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Research Article

Green Synthesis of ZnO Nanoparticles using *Abelmoschus esculenthus* L. Fruit Extract: Antioxidant, Photoprotective, Anti-inflammatory, and Antibacterial Studies

Christina Astutiningsih*, Tias Eka Rahmawati, Nanik Astutik Rahman, Meri Meri

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Abstract
Nanoparticles are extensively studied for their promising biological properties. In this study, the fruit extract of *Abelmoschus esculenthus* L. was used as a chelating agent for the synthesis of zinc oxide nanoparticles (ZnOPs-AE) using a zinc acetate solution. The prepared ZnOPs-AE were identified and characterized using UV-vis spectroscopy, Fourier-transformed infrared spectroscopy (FTIR), particle size analyzer (PSA), scanning electron microscopy (SEM), and energy dispersive spectrum (EDS). The green synthesized ZnOPs-AE were evaluated for their antioxidant, photoprotective, anti-inflammatory, and antibacterial activities. The synthesized nanoparticles showed an intensity peak at 370 nm in the UV-vis spectrum. The FTIR result shows the presence of O-H, C=O, C-O, C-OH, and C=C chelating functional groups on the surface of nanoparticles. The size of ZnOPs-AE was determined using a PSA with particle size distribution of 102.2 nm. The ZnOPs-AE were shown to be spherical by SEM analysis and composition was 82.11% and 14.79% for Zn and O, respectively. The antioxidant properties of ZnOPs-AE showed significant antioxidant potential in DPPH, ABTS, and FRAP assays compared to the quercetin standard. The photoprotection activity test showed a SPF value of 19.63, the percentage of erythema transmission was 5.98%, and the percentage of pigmentation transmission was 5.62%. The ZnOPs-AE showed good anti-inflammatory with the synthesized nanoparticle performing activity between positive control and the fruit extract of *Abelmoschus esculenthus* L. Also, the ZnOPs-AE exhibited good antibacterial activity against *Staphylococcus aureus* (20.78 mm) and *Pseudomonas aeruginosae* (11.13 mm). Overall, the results highlight the effectiveness and potential of ZnOPs-AE for biological application.

Keywords: antioxidant, anti-inflammation, antibacterial, nanoparticle, photoprotection

1. INTRODUCTION

Modern translational research makes extensive use of the technology known as nanoscience or nanotechnology. Generally, the methods for the synthesis of nanoparticles are usually classified into two categories, the physical and chemical techniques. Various methods have been reported in order to synthesis nanoparticles, but the use of toxic solvents could potentially generate unsafe and hazardous byproducts and often involve high energy consumption. The development of green processes for the synthesis of nanoparticles has been evolving into an important branch of nanotechnology as green nanotechnology deals with safe and ecofriendly methods for nanomaterials fabrication which is considered as an alternative to conventional methods [1][2].

The manufacture of metal nanoparticles using green chemistry, an ecologically friendly process, and the use of biological components including bacteria, fungi, yeast, and plants has significantly increased in recent years [3]. According to Selvan et al. [4], the addition of several phytochemical substances such as tannin, flavonoids, proteins, and polysaccharides causes the activity of the resultant nanoparticles to be higher, including antibacterial, anticancer, antioxidant, and other properties. Zinc oxide, silver, gold, copper oxide, palladium, platinum, titanium dioxide, and iron oxide are just a few of the metal nanoparticles that have been created using plant extracts [5][6].

One source of significant chemicals that can be employed in the green synthesis approach for the synthesis of nanoparticles is the fruit extract of Okra (*Abelmoschus esculentus* L.). According to Ayushi et al. [7], compounds such as tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, proteins, and polyphenols are abundant

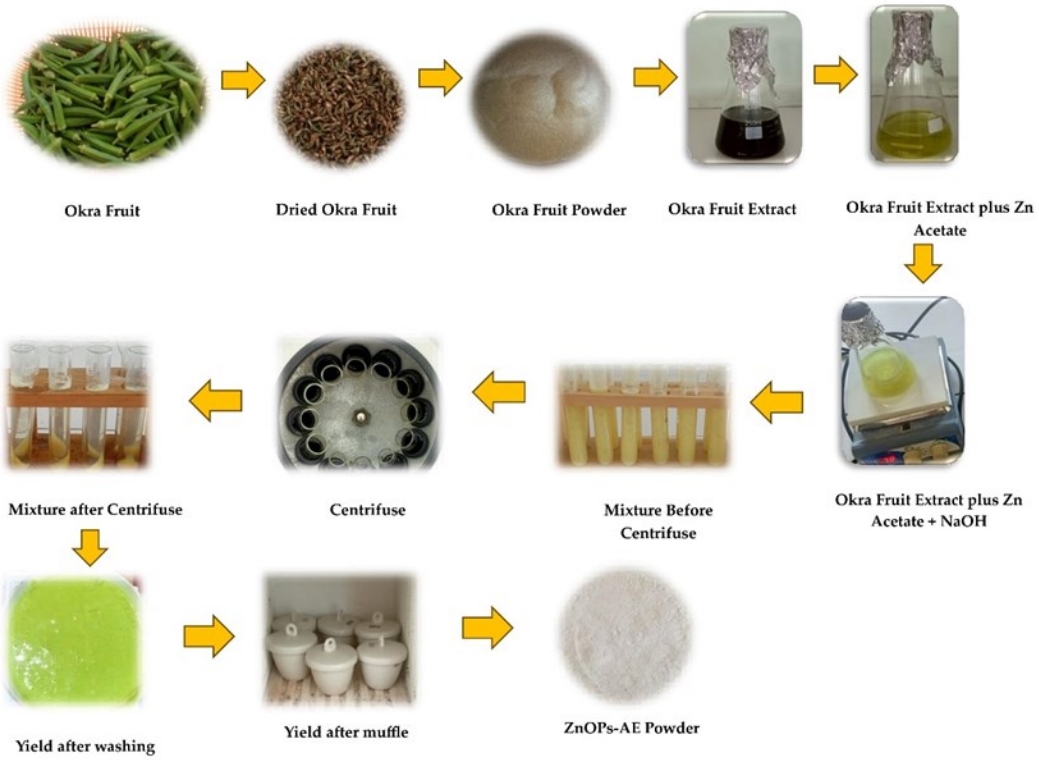


Figure 1. Green synthesis scheme of ZnOPs-AE.

in this plant. The phytocomponent in *A. esculenthus* L. that is quite influential is flavonoids, which are belong to polyphenolic compounds [8] [9].

The green synthesis in this work involves the extraction of compounds using environmentally-friendly solvents, i.e. distilled water. The fruit extract of *A. esculentus* L is then mixed with the metal precursor to produce the targeted nanoparticles. The presence of bioactive compounds in the plant extract is a key factor in the formation of nanoparticles in the green synthesis approach. These compounds have the ability to reduce the metal ions into metal nanoparticles via various oxidation processes. The functional groups in the phytochemical compounds, such as polyphenols, can chelate metal ions and form coordinated complexes, which are thermally decomposed to obtain the target nanoparticles. The phytochemicals in the plant extract can also act as a stabilizing agent to prevent the resulting nanoparticles from agglomeration [10][11]. One of the extremely promising inorganic oxides that has lately caught the attention of many scientists is zinc oxide (ZnO) [12]-[14]. Because they are simple to manufacture, affordable, and safe for both humans and animals, ZnO-NPs are of special interest in this study.

Free radicals, also known as reactive oxygen species (ROS), can cause oxidative damage, interfere, and disrupt the antioxidant system, and can be produced as a result of excessive sun exposure. Prolonged and chronic ultraviolet (UV) exposure has been shown to have negative effects on the skin, including erythema, edema, photoaging, wrinkles, sunburn, photosensitivity, immunosuppression, and carcinogenesis [15]. Skin cancer growth is linked to UV exposure, particularly UV-B radiation, and includes melanoma and nonmelanoma skin cancer [16]. Skin damage caused by sun exposure in Indonesia continues to increase [17].

Consumer needs have expanded beyond basic UV protection to include natural ingredients, however, the majority of solutions on the market today use synthetic active ingredients like titanium oxide, benzophenone, and bisdisolizole disodium, which may be dangerous when used for an extended period of time [18]. Due to their safety and capacity for a variety of biological effects on the skin, natural plant extracts are increasingly popular as protective agents added to cosmetic formulas today

[19]. Natural components have been regarded as photoprotection agent because they can inhibit the formation of UV-ROS or free radicals and the lipid peroxidation (LPO) that is associated with them. Additionally, they can act as a stimulant in the first phase of photosynthesis due to their potentially significant biological antioxidants and as anti-inflammatory agents [20].

ROS that UV causes to be created as a result of UV exposure may oxidize and damage cellular lipids, proteins, and DNA, altering and frequently destroying skin structures and impairing their normal function [21]. ROS and the body's natural antioxidant system (AO), such as reduced glutathione, are out of balance as a result of UV exposure to the skin [22][23], which leads to the production of cytokines that contribute to the development of skin inflammation [24]. Skin inflammation will expand interendothelial junctions, separate endothelial cells, produce vasodilatation, increase microvascular protein and fluid leakage into interstitium, and result in edema [25]. As a result of ZnO-NPs' "generally recognized as safe" (GRAS) certification from the FDA, it is favored for use in a variety of biological tests as an IUV photoprotection component [26][27]. Numerous uses of ZnO-NPs in diagnostics and medicine administration have also been demonstrated [28].

ZnO-NPs of leaf, stem and callus extracts of *Tabernaemontana heyneana* could exhibit potent radical scavenging activity, strong anti-inflammatory (membrane stabilization) activity, and a greater potentiality in the degradation of carcinogenic [29]. ZnO with the leaf extract of *P. odoratissimum* can as antioxidant which revealed scavenging activity, the antibacterial efficacy against four pathogenic bacterial strains, and as anti -inflammatory by improving the membrane stability of lysosome cells [30]. The nanorods were able to block irradiations in both the UV-B and UV-A range with broadband protection and antimicrobial against both Gram-positive and Gram-negative bacterial strains [31]. Due to their UV-absorbing abilities and optical clarity, ZnO-NPs have demonstrated surprising applicability in sunscreen agents. The sun emits UV radiation, a kind of electromagnetic radiation, as waves or particles with a range of wavelengths and frequencies. The three kinds of UV light are known as UV-A, UV-B, and UV-C. Before it reaches the globe, the ozone layer absorbs UV-C, but it does not completely block UV-A and UV-B rays [32].

2. MATERIALS AND METHODS

2.1. Materials

Abelmoschus esculentus L. fruit from the garden in Purwodadi, Indonesia and identified at the Biology Laboratory, College of Pharmaceutical Sciences Yayasan Pharmacy Semarang in June 2022. Laboratory grade Zinc acetate dihydrate (Zn $(CH_3COO)_2 \cdot 2H_2O$), sodium hydroxide (NaOH), quercetin, 1,1-diphenyl-2-picrylhidrazyl (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 2,2-azinobis-3-ethylbenzothiazoline-6- sulfonic acid (ABTS), *Bovine Serum Albumin* (BSA), and tris *base*, were purchased from Merck Sigma-Aldrich

Table 1. Results of phytochemical and TLC of *Abelmoschus esculentus* L. fruit extract.

Phytochemical	Chemical Identification	TLC Identification
Phenol	Black blue	+ spot Rf 0.83
Tanin	Black blue	+ spot Rf 0.83
Flavonoid	Amyl alchohol yellow or orange	+ spot Rf 0.73
Saponin	+ stabil of foam	+ spot yellow Rf 0.39
Triterpenoid	+ green	+ spot green Rf 0.31
Alkaloid	with Dragendrof formed brick-red sediment with Mayer formed white sediment with Buchardat red-brick sediment	+ spot orange brown Rf 0.74

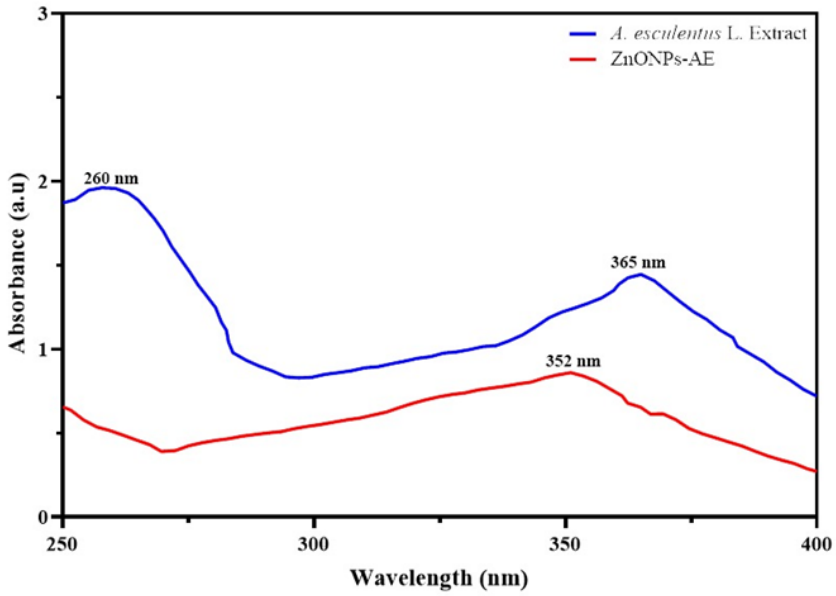


Figure 2. UV- vis spectrum of *A. esculentus* extract and ZnOPs-AE.

Reagent Pte, Singapore. While, ethanol, methanol, NaCl, sodium diclofenac (Dexa Medica), MSA media, Mac Conkey Agar (MCA) media from Oxoid, and aqua bidestillate were used in this work.

2.2. Methods

2.2.1. Preparation of Ethanol Extract

Okra fruits were collected and washed with running water and dried with drying cabinet. The dried simplisia was mashed using a blender. Extraction was carried out using 10 g of dry powder of Okra Fruit in 100 mL ethanol 80%. The mixture heated to 90 °C for 150 min and then filtered. Before use, the extract was stored at 4 °C [33].

2.2.2. Screening Flavonoid

Wistater's reagent, when the sample is mixed with concentrated HCl and magnesium powder, a yellow color will result from the presence of flavonoids. If positive flavonoids are present, the orange color will appear when the sample is mixed with a reagent containing 10% NaOH. Bate-Smite-Metcalfe reagent, Adding the sample with concentrated H_2SO_4 and putting it in a waterbath will cause red color for positive flavonoids test.

The thin-layer chromatography (TLC) test for the identification of flavonoids: the stationary phase was silica GF 254, and the mobile phase was a mixture of *n*-butanol, acetic acid, and water at a ratio of 4:1:5. Following elution, the plate is inspected using a UV lamp at 254 nm. Flavonoid stains after steaming ammonia vapor show a brownish-yellow color [34]-[36].

2.2.3. Green Synthesis of ZnOPs-AE

The method used to synthesize ZnOPs-AE is the co-precipitation method with slight adjustments (Figure 1). The liquid extract of Okra fruit (10 mL) was mixed with 0.15 M of $Zn(CH_3COO)_2{\cdot}2H_2O$ (90 mL) for 60 min in aqueous bath under continuous stirring at 70 °C. The mixture was added NaOH until pH of 8 and stirred with a magnetic stirrer on a hotplate at 70 °C for 60 min until colloidal ZnO-NPs formed [34][35]. The yellowish-white solid products were collected by centrifugation at a speed of 4,000 rpm for 10 min, washed with distillate water, dried at 100 °C in an oven for 12 h, and ZnO-NPs are obtained through a classification process. The solids are fed into the furnace at 450 °C for 4 h [36]-[38].

2.2.4. Characterization of ZnOPs-AE

The ZnOPs-AE was initially analyzed by using UV-vis spectroscopy (Shimadzu U-1700), FTIR and JEOL SEM (Hitachi, Ltd., Tokyo, Japan).

2.2.5. Determination of Antioxidant Capacity

All methods of antioxidant analysis used quercetin as a standard and used methanol for a

blank.

2.2.5.1. DPPH

As much as 2 mL of the sample was combined with 1 mL of 0.1mM of DPPH solution in methanol, and the mixture was then incubated at room temperature for 30 min in the dark. Using a UV-Vis spectrophotometer, the absorbance was measured at 517 nm [39].

2.2.5.2. ABTS

The stock solution of ABTS was diluted in methanol to produce the working solution, which had an absorbance of 0.70 at 734 nm. The sample was mixed with 2.0 mL of the ABTS solution. The mixture was then incubated for exactly 10 min in the dark at room temperature. A UV-vis spectrophotometer was used to assess the absorbance at 734 nm [40][41].

2.2.5.3. Determination of Ferric Reducing Antioxidant Power (FRAP)

As much as 0.2 mL of the sample was mixed with 4.0 mL the FRAP reagent. After that, the mixture was incubated for 30 min at 37 °C in the dark. Using a UV-vis spectrophotometer, the absorbance was measured at 593 nm [42][43].

2.2.6. Determination of UV Photoprotection

Determination of sunscreen activity *in vitro* was measured with a spectrophotometer to calculate SPF, percentage transmission of erythema and percentage transmission of pigmentation [44][45].

2.2.7. Antiinflamation

As much as 50 mL of each sample is taken. Then, a 0.2% BSA solution is added until the volume reaches 5 mL from the mixture. All solutions namely sample, positive control and negative control solutions were incubated at 25 °C for 30 min and then heated for 25 min at 23 °C [46] [47]. After cooling, the solution was vortexed and the absorbance measurements were measured with UV-Vis spectrophotometry at 660 nm [48][49].

2.2.8. Antibacterial

By using the agar well diffusion technique, the antibacterial activity of the nanoparticles was assessed against both Gram-negative and Gram-positive microbes [50]. The mannitol salt agar medium was used for *Staphylococcus aureus* while Mac Conkey agar medium was used for *Pseudomonas aeuroginosae*. In each plate, five bores were made. A sterile media containing the ZnOPs-AE with concentrations of 0.1, 1.0, and 10 mg/L; negative control (DMSO) and ciprofloxacin 1 mg/mL were transferred into the inoculated plates. The plates were held at room temperature for 3 h to allow for diffusion before being incubated upright at 37 °C for about 21 h to promote bacterial

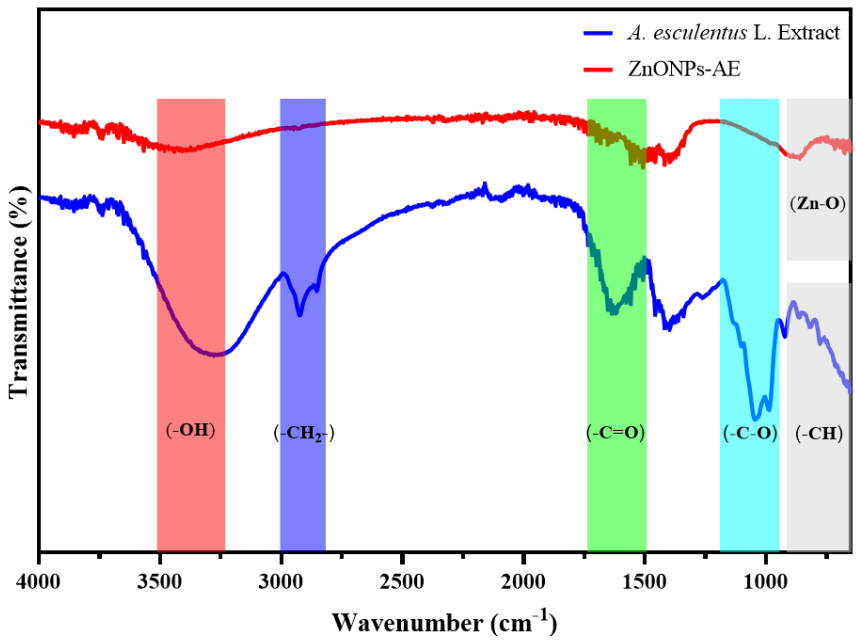


Figure 3. FTIR spectrum of *A. esculentus* extract and ZnOPs-AE.

Table 2. The particle size of ZnOPs-AE.

Sample	Polydisperse	Particle Size (nm)
ZnOPs-AE-1	0.330	118.0
ZnOPs-AE-2	0.500	105.0
ZnOPs-AE-3	0.550	98.0
ZnOPs-AE-4	0.620	81.0
ZnOPs-AE-5	0.410	109.0
Average	0.475	102.2

growth [51].

3. RESULTS AND DISCUSSIONS

3.1. Extraction and Phytochemical Screening

Liqiud extract with a concentration of 0.1 g/mL was identified for its secondary metabolite content through phytochemical and TLC tests.

The results in Table 1 are the same as the previous research [52][53] which states *Abelmoschus esculentus* L. fruit contains saponins, flavonoids, steroids, tannins, and alkaloids. Green biosynthesis is carried out by dissolving liquid extract as much as 10 mL in 90 mL of 0.15 M Zn $(CH_3COO)_2$.

3.2. Characterization of ZnONP-AE

The first important indicator to confirm the success ZnOPs-AE biosynthesis is through visual observation. When $Zn(CH_3COO)_2$ as a precursor was added to Okra fruit extract, the color of Okra fruit extract changed from green to light yellow as a result of the reaction. Colloids are formed when Zn^{2+} and OH^- reach critical solubility levels. When Zn^{2+} ions are reduced to ZnO by functional groups in the Okra fruit extract, excess OH- ions will combine with $Zn(OH)_2$ to create $Zn(OH)_4^{2+}$, which then dissociates once again to form Zn^{2+} and OH^- ions [54][55].

The maximum wavelength for Okra fruit extract was 260 nm indicating the presence of conjugated phenol chromophores and at 370 nm indicating the synamoyl functional group which is usually found at 350–380 nm [56]. The absorption spectrum showed a peak at 352 nm of the green synthesized ZnOPs-AE. The surface plasmon resonance (SPR) of ZnOPs-AE is between 310–380 nm [57]-[59]. The absorbance peak spectral value of a specific ZnOPs-AE indicates the SPR character of nano-sized particles (Figure 2). The vibration plasmon resonance that occurs will absorb the UV and visible light [60].

The FTIR spectrum was recorded for the nanoparticles synthesized and it was depicted in the range of 650–4,000 cm^{-1} in Figure 3. The synthesized ZnOPs-AE showed peak around 3,350 cm^{-1} which indicates the vibration of the O–H group characterized by polyphenolic compounds [61]. Another peak around 2,853 cm^{-1} correspond to the C–H functional group. Other peaks around 1630–1665 and 1000–1350 cm^{-1} indicate the absorption band for the C=O and C–O in polyphenolic compounds, respectively. Another peak around 600–900 cm^{-1} corresponds to the presence of C–H bending of alkene [62]. These peaks suggested the presence of different phytochemicals, i.e., phenolic, alkaloids, flavonoids, tannins, and organic acids in *A. esculentus* L extract. These compounds interact with Zn^{2+} ions via their oxygen donor atoms and adsorb on the surface of metals, which established by a decrease in peak intensities of bands observed in ZnOPs-AE. The absorption peak around 650–1,000 cm^{-1} for ZnOPs-AE sample showed the Zn–O metallic bond which confirmed the formation of ZnO nanoparticles from the *A. esculentus* extract.

Information regarding the interaction that occurs between secondary metabolite components in *Abelmoschus esculentus* L extract and precursor solution in the synthesis process of ZnOPs-AE is observed though peak shifts and intensity changes in FTIR spectral data. The suspected involvement of the O–H group in the Zn^{2+} reduction process is indicated by the decrease in the 3,350 cm^{-1}. The calcination process aimed to remove extract using a temperature of 450 °C for 2 h, but there was still weak O–H absorption in the bound 3,358 cm^{-1} [63]. In addition, the loss of an absorption band at 2,853 cm^{-1} in the FTIR spectrum of *A. esculentus* L extract indicates the reaction that occurs during the bioreduction process. Band shifts in the FTIR spectra of ZnOPs-AE indicate the involvement of polyols that have functional groups such alcohols and carboxylic acids during the bioreduction process [64]. In the FTIR spectrum of ZnOPs-AE, there is a peak shift and an increase in intensity in the 1,600 cm^{-1} region for the C=O functional group.

This can be seen from the appearance of the typical vibrations of ZnOPs-AE in the 672 cm^{-1} area. Based on the results of FTIR, it is suspected that the main groups involved in the Zn^{2+} bioreduction process are O-H, C=O, C-O, C-OH and C=C functional groups which are derivates of phenol and polysaccharide compounds contained in the extract [65]. Phenol and polysaccharide compounds in the extract with the help of NaOH reduce Zn^{2+} ions to Zn^{0}. These phenol and polysaccharide compounds are reduction agents that play a role in the Zn^{2+} bioreduction [66].

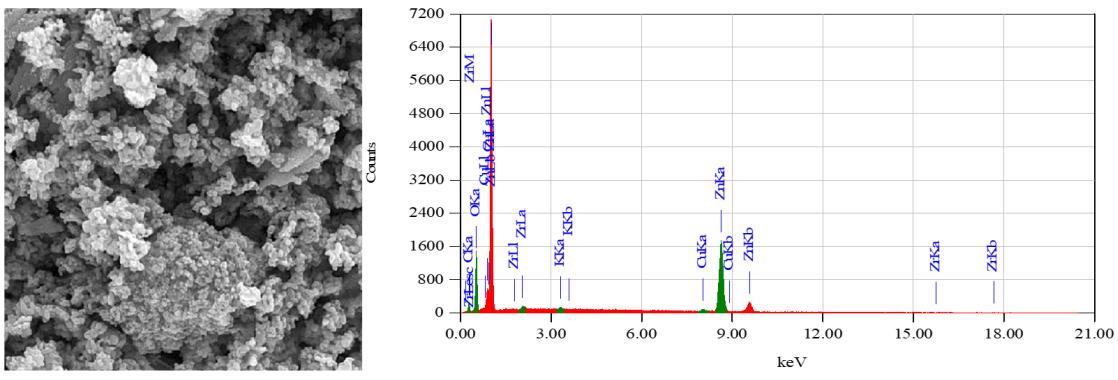
The particle size of ZnOPs-AE is shown in Table 2. The ability to form nanosized particles is strongly influenced by a strong stabilizer to prevent aggregation so that it can limit cluster growth. Thus, the Zn^{0} cluster formed does not grow into a larger size and remains in nano-sized. ZnOPs-AE formed in this study has an average particle size of 102.2 nm, which a nano-sized particle [67].

In addition to particle size, the results of PSA analysis also indicate particle distribution and uniformity. The narrower the particle size distribution, the better the particle homogeneity [68]. Based on the SEM can be known in Figure 4 (a), the results show that the ZnOPs-AE cluster shapes produced include hexagonal, cubic and spherical sheets, with spherical being cluster dominant.

This is due to the content of antioxidant polyphenol compounds found in Okra fruit extract which acts as a stabilizing agent. The nanoparticles are believed to be formed by a redox process involving polyphenols as the reducing agent [69]-[72]. It appears that the results of compound particles produced with green synthetase in this study characterized at a voltage of 10 kV show a fairly regular morphology and the structure is wide. EXD results ZnOPs-AE showed results with a composition of 82.11% Zn and 14.79% O with a ratio 6:1 in Figure 4(b). The results of the mapping analysis show evidence that the distribution of the zinc element is relatively more than that of the oxygen element.

3.3. Antioxidant Activities

The methods used to determine antioxidant activity are DPPH, ATBS, and FRAP methods [73]. In this study, three kinds of method were used to determine the results of the activity test because a sample can be said to have antioxidant activity if has been tested using at least 2 antioxidant testing methods. It was found that the DPPH radical scavenging activity of the ZnOPs-AE increased with increasing doses. DPPH and ABTS methods were chosen because they have the same mechanism in inhibiting free radicals, namely the mechanism of capturing free radicals. The third method used to determine antioxidant activity is the FRAP method. The principle of the FRAP method is a reducing agent, which is an indicator of the potential of a compound as an antioxidant. Reducing power is measured by the ability of an antioxidant to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}) so that the antioxidant power of a compound is analogous with the reducing ability of the compound. When the sample solution reacted with FRAP solution, a blue TPTZ-Fe^{2+} complex was found and then detected spectrophotometrically at 595.40 nm with an operating time of 10 min [74]. The antioxidant capability of biosynthesized ZnOPs -AE was increased in the experiments using five



(a) **(b)**

Figure 4. (a) SEM and (b) EDS results of ZnOPs-AE.

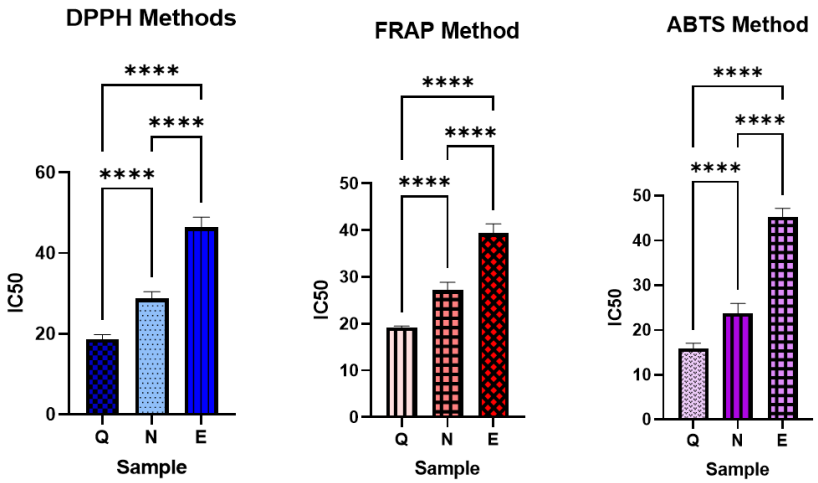


Figure 5. Antioxidant activity of quercetin standard (Q), ZnOPs-AE (N), and Extract Okra fruit extract (E).

different concentrations, i.e., 20, 40, 60, 80, and 100 mg/L. The results of potential antioxidant of ZnONP-AE as shown in Figure 5.

The IC_{50} of DPPH, ABTS and FRAP methods for standard quercetin, ZnOP-AE and *A. esculentus* L extract were below 50 mg/L, which were included in the category of very strong. Analysis of antioxidant data in this study statically using SPSS obtained normal and homogeneous data results with a significance value of higher than 0.05. Based on post ANOVA test of standards antioxidant of quercetin, ZnONP-AE, and Okra fruit extract in each method showed that there were significant differences between groups with a significant difference between groups with a significance value of less than 0.05, so it can be concluded that there are differences in results between groups in the *in vitro* antioxidant test.

3.4. Photoprotection UV Activity

Using a UV-vis spectrophotometer, the photoprotective capability of the synthesized ZnOPs-AE was examined. Along with the SPF rating, the sample sunscreen protection level may also be described by the erythema and pigmentation transmittance percentages. Sample absorbance was measured in the UV-B radiation spectrum (290–320 nm) to determine the SPF value. The primary cause of sunburn (erythema), photoaging, and photocarcinogenesis is UV-B radiation [75]. SPF values are often used to assess sunscreen protection, particularly against UV-B rays [74]. The SPF value sunscreen commercial, ZnOPs-AE, and *A. esculentus* L extract were higher at increasing concentration based on the results displayed in Table 3. The 100 mg/L ZnOPs-AE sample showed the SPF value of 15 including category medium. Another has action in preventing skin pigmentation (additional protection category), and also prevents erythema since the resulting percentage transmittance was included in the sunblock range of 290–320 nm UV rays with an additional protection category of sunscreen may be absorbed by 95% or more [76].

According to Mojeski et al. [77], the percentages of erythema or pigmentation transmission are the accumulation of UV energy that is transmitted by sunscreen on the erythema or pigmentation spectrum. Athiyah et al. [78] stated the substance referred to as having sunblock action when it can completely shield the skin against UV-A (322.5–372.5 nm) and UV-B (292.5–337.5 nm) rays, which can induce erythema and pigmentation. The sample of ZnOPs-AE gave a higher SPF level of activity in shielding the skin from UV-A radiation that causes pigmentation, according to the results of erythema and pigmentation percentages. Due to absorption, reflection, and scattering of UV and visible light, ZnOPs-AE possesses UV attenuation capabilities. According to Antoniou et al. [79], ZnO physical sunscreens are very effective, photostable, and they provide protection that extends into the UV-A and visible bands with essentially little possibility of causing irritation or sensitization. Analysis of SPF, % erythema and % pigmentation data in this study statically using SPSS obtained normal and homogeneous data results with a significance value of higher than 0.05. Based on post-ANOVA test of

commercial sunscreen, ZnOPs-AE, and Okra fruit extract in each method showed that there significant differences between groups with a significant difference between groups with a significance value of less than 0.05, so it can be concluded that there are differences in results between groups for in vitro SPF, % erythema and % pigmentation.

3.5. Anti-Inflammatory Activity

The body uses inflammation as a mechanical defence against different hazardous substances, germs, irritants, unfavorable stimuli, and damaged cells. Inhibiting protein denaturation is a test that may be used to screen for anti-inflammatory activity as protein denaturation is one of the factors that determines when inflammation starts. Therefore, developing anti-inflammatory medications will benefit from employing substances that can stop protein denaturation [80]. BSA is one protein that can be used as an anti-inflammatory test. BSA will undergo denaturation when heated. This is a marker where albumin is damaged when induced by heat so that the body is considered a foreign material therefore the body fights through inflammatory mechanisms. The absorption data obtained is then calculated as the percentage of inhibition (Figure 6). The sample can have anti-inflammatory activity if its percent inhibition is more than 20% [81].

The percent inhibition data of the positive control solution (diclofenac sodium) concentration of 25 mg/L can inhibit protein denaturation by 25%. ZnOPs-AE concentration of 60 mg/L can inhibit protein denaturation by 23%, while the extract at a concentration of 100 mg/L can inhibit protein denaturation by 25%. Based on these data, the potential inhibition of protein denaturation owned by the extract was smaller than ZnOPs-AE while ZnOPs-AE had a smaller potential of inhibition protein denaturation than positive control solution. Statistical analysis of anti-inflammatory data obtained using SPSS. The data showed normal and homogeneous data results with a significance value of higher than 0.05. Based on post-ANOVA test of diclofenac sodium and ZnOPs-AE showed that there no significant differences between groups. ZnOPs-AE and extract showed that there were no significant differences too but diclofenac sodium

Table 3. Photoprotective UV.

Sample Concentration (mg/L)	Average SPF	Average %Erythema	Average % Pigmentation
Sunscreen 100	23.9219	6.2536	5.8521
Sunscreen 80	18.5276	6.0649	5.6739
Sunscreen 60	15.9165	5.5551	5.2143
Sunscreen 40	13.4030	5.1694	4.8825
Sunscreen 20	11.6111	4.5177	4.3035
ZnONP-AE100	19.6360	5.9875	5.6233
ZnONP-AE 80	15.1792	5.1412	4.8501
ZnONP-AE 60	12.2733	4.7579	4.4568
ZnONP-AE 40	8.1615	3.9661	3.6959
ZnONP-AE 20	6.6628	3.5281	3.2906
Extract Okra Fruit 100	1.2864	83.4161	87.9416
Extract Okra Fruit 80	1.2443	83.9454	88.7063
Extract Okra Fruit 60	1.1771	88.3327	91.7871
Extract Okra Fruit 40	1.1395	89.7566	93.1190
Extract Okra Fruit 20	1.1229	89.9491	93.5026

and extract showed that there were significant difference between groups with a significance value of less than 0.05 (Figure 6).

3.6. Antibacterial Activity

By using the agar well diffusion technique, the antibacterial activity of the ZnOPs-AE was examined against a range of Gram-positive and -negative harmful bacteria. Biosynthesized ZnOPs-AE reduced *Pseudomonas aeruginosae* and *Staphylococcus aureus* growth. The different structural and chemical compositions of the bacterial cell walls can be ascribed to the variation in effectiveness [82]. According to research, Gram-negative bacteria, *Pseudomonas aeruginosae*, have external lipopolysaccharide (LPS) membranes that protect the peptidoglycan layer and help the bacteria survive. As a result, the inhibitory zone diameter produced by ZnOPs-AE is smaller for Gram-negative bacteria than for Gram-positive bacteria. The outcomes showed that all bacterial strains were significantly inhibited by the biosynthesized ZnOPs-AE. Figure 7 shows that substantial antibacterial zones against *Staphylococcus aureus* (20.79 mm) and *Pseudomonas aeruginosa* (11.13 mm) at 10 mg/L concentration.

Reddy et al. [83] have noted the same findings, highlighting how Gram-positive bacteria are more susceptible than Gram-negative bacteria. It has been suggested that changes in cell wall construction, cell physiology, metabolism, or level of contact may be responsible for Gram-positive bacteria's increased sensitivity. It is clear from these findings that ZnOPs-AE are potent antibacterial agents

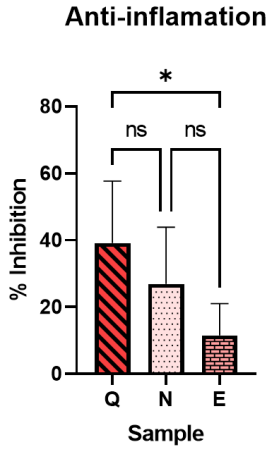


Figure 6. Anti-inflammatory activity of diclofenac sodium standard (Q), ZnOPs-AE (N), and Extract Okra fruit extract (E).

against both Gram-positive and Gram-negative bacteria. The concentrations of ZnOPs-AE used in this study are 0.1, 1.0, and 10 mg/mL. An increase in the sample concentration will increase the diameter of the resulting inhibitory zone. Statistic analysis of antibacterial data using SPSS showed normal and homogeneous data results with a significance value of higher than 0.05. Based on post-ANOVA test of ciprofloxacin and ZnOPs-AE in each method showed that there were significant differences between groups with a significant difference between groups with a significance value of less than 0.05, so it can be concluded that there are differences in results between groups in diameter inhibitory zone.

There are few bactericidal processes that have been postulated by researchers to explain ZnOPs's action. Researchers hypothesized that the released Zn from ZnOPs has hazardous qualities that are

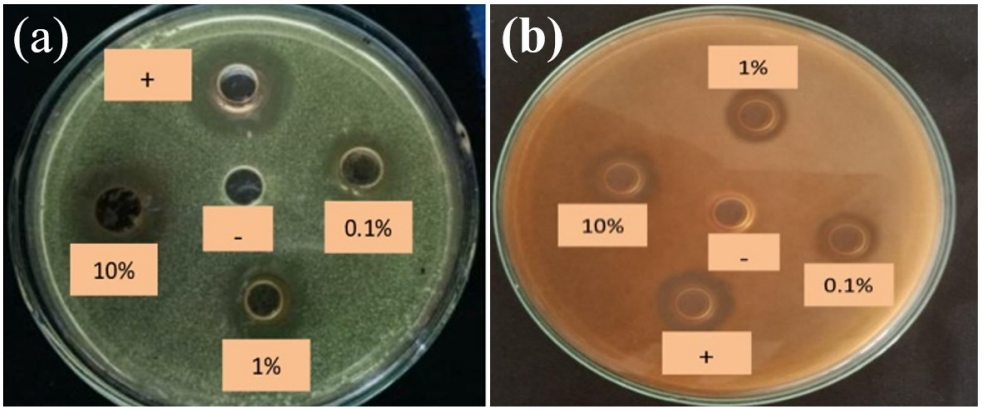


Figure 7. Antibacterial activity of ZnOPs-AE against (a) *Staphylococcus aureus* and (b) *Pseudomonas aeruginosae*.

causing it to block several bacterial cell processes including metabolism and enzyme activity, which causes bacterial cell death [83]. The creation of ROS, which triggers oxidative stress and ultimately results in cell death, is another proposed mechanism [84]. Another theory is that ZnOPs induce mortality by attaching to bacterial cell membranes, accumulating inside the cytoplasm, and compromising the integrity of the membranes. This causes the contents of the cells to seep out, causing cell death [85]. If bacterial cells are killed by these nanoparticles, especially in wounds, it can improve wound healing in the inflammatory phase, especially in wounds with bacterial infections. Dead bacterial debris and damaged cells will be phagocytosed by macrophage cells so that the wound becomes clean and can prepare for tissue growth again more quickly compared to wounds that still contain bacteria [86].

The results of antibacterial testing of metal nanoparticles synthesized through green synthesis do have potential as antibacterial agents. The results of other metal nanoparticle studies related to their antibacterial ability tested by the well diffusion method as carried out in this study have also been widely reported, including Ag metal nanoparticles with a nanoparticle size of about 100 nm with a concentration of 0.125 w/v able to provide an inhibitory zone of 10.10±0.35 mm against *Staphylococus aureus* bacteria and 9.17±0.45 nm against *Eschericia coli* bacteria [87]. Antibacteria activity of iron nanoparticles of *Lawsonia inermis* and *Gardenia jasminoides* leaves extract with a concentration size below 100 nm is able to provide approximately the same inhibitory zone of about 11 –15 mm for both bacteria [88]. It is possible that the antibacterial activity of several types of metal nanoparticles has the same tendency. The potential for Gram-positive bacteria will be greater than Gram-negative bacteria.

4. CONCLUSIONS

This work uses an Okra fruit extract to biosynthesis ZnOPs-AE in a straightforward and environmentally friendly manner. The biosynthesized ZnOPs displayed distinctive FTIR and UV-Vis signals. PSA coupled with SEM-EDX and FTIR confirmed the formation of ZnOPs-AE with an average size of 102.2 nm. ZnOPs-AE have strong antioxidant activity, medium photoprotective activity, sunblock erythema and extra protection pigmentation. Anti-inflammatory activity for sample with a concentration of 60 mg/L or higher with 23% denaturation protein inhibitors and has antibacterial activity of *Staphylococcus aureus* and *Pseudomonas aeruginosae*.

AUTHOR INFORMATION

Corresponding Author

Christina Astutiningsih — Department of Pharmaceutical and Food Analyst, STIFAR Yayasan Pharmasi, Semarang-50192 (Indonesia);
orcid.org/0000-0001-7264-1905
Email: christinaastutiningsih@gmail.com

Authors

Tias Eka Rahmawati — Department of Pharmaceutical and Food Analyst, STIFAR Yayasan Pharmasi, Semarang-50192 (Indonesia);
orcid.org/0009-0002-5043-6702

Nanik Astutik Rahman — Department of Chemical Engineering, Institut Teknologi Nasional Malang, Malang-65152 (Indonesia);
orcid.org/0000-0002-3999-0019

Meri Meri — Department of Medical Laboratory Technology, Universitas Bakti Tunas Husada, Tasikmalaya-46196 (Indonesia);
orcid.org/0000-0001-8851-7650

Author Contributions

Conceptualization:C. A.; Methodology: C. A. and T. E. R.; Software: C. A.; Validation: C. A. and T. E. R.; Formal Analysis: C. A. and T. E. R.; Investigation: C. A. and T. E. R.; Resources: C. A. and T. E. R.; Data Curation: C. A. and T. E. R.; Writing-Original Draft Preparation: C. A., T. E. R., N. A. R., and M. M.; Writing-Review & Editing: C. A., T. E. R., N. A. R., and M. M.; Visualization, C. A., T. E. R., N. A. R., and M. M.; Supervision, C. A. and T. E. R.; Project Administration: C. A.; Funding Acquisition: C. A. All the authors went through the manuscript and gave their consent for publication.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

[1] M. E. Abdel-Alim, K. Samaan, D. Guillaume, and H. Amla. (2023). "Green Synthesis of Silver Nanoparticles using Egyptian Date Palm (Phoenix dactylifera L.) Seeds and Their Antibacterial Activity Assessment". *Bioactivities.* **1** (1): 1-8. 10.47352/bioactivities.2963-654X.180.

[2] P. Dikshit, J. Kumar, A. Das, S. Sadhu, S. Sharma, S. Singh, P. Gupta, and B. Kim. (2021). "Green Synthesis of Metallic Nanoparticles: Applications and Limitations". *Catalysts.* **11** (8). 10.3390/catal11080902.

[3] H. Amrulloh, A. Fatiqin, W. Simanjuntak, H. Afriyani, and A. Annissa. (2021). "Antioxidant and Antibacterial Activities of Magnesium Oxide Nanoparticles Prepared using Aqueous Extract of Moringa Oleifera Bark as Green Agents". *Journal of Multidisciplinary Applied Natural Science.* **1** (1): 44-53. 10.47352/jmans.v1i1.9.

[4] D. Arumai Selvan, D. Mahendiran, R. Senthil Kumar, and A. Kalilur Rahiman. (2018). "Garlic, green tea and turmeric extracts-mediated green synthesis of silver nanoparticles: Phytochemical, antioxidant and in vitro cytotoxicity studies". *Journal of Photochemistry and Photobiology B: Biology.* **180** : 243-252. 10.1016/j.jphotobiol.2018.02.014.

[5] N. Kahzad and A. Salehzadeh. (2020). "Green Synthesis of CuFe(2)O(4)@Ag Nanocomposite Using the Chlorella vulgaris and Evaluation of its Effect on the Expression of norA Efflux Pump Gene Among Staphylococcus aureus Strains". *Biological Trace Element Research.* **198** (1): 359-370. 10.1007/s12011-020-02055-5.

[6] M. Khatami, H. Alijani, M. Nejad, and R. Varma. (2018). "Core@shell Nanoparticles: Greener Synthesis Using Natural Plant Products". *Applied Sciences.* **8** (3). 10.3390/app8030411.

[7] R. K. Nath, K. H. Begum, and P. Ahmed. (2020). "Integrated Pest Management of Okra (Abelmoschus esculentus L.)". *International Journal of Current Microbiology and Applied Sciences.* **9** (1): 1725-1729. 10.20546/ijcmas.2020.901.191.

[8] R. Agregan, M. Pateiro, B. M. Bohrer, M. A. Shariati, A. Nawaz, G. Gohari, and J. M. Lorenzo. (2023). "Biological activity and development of functional foods fortified with okra (Abelmoschus esculentus)". *Critical Reviews in Food Science and Nutrition.* **63** (23): 6018-6033. 10.1080/10408398.2022.2026874.

[9] J. Yang, X. Chen, S. Rao, Y. Li, Y. Zang, and B. Zhu. (2022). "Identification and Quantification of Flavonoids in Okra (Abelmoschus esculentus L. Moench) and Antiproliferative Activity In Vitro of Four Main Components Identified". *Metabolites.* **12** (6). 10.3390/metabo12060483.

[10] K. Sak. (2023). "Role of Flavonoids as Potential Plant Fungicides in Preventing Human Carcinogenesis: A Short Communication". *Bioactivities.* **1** (2): 39-42. 10.47352/bioactivities.2963-654X.187.

[11] M. E. S. Sule, E. Astuty, and R. Tahitu. (2023). "In Vitro Antibacterial Activity and Phytochemical Screening of Galoba (Hornstedtia alliaceae) Seeds Extract". *Bioactivities.* **1** (2): 81-89. 10.47352/bioactivities.2963-654X.196.

[12] A. Muthuvel, M. Jothibas, and C. Manoharan. (2020). "Effect of chemically synthesis compared to biosynthesized ZnO-NPs using Solanum nigrum leaf extract and their photocatalytic, antibacterial and in-vitro antioxidant activity". *Journal of Environmental Chemical Engineering.* **8** (2). 10.1016/j.jece.2020.103705.

[13] R. Vijayakumar, S. S. Abd Gani, U. H. Zaidan, M. I. E. Halmi, T. Karunakaran, and M. R. Hamdan. (2020). "Exploring the Potential Use of Hylocereus polyrhizus Peels as a Source of Cosmeceutical Sunscreen Agent for Its Antioxidant and Photoprotective Properties". *Evidence-Based*

Complementary and Alternative Medicine. **2020** : 7520736. 10.1155/2020/7520736.

[14] H. Mohd Yusof, R. Mohamad, U. H. Zaidan, and N. A. Abdul Rahman. (2019). "Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: a review". *Journal of Animal Science and Biotechnology.* **10** : 57. 10.1186/s40104-019-0368-z.

[15] S. Nur, M. Hanafi, H. Setiawan, and B. Elya. (2022). "In vitro ultra violet (UV) protection of curculigo latifolia extract as a sunscreen candidate". *IOP Conference Series: Earth and Environmental Science.* **1116** (1). 10.1088/1755-1315/1116/1/012009.

[16] M. S. Paulo, B. Adam, C. Akagwu, I. Akparibo, R. H. Al-Rifai, S. Bazrafshan, F. Gobba, A. C. Green, I. Ivanov, S. Kezic, N. Leppink, T. Loney, A. Modenese, F. Pega, C. E. Peters, A. M. Pruss-Ustun, T. Tenkate, Y. Ujita, M. Wittlich, and S. M. John. (2019). "WHO/ILO work-related burden of disease and injury: Protocol for systematic reviews of occupational exposure to solar ultraviolet radiation and of the effect of occupational exposure to solar ultraviolet radiation on melanoma and non-melanoma skin cancer". *Environment International.* **126** : 804-815. 10.1016/j.envint.2018.09.039.

[17] R. Rahmawati, A. Muflihunna, and M. Amalia. (2018). "Analisis aktivitas perlindungan sinar uv sari buah sirsak (annona muricata l.) berdasarkan nilai Sun Protection Factor (SPF) secara spektrofotometri UV-VIS". *Jurnal Fitofarmaka Indonesia.* **5** (2): 284-288. 10.33096/jffi.v5i2.412.

[18] O. P. Egambaram, S. Kesavan Pillai, and S. S. Ray. (2020). "Materials Science Challenges in Skin UV Protection: A Review". *Photochemistry and Photobiology.* **96** (4): 779-797. 10.1111/php.13208.

[19] M. B. Coltelli, S. Danti, K. De Clerck, A. Lazzeri, and P. Morganti. (2020). "Pullulan for Advanced Sustainable Body- and Skin-Contact Applications". *Journal of Functional Biomaterials.* **11** (1). 10.3390/jfb11010020.

[20] H. Kageyama and R. Waditee-Sirisattha. (2019). "Antioxidative, Anti-Inflammatory, and Anti-Aging Properties of Mycosporine-Like Amino Acids: Molecular and Cellular Mechanisms in the Protection of Skin-Aging". *Marine Drugs.* **17** (4). 10.3390/md17040222.

[21] W. Czarnocka and S. Karpinski. (2018). "Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to environmental stresses". *Free Radical Biology and Medicine.* **122** : 4-20. 10.1016/j.freeradbiomed.2018.01.011.

[22] M. Emanuelli, D. Sartini, E. Molinelli, R. Campagna, V. Pozzi, E. Salvolini, O. Simonetti, A. Campanati, and A. Offidani. (2022). "The Double-Edged Sword of Oxidative Stress in Skin Damage and Melanoma: From Physiopathology to Therapeutical Approaches". *Antioxidants (Basel).* **11** (4). 10.3390/antiox11040612.

[23] D. Xu, M. J. Hu, Y. Q. Wang, and Y. L. Cui. (2019). "Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application". *Molecules.* **24** (6). 10.3390/molecules24061123.

[24] K. Nakai and D. Tsuruta. (2021). "What Are Reactive Oxygen Species, Free Radicals, and Oxidative Stress in Skin Diseases?". *International Journal of Molecular Sciences.* **22** (19). 10.3390/ijms221910799.

[25] S. Weinbaum, L. M. Cancel, B. M. Fu, and J. M. Tarbell. (2021). "The Glycocalyx and Its Role in Vascular Physiology and Vascular Related Diseases". *Cardiovascular Engineering and Technology.* **12** (1): 37-71. 10.1007/s13239-020-00485-9.

[26] M. G. Tosato, D. E. Orallo, M. F. Fangio, V. Diz, L. E. Dicelio, and M. S. Churio. (2016). In: "Surface Chemistry of Nanobiomaterials". 359-392. 10.1016/b978-0-323-42861-3.00012-1.

[27] A. Aditya, S. Chattopadhyay, N. Gupta, S. Alam, A. P. Veedu, M. Pal, A. Singh, D. Santhiya, K. M. Ansari, and M. Ganguli. (2019). "ZnO Nanoparticles Modified with

an Amphipathic Peptide Show Improved Photoprotection in Skin". *ACS Applied Materials & Interfaces.* **11** (1): 56-72. 10.1021/acsami.8b08431.

[28] D. J. Manasa, K. R. Chandrashekar, M. A. Pavan Kumar, D. Suresh, D. J. Madhu Kumar, C. R. Ravikumar, T. Bhattacharya, and H. C. Ananda Murthy. (2021). "Proficient synthesis of zinc oxide nanoparticles from Tabernaemontana heyneana Wall. via green combustion method: Antioxidant, anti-inflammatory, antidiabetic, anticancer and photocatalytic activities". *Results in Chemistry.* **3**. 10.1016/j.rechem.2021.100178.

[29] A. S. Abdelbaky, T. A. Abd El-Mageed, A. O. Babalghith, S. Selim, and A. Mohamed. (2022). "Green Synthesis and Characterization of ZnO Nanoparticles Using Pelargonium odoratissimum (L.) Aqueous Leaf Extract and Their Antioxidant, Antibacterial and Anti-inflammatory Activities". *Antioxidants (Basel).* **11** (8). 10.3390/antiox11081444.

[30] S. M. Taghizadeh, N. Lal, A. Ebrahiminezhad, F. Moeini, M. Seifan, Y. Ghasemi, and A. Berenjian. (2020). "Green and Economic Fabrication of Zinc Oxide (ZnO) Nanorods as a Broadband UV Blocker and Antimicrobial Agent". *Nanomaterials (Basel).* **10** (3). 10.3390/nano10030530.

[31] D. McDaniel, P. Farris, and G. Valacchi. (2018). "Atmospheric skin aging-Contributors and inhibitors". *Journal of Cosmetic Dermatology.* **17** (2): 124-137. 10.1111/jocd.12518.

[32] C. Astutiningsih. (2022). "Isolation And Inhibition Test Of Quercetin Compound From Okra Fruit (Abelmoschus esculentus L.)". *Jurnal Farmasi Sains dan Praktis.* **7** (3): 356-364. 10.31603/pharmacy.v7i3.6203.

[33] P. I. Rajan, J. J. Vijaya, S. K. Jesudoss, K. Kaviyarasu, L. J. Kennedy, R. Jothiramalingam, H. A. Al-Lohedan, and M.-A. Vaali-Mohammed. (2017). "Green-fuel-mediated synthesis of self-assembled NiO nano-sticks for dual applications—photocatalytic activity on Rose Bengal dye and antimicrobial action on bacterial strains". *Materials Research Express.* **4** (8). 10.1088/2053-1591/aa7e3c.

[34] H. Ou-Yang and R. Rzendzian. (2017). "Sunburn Protection by Sunscreen Sprays at Beach". *Cosmetics.* **4** (1). 10.3390/cosmetics4010010.

[35] D. Saravanakkumar, S. Sivaranjani, K. Kaviyarasu, A. Ayeshamariam, B. Ravikumar, S. Pandiarajan, C. Veeralakshmi, M. Jayachandran, and M. Maaza. (2018). "Synthesis and characterization of ZnO–CuO nanocomposites powder by modified perfume spray pyrolysis method and its antimicrobial investigation". *Journal of Semiconductors.* **39** (3). 10.1088/1674-4926/39/3/033001.

[36] Sumaira, M. Siddique Afridi, S. Salman Hashmi, G. S. Ali, M. Zia, and B. Haider Abbasi. (2018). "Comparative antileishmanial efficacy of the biosynthesised ZnO NPs from genus Verbena". *IET Nanobiotechnology.* **12** (8): 1067-1073. 10.1049/iet-nbt.2018.5076.

[37] S. Azizi, M. B. Ahmad, F. Namvar, and R. Mohamad. (2014). "Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalga Sargassum muticum aqueous extract". *Materials Letters.* **116** : 275-277. 10.1016/j.matlet.2013.11.038.

[38] S. Azizi, F. Namvar, M. Mahdavi, M. B. Ahmad, and R. Mohamad. (2013). "Biosynthesis of Silver Nanoparticles Using Brown Marine Macroalga, Sargassum Muticum Aqueous Extract". *Materials (Basel).* **6** (12): 5942-5950. 10.3390/ma6125942.

[39] M. O. Kyene, E. K. Droepenu, F. Ayertey, G. N. Yeboah, M.-A. Archer, D. Kumadoh, S. O. Mintah, P. K. Gordon, and A. A. Appiah. (2023). "Synthesis and characterization of ZnO nanomaterial from Cassia sieberiana and determination of its anti-inflammatory, antioxidant and antimicrobial activities". *Scientific African.* **19**. 10.1016/j.sciaf.2022.e01452.

[40] A. Ilangovan, A. Venkatramanan, P. Thangarajan, A. Saravanan, S. Rajendran, and K. Kaveri. (2021). "Green Synthesis of

Zinc Oxide Nanoparticles (ZnO NPs) using Aqueous Extract of Tagetes Erecta flower and Evaluation of its Antioxidant, Antimicrobial, and Cytotoxic activities on HeLa cell line". *Current Biotechnology.* **10** (1): 61-76. 10.2174/2211550109999201202123939.

[41] M. Singh, K. E. Lee, R. Vinayagam, and S. G. Kang. (2021). "Antioxidant and Antibacterial Profiling of Pomegranate-pericarp Extract Functionalized-zinc Oxide Nanocomposite". *Biotechnology and Bioprocess Engineering.* **26** (5): 728-737. 10.1007/s12257-021-0211-1.

[42] M. Spiegel, K. Kapusta, W. Kolodziejczyk, J. Saloni, B. Zbikowska, G. A. Hill, and Z. Sroka. (2020). "Antioxidant Activity of Selected Phenolic Acids-Ferric Reducing Antioxidant Power Assay and QSAR Analysis of the Structural Features". *Molecules.* **25** (13). 10.3390/molecules25133088.

[43] T. Suktham, A. Jones, A. Soliven, G. R. Dennis, and R. A. Shalliker. (2019). "A comparison of the performance of the cupric reducing antioxidant potential assay and the ferric reducing antioxidant power assay for the analysis of antioxidants using reaction flow chromatography". *Microchemical Journal.* **149**. 10.1016/j.microc.2019.104046.

[44] F. Y. D. Mutmainah, I. Puspitaningrum, and L. Kusmita. (2020). "Sunscreen activity on fruit skin extract of Annatto (Bixa orellana L.) in vitro". *Indian Journal of Science and Technology.* **13** (45): 4506-4512. 10.17485/IJST/v13i45.2143.

[45] H. W. Lim, I. Kohli, C. Granger, C. Trullas, J. Piquero-Casals, M. Narda, P. Masson, J. Krutmann, and T. Passeron. (2021). "Photoprotection of the Skin from Visible Light–Induced Pigmentation: Current Testing Methods and Proposed Harmonization". *Journal of Investigative Dermatology.* **141** (11): 2569-2576. 10.1016/j.jid.2021.03.012.

[46] T. T. Akhil and P. Prabhu. (2013). "Evaluation of anti-oxidant, anti-inflammatory and cytotoxicity potential of Hemigraphis colorata". *International Journal of Pharmaceutical Sciences and Research.* **4** (9). 10.13040/ijpsr.0975-8232.4(9).3477-83.

[47] S. Murthuza and B. K. Manjunatha. (2018). "In vitro and in vivo evaluation of anti-inflammatory potency of Mesua ferrea, Saraca asoca, Viscum album & Anthocephalus cadamba in murine macrophages raw 264.7 cell lines and Wistar albino rats". *Beni-Suef University Journal of Basic and Applied Sciences.* **7** (4): 719-723. 10.1016/j.bjbas.2018.10.001.

[48] S. V. Djova, M. A. Nyegue, A. N. Messi, A. D. Afagnigni, and F. X. Etoa. (2019). "Phytochemical Study of Aqueous Extract of Ochna schweinfurthiana F. Hoffm Powder Bark and Evaluation of Their Anti-Inflammatory, Cytotoxic, and Genotoxic Properties". *Evidence-Based Complementary and Alternative Medicine.* **2019** : 8908343. 10.1155/2019/8908343.

[49] M. Hamoudi, D. Amroun, A. Baghiani, S. Khennouf, and S. Dahamna. (2021). "Antioxidant, Anti-inflammatory, and Analgesic Activities of Alcoholic Extracts of Ephedra nebrodensis From Eastern Algeria". *Turkish Journal Of Pharmaceutical Sciences.* **18** (5): 574-580. 10.4274/tjps.galenos.2021.24571.

[50] M. D, K. P, and V. R. Kolli. (2018). "Characterization and antibacterial activity of ZnO nanoparticles synthesized by co-precipitation method". *International Journal of Applied Pharmaceutics.* **10** (6). 10.22159/ijap.2018v10i6.29376.

[51] I. K. Chukwudozie and I. M. Ezeonu. (2022). "Antimicrobial properties and acute toxicity evaluation of Pycnanthus angolensis stem bark". *Scientific African.* **16**. 10.1016/j.sciaf.2022.e01185.

[52] M. Nurfatwa. (2018). "Uji Toksisitas Akut Ekstrak Buah Okra (Abelmoschus esculatus L.Moench) Terhadap Parameter Kadar Sgot Dan Sgpt Serta Histopatologi Hepar Tikus Galur Wistar". *Journal of Pharmacopolium.* **1** (2). 10.36465/jop.v1i2.330.

[53] J. Tandi, B. Melinda, A. Purwantari, and A. Widodo. (2020). "Analisis Kualitatif dan

Kuantitatif Metabolit Sekunder Ekstrak Etanol Buah Okra (Abelmoschus esculentus L. Moench) dengan Metode Spektrofotometri UV-Vis". *KOVALEN: Jurnal Riset Kimia.* **6** (1): 74-80. 10.22487/kovalen.2020.v6.i1.15044.

[54] W. Chen, T. Li, X. Yan, X. Wu, Y. Zhang, X. Wang, F. Zhang, S. Zhang, and G. He. (2021). "Constructing ionic channels in anion exchange membrane via a Zn2+ soft template: Experiment and molecular dynamics simulation". *Journal of Membrane Science.* **629**. 10.1016/j.memsci.2021.119293.

[55] K. M. Ezealisiji, X. Siwe-Noundou, B. Maduelosi, N. Nwachukwu, and R. W. M. Krause. (2019). "Green synthesis of zinc oxide nanoparticles using Solanum torvum (L) leaf extract and evaluation of the toxicological profile of the ZnO nanoparticles –hydrogel composite in Wistar albino rats". *International Nano Letters.* **9** (2): 99-107. 10.1007/s40089-018-0263-1.

[56] S. Faisal, H. Jan, S. A. Shah, S. Shah, A. Khan, M. T. Akbar, M. Rizwan, F. Jan, Wajidullah, N. Akhtar, A. Khattak, and S. Syed. (2021). "Green Synthesis of Zinc Oxide (ZnO) Nanoparticles Using Aqueous Fruit Extracts of Myristica fragrans: Their Characterizations and Biological and Environmental Applications". *ACS Omega.* **6** (14): 9709-9722. 10.1021/acsomega.1c00310.

[57] S. Fakhari, M. Jamzad, and H. Kabiri Fard. (2019). "Green synthesis of zinc oxide nanoparticles: a comparison". *Green Chemistry Letters and Reviews.* **12** (1): 19-24. 10.1080/17518253.2018.1547925.

[58] Farhana, M. F. H. Munis, K. H. Alamer, A. T. Althobaiti, A. Kamal, F. Liaquat, U. Haroon, J. Ahmed, H. J. Chaudhary, and H. Attia. (2022). "ZnO Nanoparticle-Mediated Seed Priming Induces Biochemical and Antioxidant Changes in Chickpea to Alleviate Fusarium Wilt". *Journal of Fungi.* **8** (7). 10.3390/jof8070753.

[59] G. S. Mei, P. S. Menon, and G. Hegde. (2020). "ZnO for performance enhancement of surface plasmon resonance biosensor: a review". *Materials Research Express.* **7** (1). 10.1088/2053-1591/ab66a7.

[60] Y. Bouramdane, S. Fellak, F. El Mansouri, and A. Boukir. (2022). "Impact of Natural Degradation on the Aged Lignocellulose Fibers of Moroccan Cedar Softwood: Structural Elucidation by Infrared Spectroscopy (ATR-FTIR) and X-ray Diffraction (XRD)". *Fermentation.* **8** (12). 10.3390/fermentation8120698.

[61] A. Maimulyanti, I. Nurhidayati, B. Mellisani, F. Amelia Rachmawati Putri, F. Puspita, and A. Restu Prihadi. (2023). "Development of natural deep eutectic solvent (NADES) based on choline chloride as a green solvent to extract phenolic compound from coffee husk waste". *Arabian Journal of Chemistry.* **16** (4). 10.1016/j.arabjc.2023.104634.

[62] S. S. K, V. Vellora Thekkae Padil, C. Senan, R. Pilankatta, S. K, B. George, S. Waclawek, and M. Cernik. (2018). "Green Synthesis of High Temperature Stable Anatase Titanium Dioxide Nanoparticles Using Gum Kondagogu: Characterization and Solar Driven Photocatalytic Degradation of Organic Dye". *Nanomaterials (Basel).* **8** (12). 10.3390/nano8121002.

[63] N. Saridewi, H. T. Syaputro, I. Aziz, D. Dasumiati, and B. N. Kumila. (2021). "Synthesis and characterization of ZnO nanoparticles using pumpkin seed extract (Cucurbita moschata) by the sol-gel method". *the 4th International Seminar on Chemistry*. 10.1063/5.0051826.

[64] G. Gong, Y. Zhang, and H. Zheng. (2022). "Extraction and characterization of coal-based fulvic acid from Inner Mongolia lignite by hydrogen peroxide-glacial acetic acid". *Chemical Papers.* **76** (3): 1665-1674. 10.1007/s11696-021-01986-0.

[65] R. Khan, M. Inam, M. Iqbal, M. Shoaib, D. Park, K. Lee, S. Shin, S. Khan, and I. Yeom. (2018). "Removal of ZnO Nanoparticles from Natural Waters by Coagulation-Flocculation Process: Influence of Surfactant Type on Aggregation, Dissolution and Colloidal Stability". *Sustainability.* **11** (1). 10.3390/

su11010017.

[66] Z. Eskandari, F. Bahadori, B. Celik, and H. Onyuksel. (2020). "Targeted Nanomedicines for Cancer Therapy, From Basics to Clinical Trials". *Journal of Pharmacy & Pharmaceutical Sciences.* **23** (1): 132-157. 10.18433/jpps30583.

[67] C. Mendez Torrecillas, G. W. Halbert, and D. A. Lamprou. (2017). "A novel methodology to study polymodal particle size distributions produced during continuous wet granulation". *International Journal of Pharmaceutics.* **519** (1-2): 230-239. 10.1016/j.ijpharm.2017.01.023.

[68] M. Nasrollahzadeh and S. M. Sajadi. (2015). "Synthesis and characterization of titanium dioxide nanoparticles using Euphorbia heteradena Jaub root extract and evaluation of their stability". *Ceramics International.* **41** (10): 14435-14439. 10.1016/j.ceramint.2015.07.079.

[69] M. Sathishkumar, K. Sneha, I. S. Kwak, J. Mao, S. J. Tripathy, and Y. S. Yun. (2009). "Phyto-crystallization of palladium through reduction process using Cinnamom zeylanicum bark extract". *Journal of Hazardous Materials.* **171** (1-3): 400-4. 10.1016/j.jhazmat.2009.06.014.

[70] V. Sharma and P. Janmeda. (2017). "Extraction, isolation and identification of flavonoid from Euphorbia neriifolia leaves". *Arabian Journal of Chemistry.* **10** (4): 509-514. 10.1016/j.arabjc.2014.08.019.

[71] U. R. Shwetha, M. S. Latha, C. R. Rajith Kumar, M. S. Kiran, and V. S. Betageri. (2020). "Facile Synthesis of Zinc Oxide Nanoparticles Using Novel Areca catechu Leaves Extract and Their In Vitro Antidiabetic and Anticancer Studies". *Journal of Inorganic and Organometallic Polymers and Materials.* **30** (12): 4876-4883. 10.1007/s10904-020-01575-w.

[72] O. Chongsrimsirisakhol and T. Pirak. (2022). "Total polyphenol content and antioxidant properties of cold brew coffee extracts as affected by ultrasound treatment and their application in low fat pork sausage". *International Journal of Food Properties.* **25** (1): 813-826. 10.1080/10942912.2022.2056197.

[73] V. Areekul and W. Krungkri. (2019). "Effect of Heating Condition and pH on Stability of Total Phenolic Content and Antioxidant Activities of Samui (Micromelum minutum) Extract". *the Proceedings of the 16th ASEAN Food Conference*. 10.5220/0009980801260132.

[74] A. M. Espinosa-Gonzalez, E. A. Estrella-Parra, E. Nolasco-Ontiveros, A. M. Garcia-Bores, R. Garcia-Hernandez, E. Lopez-Urrutia, J. E. Campos-Contreras, M. D. R. Gonzalez-Valle, J. D. C. Benitez-Flores, C. L. Cespedes-Acuna, J. Alarcon-Enos, J. C. Rivera-Cabrera, and J. G. Avila-Acevedo. (2021). "Hyptis mociniana: phytochemical fingerprint and photochemoprotective effect against UV-B radiation-induced erythema and skin carcinogenesis". *Food and Chemical Toxicology.* **151** : 112095. 10.1016/j.fct.2021.112095.

[75] R. Pandel, B. Poljsak, A. Godic, and R. Dahmane. (2013). "Skin photoaging and the role of antioxidants in its prevention". *ISRN Dermatology.* **2013** : 930164. 10.1155/2013/930164.

[76] S. Gonzalez, J. Aguilera, B. Berman, P. Calzavara-Pinton, Y. Gilaberte, C. L. Goh, H. W. Lim, S. Schalka, F. Stengel, P. Wolf, and F. Xiang. (2022). "Expert Recommendations on the Evaluation of Sunscreen Efficacy and the Beneficial Role of Non-filtering Ingredients". *Frontiers in Medicine.* **9** : 790207. 10.3389/fmed.2022.790207.

[77] J. A. Mojeski, M. Almashali, P. Jowdy, M. E. Fitzgerald, K. L. Brady, N. C. Zeitouni, O. R. Colegio, and G. Paragh. (2020). "Ultraviolet imaging in dermatology". *Photodiagnosis and Photodynamic Therapy.* **30** : 101743. 10.1016/j.pdpdt.2020.101743.

[78] M. Athiyah, I. Ahmad, and L. Rijai. (2015). "Aktivitas Tabir Surya Ekstrak Akar Bandotan (Ageratum Conyzoides L.)". *Jurnal Sains dan Kesehatan.* **1** (4): 181-187. 10.25026/jsk.v1i4.37.

[79] C. Antoniou, M. G. Kosmadaki, A. J. Stratigos, and A. D. Katsambas. (2008).

"Sunscreens – what's important to know". *Journal of the European Academy of Dermatology and Venereology.* **22** (9): 1110-1119. 10.1111/j.1468-3083.2007.02580.x.

[80] U. Bhattacharya, A. Hackethal, S. Kaesler, B. Loos, and S. Meyer. (2012). "Is Unbiased Financial Advice to Retail Investors Sufficient? Answers from a Large Field Study". *Review of Financial Studies.* **25** (4): 975-1032. 10.1093/rfs/hhr127.

[81] M. Mueller, S. Hobiger, and A. Jungbauer. (2010). "Anti-inflammatory activity of extracts from fruits, herbs and spices". *Food Chemistry.* **122** (4): 987-996. 10.1016/j.foodchem.2010.03.041.

[82] A. Khakpour, N. A. Shadmehri, H. Amrulloh, and H. Kioumarsi. (2023). "Antibacterial Effect of Juglans regia, Citrus sinensis, Vicia faba, and Urtica urens Extracts under In vitro Conditions". *Bioactivities.* **1** (2): 74-80. 10.47352/bioactivities.2963-654X.195.

[83] K. M. Reddy, K. Feris, J. Bell, D. G. Wingett, C. Hanley, and A. Punnoose. (2007). "Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems". *Applied Physics Letters.* **90** (213902): 2139021-2139023. 10.1063/1.2742324.

[84] M. Godoy-Gallardo, U. Eckhard, L. M. Delgado, Y. J. D. de Roo Puente, M. Hoyos-Nogues, F. J. Gil, and R. A. Perez. (2021). "Antibacterial approaches in tissue engineering using metal ions and nanoparticles: From mechanisms to applications". *Bioactive Materials.* **6** (12): 4470-4490. 10.1016/j.bioactmat.2021.04.033.

[85] Y. Hong, J. Zeng, X. Wang, K. Drlica, and X. Zhao. (2019). "Post-stress bacterial cell death mediated by reactive oxygen species". *Proceedings of the National Academy of Sciences.* **116** (20): 10064-10071. 10.1073/pnas.1901730116.

[86] K. Khorsandi, S. Keyvani-Ghamsari, F. Khatibi Shahidi, R. Hosseinzadeh, and S. Kanwal. (2021). "A mechanistic perspective on targeting bacterial drug resistance with nanoparticles". *Journal of Drug Targeting.* **29** (9): 941-959. 10.1080/1061186X.2021.1895818.

[87] T. P. Lestari, F. A. Tahlib, J. Sukweenadhi, K. Kartini, and C. Avanti. (2019). "Physical Characteristic and Antibacterial Activity of Silver Nanoparticles from Green Synthesis Using Ethanol Extracts of Phaleria macrocarpa (Scheff.) Boerl Leaves". *Majalah Obat Tradisional.* **24** (1). 10.22146/mot.37956.

[88] T. Naseem and M. A. Farrukh. (2015). "Antibacterial Activity of Green Synthesis of Iron Nanoparticles UsingLawsonia inermisandGardenia jasminoidesLeaves Extract". *Journal of Chemistry.* **2015** : 1-7. 10.1155/2015/912342.