visible analysis has been named to be the simplest and fastest method for the confirmation of silver nanoparticle synthesis [42], the reaction mixtures of $AgNO_3$ + CHAE and $AgNO_3$+CBLE were both subjected to UV-visible analysis. No peak was observed for $AgNO_3$ + CHAE **Fig. 2A** but was observed for $AgNO_3$+CBLE at 412 nm **Fig. 2B**. This similar peak value has been reported for several biosynthesized AgNPs [19, 43].

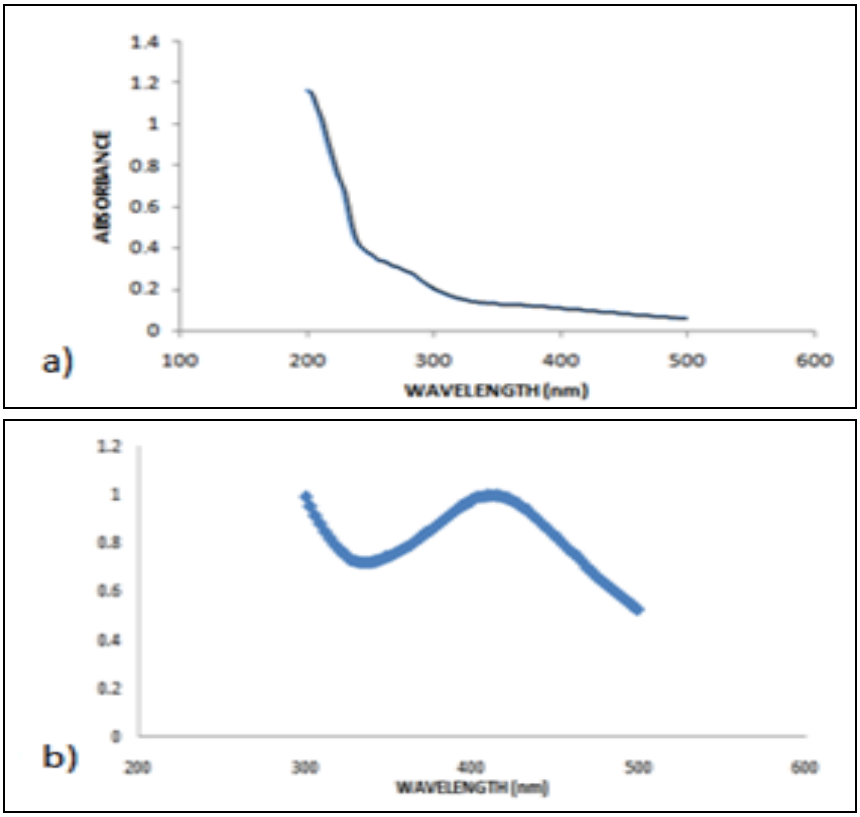

FIG. 2: RESULT OF UV- VIS SPECTROSCOPY OF (A) $AGNO_3$ + CHAE AND (B) AGNPS + CBLE

FTIR (Fourier Transform Infrared) Spectroscopy: The FTIR spectrum of the biosynthesized AgNPs showed major absorption peaks at ~3300 cm^{-1}, 2100 cm^{-1}, 1630 cm^{-1} revealing the presence of OH– stretching, C=N– stretching, and NH–stretching respectively **Fig. 3**. The OH–bond stretching establishes explicitly the involvement of phenolic compounds in the bioreduction of Ag^{+} as well as the capping/ stabilization of the biosynthesized nanoparticles [44, 45]

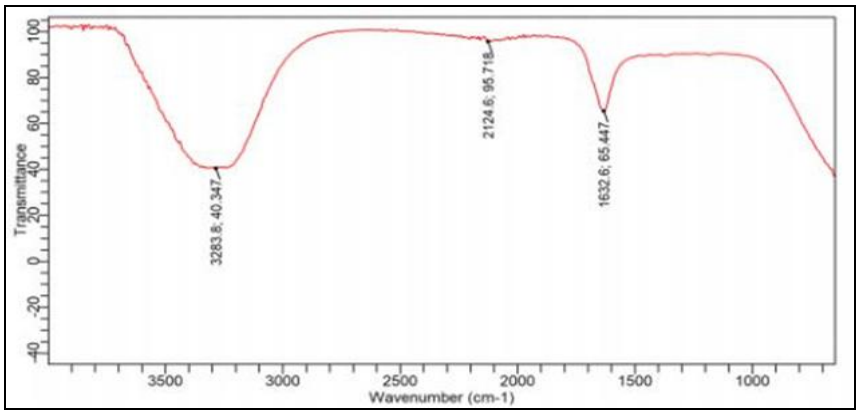

FIG. 3: FOURIER TRANSFORMED INFRARED SPECTRUM OF AGNPS-CBLE

Scanning Electron Microscopy: The SEM micrograph revealed that AgNPs-CBLE were irregularly granulated and highly aggregated.

The sizes of AgNPs-CBLE from the SEM analysis were found to be 20-30 nm **Fig. 4**. This nanoparticle range was reported earlier in literature [46, 47].

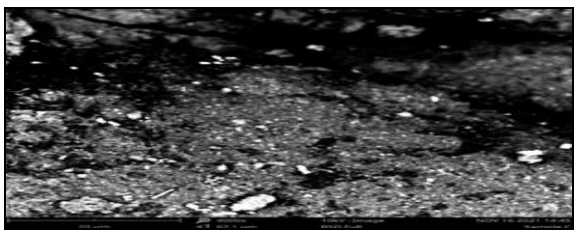

FIG. 4: RESULT SHOWING SCANNING ELECTRON MICROSCOPY OF AGNPS-CBLE

Antioxidant Activity:

Scavenging Capacity of DPPH Radical: The test samples' scavenging capacity of DPPH radical is shown in **Fig. 5** below. Radical scavenging capacity increases with a concentration in all of the samples.

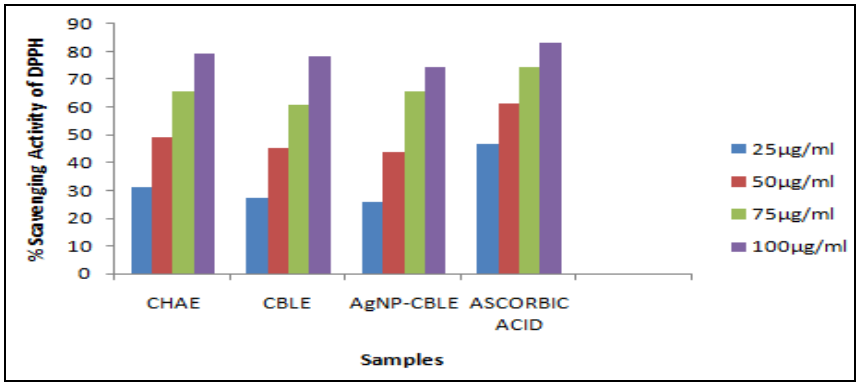

FIG. 5: SCAVENGING CAPACITY OF DPPH OF TEST SAMPLES; COMBINED HOT AQUEOUS EXTRACT OF GINGER AND TURMERIC (CHAE), COMBINED BLENDED EXTRACT OF GINGER AND TURMERIC (CBLE), AGNPS-CBLE, AND ASCORBIC ACID. RESULTS ARE REPORTED AT CONCENTRATIONS OF 25, 50, 75 AND 100 µg/mL AS THE MEAN ± STANDARD DEVIATION (N = 3)

Reducing Power Activity: The test samples' capacity to donate an electron is depicted below in **Fig. 6**. The capacity of all the samples rises with concentration.

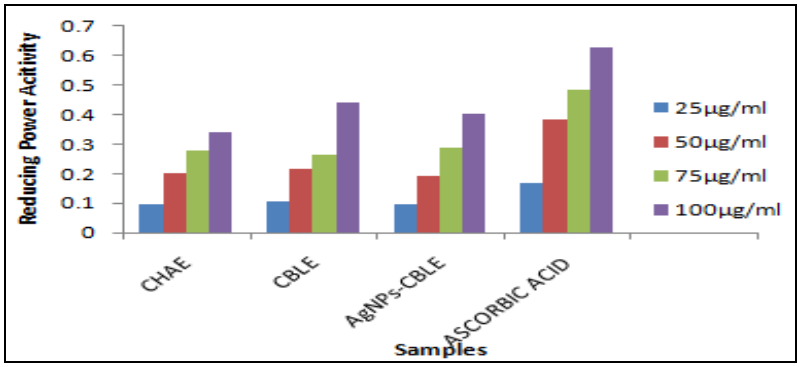

FIG. 6: REDUCING POWER ACTIVITY OF COMBINED HOT AQUEOUS EXTRACT OF GINGER AND TURMERIC (CHAE), COMBINED BLENDED EXTRACT OF GINGER AND TURMERIC (CBLE), AGNPS-CBLE, AND ASCORBIC ACID. RESULTS ARE REPORTED AT CONCENTRATIONS OF 25, 50, 75 AND 100 µg/mL AS THE MEAN ± STANDARD DEVIATION (N = 3)

Scavenging Capacity of Nitric Oxide: The test samples' scavenging capacity of nitric oxide is shown in **Fig. 7** below. The scavenging capacity of all the samples rises with concentration.

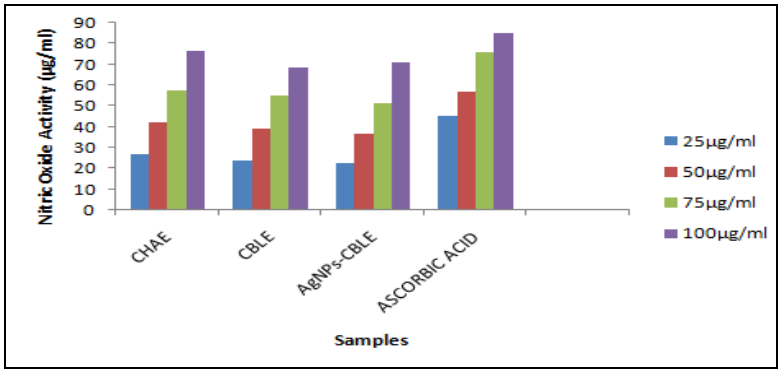

FIG. 7: SCAVENGING CAPACITY OF NITRIC OXIDE RADICAL OF TEST SAMPLES; COMBINED HOT AQUEOUS EXTRACT OF GINGER AND TURMERIC (CHAE), COMBINED BLENDED EXTRACT OF GINGER AND TURMERIC (CBLE), AGNPS-CBLE AND ASCORBIC ACID. RESULTS ARE REPORTED AT CONCENTRATIONS OF 25, 50, 75 AND 100 µg/mL AS THE MEAN ± STANDARD DEVIATION (N=3)

Antibacterial Activity: The antibacterial result revealed that CHAE had no zone of inhibition against the test organism used (*Salmonella typhi* and *Bacillus substilis*). At the same time, CBLE exhibited antibacterial activity against *Salmonella typhi* with a zone of inhibition of 20 mm and none against *Bacillus subtilis*. However, AgNP-CBLE exhibited a greater antibacterial activity against *Bacillus subtilis* and a significant antibacterial activity against *Salmonella typhi* compared to the standard Levofloxacin (**Fig. 8.** The result of the antibacterial activity supports the motion that biosynthesized AgNPs have enhanced therapeutic action when compared with plant extracts alone [48].

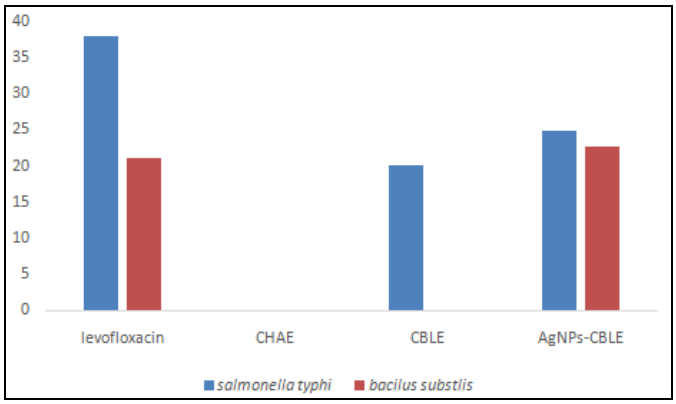

FIG. 8: COMPARISON OF INHIBITION ZONE DIAMETER (MM) OF CHAE, CBLE, AGNPS-CBLE AND LEVOFLOXACIN ON *SALMONELLA TYPHI* AND *BACILLUS SUBTILIS*

CONCLUSION: The result of this study indicates that the method of extract preparation of ginger and turmeric combination (blending and hot aqueous extraction) influenced their phytochemical contents, their use for silver nanoparticle synthesis, and consequently, their medicinal property. Blending is therefore considered a remarkable approach for preparing ginger and turmeric based on the findings of this research. In addition, the use of blended ginger and turmeric combinations could be optimized for synthesizing silver nanoparticles.

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CONFLICTS OF INTEREST: Nil

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