Antioxidant and anti-inflammatory activities of silver nanoparticles biosynthesized from aqueous leaves extracts of four *Terminalia* species

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Abstract

The environmentally friendly synthesis of nanoparticles process is a revolutionary step in the field of nanotechnology. In recent years plant mediated biological synthesis of nanoparticles has been gaining importance due to its simplicity and eco-friendliness. In this study, a simple and an efficient eco-friendly approach for the biosynthesis of stable, monodisperse silver nanoparticles using aqueous extracts of four *Terminalia* species, namely, *Terminalia catappa*, *Terminalia melluera*, *Terminalia bentazoe* and *Terminalia bellerica* were described. The silver nanoparticles were characterized in terms of synthesis, capping functionalities (polysaccharides, phenolics and flavonoidal compounds) and microscopic evaluation by UV-visible spectroscopy, Fourier transform infrared spectroscopy and transmission electron microscopy. The results showed a simple and feasible approach for obtaining stable aqueous monodisperse silver nanoparticles. Furthermore, biological activity of the biosynthesized silver nanoparticles was examined. Concerning this, dose-dependent antioxidant activity of silver nanoparticles imparted by the plant phenolic and flavonoidal components was evaluated using *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and found to be comparable to standard ascorbic acid. The same holds true for the anti-inflammatory activity where *Terminalia catappa* and *Terminalia melluera* have a high-test inhibition percentage better than that of ascorbic acid in the carrageenan induced hind paw edema. The results also revealed that the aqueous extract of *Terminalia catappa* and its silver nanoparticles recorded the most potent *in vivo* antioxidant effect.

Keywords: silver nanoparticles, plant extracts, *Terminalia* species, antioxidant activity, anti-inflammatory activity

Classification numbers: 2.04, 2.05, 4.02

1. Introduction

The genus *Terminalia* is the second largest genus in the Combretaceae which is widespread in Egypt and other subtropical and tropical regions of the world [1]. The family Combretaceae is comprised of 20 genera and about 475 species [2]. Of these, about 200 belong to the genus *Terminalia*, making it the second largest genus of the family after *Combretum* [3]. About 30 species of *Terminalia* are found in Africa [4]. Species of *Terminalia* vary greatly in morphology, anatomy and karyotype evidence [5, 6].

It has been reported that the plant extract of the genus *Terminalia* is commonly rich in phenolics, flavonoids, alkaloids, triterpenoids, tannins and other compounds [7–12]. Because of these phytoconstituents, the majority of *Terminalia* species have multiple biological, pharmacological and medicinal activities [13–20].

For the last decade, many researchers have directed their attention to develop new, inexpensive and most effective...
drugs from plant sources based on nanotechnology [21–23]. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology. Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction at cell level between synthetic and biological materials with the help of nanoparticles. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The biological synthesis of nanoparticles is emerging as an eco-friendly and exciting approach in the field of nanotechnology. Use of plants for the biosynthesis of nanoparticles does not require high energy or temperatures, and it is easily scaled up for large-scale synthesis, and it is cost effective too [24, 25].

A survey of literature revealed that very limited species of the genus Terminalia are used for the biosynthesis of metal nanoparticles [26–28]. To fill this gap, this work was undertaken with the objectives of (a) biosynthesizing silver nanoparticles using the aqueous extracts of the four Terminalia catappa, bellerica, bentzoe and muelleri species; (b) searching the proper conditions for synthesizing these particles; (c) characterizing the formed silver nanoparticles (AgNPs); and (d) evaluating the antioxidant and anti-inflammatory activities of the biosynthesized AgNPs.

2. Experimental

2.1. Plant material

Green leaves of Terminalia catappa (T. catappa), Terminalia bellerica (T. bellerica), Terminalia bentzoe (T. bentzoe) and Terminalia muelleri (T. muelleri) were collected from the Giza Zoo Garden, Cairo, Egypt, in March 2012. The plant leaves were washed thoroughly with tap water to remove all attached materials and then rinsed with distilled water and air dried under shade at room temperature. The dried leaves were finely powdered using an electric grinder and used for aqueous extraction.

2.2. Extraction

The air-dried powdered leaves of T. catappa, T. bellerica, T. bentzoe and T. muelleri (5 g of each) were mixed with 100 ml of deionized water and heated at 90 °C on temperature controlled water bath for 1 hr. The aqueous extracts were filtered and the filtrates were individually stored at 4 °C.

2.3. Chemical analysis of the extracts

2.3.1. Phytochemical screening. The preliminary phytochemical screening tests were carried out to identify the leaves constituents by standard methods [29–31].

2.3.2. Moisture, total ash and protein contents. Using weight difference, moisture and total ash were done using the method reported by association of analytical community (AOAC) [32]. The protein contents were estimated as total nitrogen by the procedure adopted by Pearson using micro-Kjeldhal method [33]. Crude protein was subsequently calculated by multiplying the nitrogen content by 100/16 or 6.25. The factor 6.25 is derived from the generalization that most proteins contain 16% nitrogen.

2.3.3. Extraction of water soluble polysaccharides. 50 g of the dried powdered leaves were extracted in distilled water at 80 °C for 3 hrs. The extracts were filtered and concentrated under reduced pressure at a temperature not exceeding 40 °C. The hot water soluble polysaccharides were precipitated by the addition of 4-fold volume of 95% (v/v) ethanol and the precipitated polysaccharides were filtered, washed twice with absolute ethanol and oven-dried at 40 °C to obtain the crude hot water soluble polysaccharides (chwp).

2.3.4. Acid hydrolysis and silylation of polysaccharides. The precipitated polysaccharides (0.1 g) of each studied plant were added to 10 ml 1 N H2SO4 and heated in a boiling water bath for 5 h to hydrolyze polysaccharides. BaCO3 was added then centrifuged and the precipitate was washed twice by water, then the solution was evaporated until the volume reached 2 ml hydrolyzate. A part of the hydrolyzate solution (0.5 ml) was evaporated in small screw-topped septum vials to dryness under a stream of nitrogen at 40 °C. When almost dry, 0.5 ml isopropanol was added and the drying was completed under a stream of nitrogen until a dry solid residue remained. To this residue, 0.5 ml of 2.5% hydroxylamine hydrochloride in pyridine was added, mixed and heated for 30 min at 80 °C then allowed to cool. 1 ml of trimethylchlorosilane: N,O-bis-(trimethylsilyl) acetamide (1:5 by volume) was added, mixed and heated for 30 min at 80 °C.

2.3.5. Gas-liquid chromatography (GLC) of the polysaccharide hydrolyzate. 1 μl of the silylated sugars was subjected to GLC analysis adopting the conditions listed in table 1.

2.3.6. Total phenolic assay. The concentration of phenolics in each plant leaves extract was determined using the Folin–Ciocalteu method [34]. 0.5 ml of the plant leaves extract solution was added to 2.5 ml of freshly prepared Folin–Ciocalteu reagent (0.2 N) and mixed for 5 min, followed by the addition of 2 ml of the 75 g l−1 sodium

Table 1. Conditions of GLC analysis of the polysaccharide hydrolyzate.

| Column            | ZB-1701, 30 m × 0.25 m × 0.25 μm |
|-------------------|----------------------------------|
| Stationary        | 14% cyanopropyl phenyl methyl    |
| Carrier gas       | Helium at 1.2 ml min⁻¹, 10.6 psi |
| Temperature       | Initial temperature: 150 °C for 2 min with a rate 7 °C min⁻¹, final temperature: 200 °C for 20 min. |
| Injector chamber  | 250 °C                           |
| Detector          | FID at 250 °C                    |
carbonate ($\text{Na}_2\text{CO}_3$). After incubation at room temperature for 2 hrs, the absorbance of the reaction mixture was measured at 760 nm against methanol blank. Gallic acid was used as standard to produce the calibration curve. The total phenolic content was expressed in g of gallic acid equivalents (GAE)/100 g of plant.

2.3.7. Total flavonoid assay. The total flavonoid content (expressed as quercetin equivalent, g QE/100 g) in the examined plant extracts was determined using the modified Quettier–Deleu et al method [35]. 2% AlCl$_3$ solution in methanol was mixed with the leaves extract solution. Absorbance readings at 415 nm were taken after 10 min against a blank sample consisting of a 5 ml leaves extract solution with 5 ml methanol without AlCl$_3$. The total flavonoid content was determined using a standard curve with quercetin as a standard. The total flavonoid content was expressed in g of quercetin equivalents (QE)/100 g of plant leaves extract.

2.4. Synthesis of AgNPs

The aqueous leaves extracts prepared from the four $Terninalia$ species were used for synthesis of AgNPs according to the following procedure: 10 ml of the prepared extract was added to a defined volume of distilled water, the pH of the solution was adjusted to 11, which is the optimum pH as the results of the preliminary experiments made in this work indicated. To this solution, 0.017 g of Ag NO$_3$ dissolved in 10 ml of distilled water was added and the total volume was adjusted to 100 ml with distilled water. The reaction mixture was kept under continued stirring at 60 °C using a magnetic stirrer for different durations (15, 30, 45, 60 and 75 min). The visual change of colour from yellow to reddish brown indicated the formation of silver nanoparticles (AgNPs). The coloured colloidal solution formed was checked by diluting HCl for complete conversion of AgNO$_3$ to AgNPs where no white precipitate is formed.

2.5. Characterization of AgNPs

2.5.1. UV–Vis spectroscopy. The initial characterization of the synthesized AgNPs was carried out using UV–Vis spectroscopy. The reduction of silver ions was monitored from 250 to 500 nm by Jasco V-670 UV-V after 5-fold diluting the sample with distilled water against distilled water as blank. The measurements were carried out as a function of reaction time at room temperature.

2.5.2. Fourier transform infrared (FTIR) spectroscopy. The binding properties of AgNPs synthesized by different $T$. catappa, $T$. bellerica, $T$. bentzoe and $T$. muelleri leaves extracts were investigated by FTIR analysis using a JASCO FT-IR 4100 instrument in the diffuse reflectance mode at a resolution of 4 cm$^{-1}$ in KBr pellets. Prior to analysis, AgNPs were purified, dried and palleted with potassium bromide. For comparison, $T$. catappa, $T$. bellerica, $T$. bentzoe and $T$. muelleri leaves extracts were freeze dried, palletized to be used as a control.

2.5.3. Transmission electron microscopy (TEM). The size and morphology of the synthesized AgNPs were determined by high resolution transmission electron microscopy (HR-TEM, JEOL JEM 2100). The sample for TEM studies was prepared as follows. 1 ml of the reaction mixture containing AgNPs was diluted to 10 ml, sonicated using ultrasonic bath and a drop of it was placed on a Cu grid with ultrathin Cu on holey C-film and it was allowed to dry in a vacuum. The instrument was operated with an acceleration voltage of 200 kV.

2.6. Anti-oxidant activity

2.6.1. In vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. In order to evaluate the in vitro free radical scavenging activity of each aqueous plant leaves extract (blank) as well as the corresponding AgNPs colloidal solutions, DPPH assay [36] was used. 2 ml of DPPH solution in ethanol (100 $\mu$M) was separately mixed with 2 ml of the plant extract and its own AgNPs colloidal solution (100 $\mu$g ml$^{-1}$). The effective test concentrations of DPPH and the plant extract or its AgNPs solution were 50 $\mu$M and 50 $\mu$g ml$^{-1}$, respectively. The reaction mixture with different dilutions (10, 50 and 100 $\mu$g ml$^{-1}$) for different concentrations was incubated in the dark for 15 min and thereafter the optical density was recorded at 517 nm against blank. For control, 2 ml of ethanol was added instead of plant mixture and run simultaneously with the test. The scavenging percentages of DPPH with plant extracts and nanoparticles were calculated by the following formula:

$$S_a(\%) = \frac{A_c - A_t}{A_c} \times 100\%,$$

where $S_a$ is free radical scavenging, $A_c$ is the absorbance of the control, while $A_t$ is the absorbance obtained from the sample.

2.6.2. In vivo anti-oxidant activity. The extract that recorded the highest in vitro antioxidant effect was subjected to in vivo antioxidant evaluation.

Animals. Male Wistar albino rats (100:120 g) were selected for this study. They were obtained from the animal house, National Research Center, Egypt. All animals were kept in a controlled environment of air and temperature with the access of water and diet ad libitum.

Ethics. Anesthetic procedures and handling with animals complied with the ethical guidelines of the Medical Ethical Committee of National Research Centre in Egypt.

2.6.3. In vivo antioxidant effect of $Terninalia$ catappa ($T$. catappa) in normal rats. As $T$. catappa showed the highest in vitro antioxidant effect, it is subjected to extra in vivo antioxidant evaluation. Its ethanolic extract, polysaccharides and silver
nanoparticles were examined. Three groups of rats (5 rats each) were orally given T. catapatal ethanol extract, polysaccharides and its silver nanoparticles at a dose of 250 mg kg\(^{-1}\) body weight [37]. A fourth group served as a control. 24 h after administration, rats were sacrificed and dissected by abdominal midline incision using diethyl ether. The oxidative stress markers (glutathione, malondialdehyde, and superoxide dismutase) were estimated in the liver tissue.

Glutathione (GSH) was assayed according to the method of Moron et al. [38]. The method is based on the development of a relatively stable yellow colour when 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) is added to sulphydryl compounds at 412 nm.

Malondialdehyde was assayed according to the method of Buege and Aust [39]. Malondialdehyde is an unstable compound that decomposed to form a complex series of reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generated malondialdehyde (MDA) which has been used as an indicator of lipid peroxidation process. Its concentration was calculated using the extinction coefficient value 1.56 \times 10^5 \text{M}^{-1} \text{cm}^{-1} and read at 535 nm.

Superoxide dismutase (SOD) in liver tissue was assayed according to Nishikimi et al. [40]. SOD determination is based on the oxidation of nicotinamide adenine dinucleotide reduced disodium salt (NADH) mediated by superoxide radical through a free radical chain of reactions involving thiol oxidation and univalent \(O_2\) reduction. The increase in NADH oxidation was measured at 560 nm using its molar extinction coefficient 6.22 \times 10^3 \text{M}^{-1} \text{cm}^{-1}.

### 2.7. Anti-inflammatory studies

Anti-inflammatory activities of the aqueous leaves extracts and their AgNPs colloidal solutions were evaluated by using carrageenan induced hind paw edema model [41]. The 36 Wistar albino rats were divided into six groups comprising six animals in each group \((n = 6)\) as shown in Table 2. The animals were starved overnight and deprived of water only during the experiment. Inflammation of the hind paw was induced by injecting 0.1 ml of 1% w \(v^{-1}\) carrageenan suspension prepared with 1 ml saline into the sub-plantar surface of the right hind paw. The negative control group was treated with a dose of 1 ml kg\(^{-1}\) body weight. The positive control group was treated with Indomethacin (20 mg kg\(^{-1}\) body weight). The remaining four groups received the aqueous leaves extracts of four Terminalia species at the dose of 50 mg kg\(^{-1}\) body weight. All the treatments were given one hour before the carrageenan injection. Four hours after oral aminstraion, the rats were sacrificed; both hind paws were, separately, excised and weighed. The oedema (%) and oedema inhibition (%) are defined as follows:

\[
\text{Oedema} (\%) = \frac{W_l - W_r}{W_r} \times 100%,
\]

\[
\text{Oedema inhibition} (\%) = \frac{M_r - M_c}{M_c} \times 100%,
\]

where \(W_l\) is weight of right paw, \(W_r\) is weight of left paw, \(M_r\) is the mean oedema (%) in control group and \(M_c\) is the mean oedema (%) in the drug—treated group.

### 3. Results and discussion

#### 3.1. Phytoconstituents

Qualitative screening for the presence of various phytochemical compounds was performed using aqueous leaves extract. The preliminary phytochemical results obtained showed the presence of some phytochemicals in the four Terminalia species under investigation. These embrace carbohydrates, terpenoids, steroids flavonoids, phenols, tannins, proteins, amino acids and alkaloids (table 2). Data of table 2 show also that fats/oils, saponins, cardiac glycosids and anthraquinones were not detected in any extract.

The quantitative analysis of these constituents (table 3) revealed that the highest yield (%) of the hot water extract (HWE) are observed in T. melliueri (12%), T. bentazo (11.8%), followed by T. bellerica (11%) and T catapata (10%). Meanwhile the highest phenolic and flavonoid contents are observed in T. melliueri aqueous leaves extract (15.86%, 11.86%), followed by the T. catapata (12.2%, 9.5%), T. bentazo (11.34%, 8.22%) and T. bellerica (7.11%, 4.8%), respectively. Table 3 discloses that the highest percent yield of the polysaccharides (PS) is present in the T. melliueri (7.2%). Data of this table shows also that the order obtained from the total protein content is T. bellerica > T. bentazo > T. catapata > T. melliueri extract. The T. bellerica extract presented a significant protein content being clearly distinct from all the other species extracts. A close variation between the contents was given by the T. bentazo and T.catapata extracts.

GLC analysis of the polysaccharide hydrolyzate indicates that T. catapata, T. melliueri and T. bentazoz contain glucose as the main aldohexose sugar in their hydrolyzate (33.03%, 32.83% and 32.03%, respectively), meanwhile T. bellerica contains xylene and mannose as the main sugars in its hydrolyzate (table 4).

### Table 2. Phytochemical constituents detected in aqueous leaves extracts of Terminalia species.

| Phytochemical constituents | T. catapata | T. bellerica | T. muelleri | T. bentze |
|----------------------------|-------------|-------------|-------------|-----------|
| Carbohydrates              | +           | +           | +           | +         |
| Fats/Oils                  | –           | –           | –           | –         |
| Saponins                   | –           | –           | –           | –         |
| Terpenoids                 | +           | +           | +           | +         |
| Steroids                   | +           | +           | +           | +         |
| Flavenoids                 | +           | +           | +           | +         |
| Phenolics/Tannins          | +           | +           | +           | +         |
| Cardiac glycosids          | –           | –           | –           | –         |
| Proteins/Amino acids       | +           | +           | +           | +         |
| Alkaloids                  | +           | +           | +           | +         |
| Anthraquinones             | –           | –           | –           | –         |

+ present, – absent.
The qualitative and quantitative phytochemical analyses of the aqueous leaves extracts of the four *Terminalia* species in this work have shed light on their antioxidant and anti-inflammatory propensities. Many useful chemical constituents that have vast pharmacological properties has been screened out, which thereby paved the way to estimating their free radical scavenging and anti-inflammatory activities.

### 3.2. Visual inspection and UV-Vis spectroscopy analysis

Biosynthesis of AgNPs at pH11 from aqueous solution of AgNO₃ with the aqueous leaves extracts of the four *Terminalia* species was confirmed by UV–Vis spectral analysis. The color of the reaction mixture changed from pale green to brown due to excitation of surface plasmon resonance (SPR) vibration of AgNPs. Figure 1 showed that formation of AgNPs started within 15 min of the reaction time. SPR peaks centered at 401, 433, 445 and 455 nm for the AgNPs prepared from *T. catapa*, *T. bellerica*, *T. bentazoe* and *T. mellueri*, respectively. Irrespective of the *Terminalia* species used, the intensity of SPR and absorbance increases as the reaction time increased up to 75 min from 250 nm to 500 nm. There was no change in SPR means that the stability of the colloidal solution of AgNPs within the reaction time period (15–75 min).

The stability of the AgNPs was studied for a period of three months. UV-Vis spectral analysis of the lyophilized silver nanoparticles was carried out to check its stability after dispersion in water. The results showed similar mono-dispersity and chemical stability of the nanoparticles which are well dispersed in the solution without any aggregation.

### 3.3. FTIR analysis

It has been reported [42] that plant constituents involved in the reduction and capping of nanoparticle can be identified by FTIR technique. The FTIR measurements of the biosynthesized AgNPs were carried out to identify the interaction between bio-organics of aqueous leaves extracts and nanoparticles.

FTIR spectra of AgNPs in figures 2–5 showed absorption peaks located at 3411, 2925, 1617, 1389, 1049, 835 and 699 cm⁻¹ for all formed AgNPs most of these bands were found to be corresponding to peaks in the aqueous leaves extracts spectra. The very intense broad band located at 3411 cm⁻¹ position in the spectrum corresponds to –OH structural polymeric association stretching. The stretch located at 2925 cm⁻¹ represents the C–H stretching of the alkyl group or chelating compounds while the stretching vibration at the absorption peaks located at 1617 cm⁻¹ in all spectra corresponds to –CONH stretch. The presence of 1389 cm⁻¹ stretch in the FTIR spectra indicated the presence of the C–N stretching vibration of the aromatic amines or germinal methyls, while the band appearing at 1049 cm⁻¹ represents to the C–N stretching vibration of aliphatic amines or ether linkages. The presence of the N–H wags of primary and secondary amine confirmed by the band at 699 cm⁻¹ stretch in all spectra and the band at 579 cm⁻¹ represents the C–C groups or aromatic rings. These stretching vibrations indicated that the different terpenoids and proteins were abundant in aqueous leaves extract and are responsible for the bioreduction of Ag ions. The bioreduction reaction, in the present study, was done in the aqueous medium. Therefore, it was inferred that some of the bio-organics of the aqueous leaves extract such as proteins, flavenoids, phenols and polysaccharides bind to Ag atoms and are responsible for the synthesis and stabilization of AgNPs. Such interactions between metal nanoparticles and plant constituents have been reported in the literature [43].

### 3.4. Transmission electron microscopy (TEM) imaging

TEM images of AgNPs are shown in figures 6(a)–(d). The synthesized NPs were nearly spherical with few irregular-shaped particles obtained. The major sizes of AgNPs prepared from *T. bentazoe*, *T. bellerica*, *T. bentazoe* and *Terminalia catapa* leaves extracts were 7, 11, 10 and 10 nm, respectively. The synthesized AgNPs were capped by the plant constituents (polysaccharides, protein, polyphenolic and flavonoidal compounds) that prevented their aggregation. Inherent capping offers the additional advantage of the stability in the green chemical synthesis [44].

### 3.5. In vitro and in vivo antioxidant activities

#### 3.5.1. DPPH assay analysis

Plants contain specific metabolites that are acknowledged to perform a range of purposeful activities. Phenolic and flavonoidal compounds, the biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of these compounds to scavenge radicals may be explained by their phenolic hydroxyl groups [45].

It is well known and also reported in the literature that plant mediated nanoparticles synthesis involves sequential reduction followed by capping with these constituents of plants [46]. The results obtained indicated that the antioxidant activity of AgNPs capped with plant constituents possessing free radical scavenging activity. The antioxidant activity of

### Table 3. Estimation of various contents of leaves hot water extract (HWE) of *Terminalia* species.

| T. species | yield of HWE (%) | Moisture content | Total phenolics (GAE/100 g) | Total flavonoids (GAE/100 g) | Protein | Yield of PS (%) |
|------------|-----------------|-----------------|-----------------------------|-----------------------------|---------|----------------|
| *T. catapa* | 10              | 5.6             | 12.2                        | 9.5                         | 13.2    | 5.9            |
| *T. bellerica* | 11              | 6.4             | 11.7                        | 4.8                         | 16.0    | 5.0            |
| *T. mellueri* | 12              | 4.8             | 15.86                       | 11.86                       | 12.5    | 7.2            |
| *T. bentazoe* | 11.8            | 5.4             | 11.34                       | 8.22                        | 13.69   | 5.3            |

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Table 4. Sugar composition of the polysaccharides hydrolysate of *Terminalia* species calculated as the area percentage from total chromatogram of GLC.

| T. species | Xylose | Glucose | Mannose | Ribose | Rhaminose | Arabinose | Galactose | Fructose | Sorbitol | Mannitol | Glucoronic |
|------------|--------|---------|---------|--------|-----------|-----------|-----------|----------|----------|----------|------------|
| *T. catapa* | 3.90   | 33.03   | 2.21    | 0.70   | 2.43      | 7.26      | 7.44      | 0.37     | —        | 0.40     | 0.03       |
| *T. bellerica* | 19.90  | 16.94   | 14.0    | 0.60   | 1.90      | 14.90     | 4.82      | 0.33     | 0.07     | 4.14     | 0.32       |
| *T. melluert* | 2.31   | 32.83   | 4.75    | 0.38   | 2.60      | 6.22      | 2.17      | 0.28     | —        | 0.21     | 0.07       |
| *T. bentazoe* | 4.60   | 32.03   | 3.0     | 0.74   | 3.03      | 11.73     | 10.05     | 0.12     | 0.08     | 2.09     | 0.07       |
Figure 1. UV-Vis absorption spectrum recorded from leaf extracts-reduced silver nanoparticles of: (a) *T. catapa*, (b) *T. bellerica*, (c) *T. bentzoe* and (d) *T. muelleri* with different reaction times 15, 30, 45, 60 and 75 min. — 15 min, — 30 min, — 45 min, — 60 min, — 75 min.

Figure 2. FTIR spectrum recorded from *Terminalia catapa* leaves extract (a) and their leaves extract-reduced silver nanoparticles (b).

Figure 3. FTIR spectrum recorded from *Terminalia bentzoe* leaves extract (a) and their leaves extract-reduced silver nanoparticles (b).

Figure 4. FTIR spectrum recorded from *Terminalia mellueri* leaves extract (a) and their leaves extract-reduced silver nanoparticles (b).

Figure 5. FTIR spectrum recorded from *Terminalia bellarica* leaves extract (a) and their leaves extract-reduced silver nanoparticles (b).
AgNPs is shown as DPPH inhibition % in figure 7. As displayed in the results, capped silver nanoparticles of T. catappa and T. mellueri leaves extracts were found to be a potent free radical scavenger when compared to both T. bentzoe and T. bellerica extracts as well as the standard ascorbic acid. An antioxidant works in stopping the oxidation by neutralizing the free radicals produced. In order to neutralize the free radicals, the antioxidant itself undergoes oxidation. The activity and stability of the silver nanoparticles are affected during the antioxidation process and also they are oxidized in the presence of air. This antioxidant activity may be due to the capping constituents present in plant extract and present on the metal surface.

3.5.2 In vivo antioxidant effect of Terminalia catappa. Table 5 recorded the effect of hot water extract of the leaves (HWE) and the prepared silver nanoparticles of T. catapa on the oxidative stress biomarkers. Concerning to the prepared silver nano-particles, the present results recorded its pronounced antioxidant effect as it increases glutathione level by 34.49%
and decrease malondialdehyde and superoxide dismutase by 27.27% and 32.29%, respectively. In case of aqueous hot water extract of *T. catapa*, it recorded less antioxidant effect as it revealed insignificant changes in glutathione and superoxide dimutase, while it showed significant decrease in malondialdehyde by 21.59%.

### 3.6. Anti-inflammatory activity

The carrageenan-induced hind paw oedema model in rats is known to be the acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAID), which primarily inhibit the cyclooxygenase involved in prostaglandin (PG) synthesis. Therefore, it can be inferred that the inhibitory effect of the extract on the carrageenan induced inflammation could be due to the inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

The anti-inflammatory effect of the aqueous leaves extracts, the prepared AgNPs and the reference drug (Indomethacin) in carrageenan induced paw edema model in rats are illustrated in table 6. After carrageenan administration, paw edema in rats reached a peak value at 3 hrs. Maximum percentage inhibition of edema exhibited by *T. catapa*, *T. mellueri* and *T. bellerica/AgNPs at 50 mg kg$^{-1}$ body weight concentration are 95.7%, 93% and 92.13%, respectively, and the effect is comparable to that of the standard drug Indomethacin 92.1%.

It is well-known that in chronic and sub-acute inflammation, reactive oxygen species (ROS) play an important role in modulating the extent of the inflammatory response, consequent tissue and cell injury; antioxidants are considered as possible protecting agents reducing oxidative damage of the human body from ROS and retarding the progress of many diseases [47]. The results of previous *in vitro* antioxidant studies and quantitative determination of the total phenolic and flavonoidal compounds, strongly support the high anti-inflammatory activity of AgNPs of aqueous leaves extract of *T. catapa*, *T. mellueri* and *T. bellerica* due to free radical scavenging activity in carrageenan-induced paw edema model [48].

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**Table 5. Effect of HWE and the prepared silver nanoparticles of *Terminalia catapa* on oxidative stress biomarkers(**).

| Parameters          | Control      | HWE          | AgNPs         |
|---------------------|--------------|--------------|---------------|
| Glutathione         | 37.59b ± 0.99| 44.45ab ± 6.89| 46.76a ± 5.14 |
| Malondialdehyde     | —            | (+18.24)     | (+34.39)      |
| Superoxide dimutase | 47.01a ± 7.43| 40.23ab ± 1.54| 31.83b ± 6.33 |

(*) - Data are means ±SD of five rats in each group.
- Data are expressed as μg mg$^{-1}$ protein for glutathione, μmol/mg protein for malondialdehyde and superoxide dimutase.
- Unshared letters (a, b and ab) between groups are the significance values at p < 0.0148 for glutathione, p < 0.0005 for malondialdehyde and p < 0.0159 for superoxide dimutase.
- Values between brackets are percentage change over the control group.

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**Figure 7.** Antioxidant activities of *Terminalia* leaves extracts and their corresponding AgNPs colloidal solutions: (a) *T. catapa*, (b) *T. bellerica*, (c) *T. bentzoe* and (d) *T. mellueri*.
Table 6. Acute anti-inflammatory activity of the aqueous leaves extracts of the four *Terminalia* species in male albino rats (n=6)a.

| Group                  | Dose (mg kg⁻¹) | Oedema (%) | Inhibition (%) | Relative potency |
|------------------------|----------------|------------|----------------|-----------------|
| Control                | 1 ml saline    | 62.3 ± 2.1 | —              | —               |
| Indomethacine          |                |            |                |                 |
| *T. catapa* aqueous leaves extract | 50 | 21.4 ± 0.3b | 92.1 | 100 |
| *T. bellarrica* aqueous leaves extract | 50 | 31.4 ± 1.1b | 84 | 91.2 |
| *T. bentzoe* aqueous leaves extract | 50 | 33.6 ± 0.8b | 80.4 | 87.3 |
| *T. mellueri* aqueous leaves extract | 50 | 32.8 ± 0.9b | 81.7 | 88.7 |
| AgNPs prepared from *T. catapa* aqueous leaves extract | 50 | 26.3 ± 0.0b | 92.13 | 100 |
| AgNPs prepared from *T. bellarrica* aqueous leaves extract | 50 | 28.6 ± 0.9b | 88 | 95.6 |
| AgNPs prepared from *T. bentzoe* aqueous leaves extract | 50 | 25.7 ± 0.4b | 93 | 101 |

a Values are expressed as mean ± SE of n=6 animals in each group. b Significantly different from control at p<0.01.

4. Conclusion

*Terminalia* species mediated synthesis of silver nanoparticles approach is a fast, green and economical method which produces highly stable AgNPs. The examined *Terminalia* species embracing *T. catappa*, *T. mellieri*, *T. bentzoe* and *T. bellarrica*, was found to contain flavonoids, phenolic, protein and polysaccharides compounds that seem to play an important role in synthesis as well as stabilization of the AgNPs. The synthesized nanoparticles have antioxidant activity due to capped phenolic and flavonoid compounds and can be used against deleterious effects of free radicals as well as being powerfully anti-inflammatory.

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