UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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Green synthesis of light-assisted silver nanoparticles using different agro-industrial waste extracts.

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HOJA DE CALIFICACIÓN DE TRABAJO DE FIN DE CARRERA

Green synthesis of silver nanoparticles in absence and presence of LED light

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RESUMEN

Las nanopartículas de plata son un nanomaterial interesante entre una variedad de nanopartículas metálicas que pueden estar involucradas en aplicaciones biomédicas y en varios campos de la industria. El presente trabajo detalla la síntesis verde de nanopartículas de plata asistidas por luz (AgNPs) usando como solventes agua y solvente eutéctico profundo natural (NADES) con extractos de semilla de aguacate, cáscara de cacao y cáscara de naranja utilizando métodos de maceración y Soxhlet para la extracción de los metabolitos presentes en la planta. La absorción UV-Vis mostró la banda de resonancia plasmónica característica de las AgNP a 435 nm. El TEM mostró el tamaño de las AgNPs con tendencia a una morfología esférica. Y se utilizó la caracterización FTIR para obtener información sobre los grupos funcionales presentes en las NPs y en los extractos. El NADES mostró una aglomeración de las NPs, y se discuten las razones. Se concluyó que las AgNPs tienen una distribución homogénea, que la materia prima utilizada para la obtención de los extractos fue la mejor debido a que se desempeñaron como agentes estabilizantes, de recubrimiento y reductores. Finalmente, se discute el trabajo futuro que se podría realizar a partir de esta investigación para corregir y mejorar el tamaño y forma de las nanopartículas.

Palabras clave: Disolvente natural eutéctico profundo, extracción Soxhlet, maceración, metabolitos vegetales, semillas de aguacate, cascarilla de cacao, cáscara de naranja, extracción verde, síntesis, nanopartículas de plata, caracterización.

ABSTACT

Silver nanoparticles are an interesting nanomaterial among a variety of metallic nanoparticles that can be involved in biomedical applications and in several fields of the industry. The present work details the green synthesis of light-assisted silver nanoparticles (AgNPs) using as solvents water and natural deep eutectic solvent (NADES) with avocado seeds, cacao pod husks and orange peel extracts using maceration and Soxhlet methods for extraction of its plant metabolites. The UV-Vis absorption showed the characteristic plasmon resonance band of AgNPs at 435 nm. The TEM showed the size of the AgNPs with a tendency to a spherical morphology. And the FTIR characterization was used to obtain information about the functional groups present in the NPs and in the extracts. The NADES showed an agglomeration of the NPs, and the reasons are discussed. We conclude that the AgNPs have a homogeneous distribution, that the raw material used for obtaining the extracts was the best because they performed as stabilizing, capping, and reduction agents. Finally, is discussed the future work that could be done from this investigation to correct and improve size and shape of the nanoparticles.

Keywords: Natural Deep eutectic solvent, Soxhlet extraction, maceration, plant metabolites, Avocado seeds, cacao pod husks, orange peel, green extraction, synthesis, silver nanoparticles, characterization.

CONTENT

TABLES

FIGURES

1. INTRODUCTION

1.1. Background

Nanotechnology involves the production of materials with sizes between 1 and 100 nm in at least two dimensions [1], [2]. Metal nanoparticles (MNPs) have several applications for the food industry, environment and in the field of healthcare [3], [4]. AgNPs have been widely used as antimicrobial agents, drug-delivers, biomedical device coatings, among others, due to their antibacterial and antiviral properties [4].

However, there are a handful of reports showing that chemically synthesized AgNPs have cytotoxic and genotoxic effects that cause oxidative cell and DNA damage, inhibition of cell proliferation, etc. This, due to the nature of the reducing agent and capping material used [5]. NPs are synthesized by a variety of methods such as physical and chemical ones; these methods use harsh conditions that lead to health risks, so the biological synthesis appears as a good solution [1].

In this sense, a variety of MNPs have been produced through green synthesis using medicinal plants and other type of plant species. Several studies have shown the successful synthesis of MNPs using plant extracts [3]. For instance, south African medicinal plants such as *Salvia Africana-lutea*, *Galenia Africana*, and many others have been successfully used for NPs synthesis in the range between 5-50 nm [3]. A better-known example of plant extract is the *Musa paradisiaca* using its peel to synthesize spherical AgNPs with a size of 23.7 nm and was tested for a great number of microorganisms [6].

The plant secondary metabolites extracted from different parts of the plant, make the NPs present different morphological and physicochemical characteristics depending on the metal and the part of the plant used for the extraction of them [4], [7], [8], [9]. For instance, the terpenoids act as capping and reducing agents for the formation of uniform nanoparticles [10] and the polyphenolic compounds have a great number of bioactive properties [11].

Vegetables are consumed all over the world, and their residues are usually thrown away [4], [7], [9], [10], [11], [12], [13]. The processing of fruits and vegetables generates around 25-30% of total global waste, this waste includes mainly: seeds, peels, pomace that have valuable bioactive compounds [14]. For instance, the production of oranges (*Citrus sinensis*) is about 70% of the citrus world production and consumption, and 50% of the fruit mass is the citrus peel that is discharged [15]. Figure 1 shows the production of citric fruits around the globe, and how it is distributed to conform the total amount of the principal citrus global production, including oranges, tangerines, lemon, and grapefruit [15].

The 16% of total fruit production world-wide is banana (*Musa*), where the 30-40% (w/w) of the fruit is its peel [16]. The Food and Agriculture Organization of the United Nations (FAO) reported that 7,0 million tons of avocado (*Persea americana)* are produced, and half of this production is lost on the process [17]. Cocoa Pod Husk constitute approximately 76% of the cocoa fruit weight, 95% of cacao produced worldwide comes mainly from Brazil, Ecuador, Nigeria, Indonesia, Ghana, Malaysia, Côte D'Ivoire, the largest producing countries.

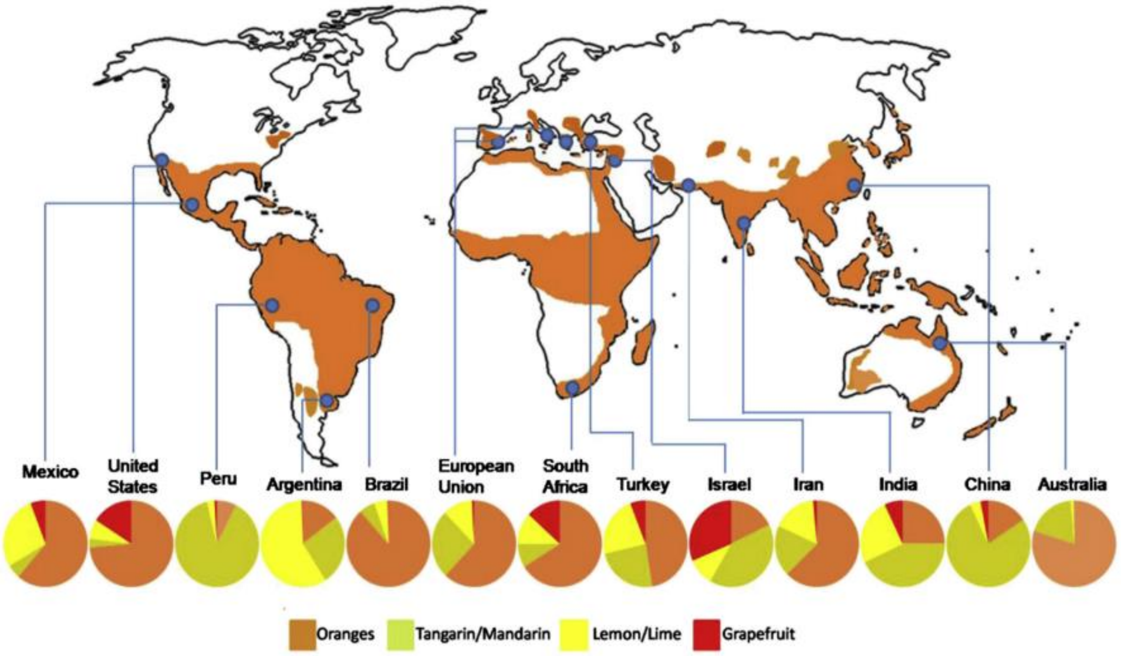

Figure 1: Production of citric fruits around the globe.

This figure above shows the production of citric fruits around the globe, and how it is distributed to conform the total amount of the principal citrus global production, including oranges, tangerines, lemon, and grapefruit [15].

Extracts from agro-industrial residues as avocado seeds, cacao pod husks, orange peels and banana peels are used as possible reducing and capping agents for NPs synthesis, as it has been reported in the literature. There are studies that show the synthesis of AgNPs by a green route using banana peels as stabilizer, obtaining a new range of antibacterial agents [7]; cacao pod husks, that contain oxalic acid and acts as reducing agent and contain flavonoids as well, that act as capping agents [13]. The use of avocado seeds also has been studied globally, due to its content of carotenoids that have antioxidant properties and help in the synthesis of the NPs [4]. Utilization of peel extracts as reducing agents for the synthesis of AgNPs has increased and the NPs obtained have been tested against several bacterial strains, showing their remarkable antibacterial activity [18].

Due to the antimicrobial resistance (AMR) threatening the future, with a growing number of annual deaths (with a projection of 10 million deaths by 2050), the interest of finding alternatives to the actual antimicrobials has increased [19]. Plant extracts play a key role for the green synthesis of metal particles with enhanced antimicrobial properties. Thus, their extraction using green solvents such as deep eutectic solvents (NADESs) has been of great interest for scientific research. There is no published information about the activity that eutectic solvents may have in the MNPs morphology and how much affinity with the biomass will have the metals studied.

The use of green solvents for plant extraction has been of great interest for scientific research to help the industries that want to change the MNPs produced chemically with green synthesized ones due to their nontoxic nature [5]. The use of green chemistry has increased in the past few years, because of its capability of reducing/eliminating hazardous substances in the processes and the residues generated in those processes [11]. Plant based MNPs are safer and eco-friendly in comparison with the chemically synthesized ones [7], [9]. Undeniably, it needs more time until the industry changes fully to greener routes because of the cost it represents.

The use of ethanolic and aqueous solvents is safe, but since 2004, there is this whole new generation of DESs and from 2013 until now, NADESs (Natural Deep Eutectic Solvents), also work as a green extraction method. The DESs are mixtures of many quaternary ammonium salts and carboxylic acids forming a mixture that shows similar properties to ionic liquids but are easier to synthesize and biodegradable so are an alternative to ionic liquids [20].

In the other hand, NADES have shown great potential because of their performance on the extraction of plant metabolites. They are easily prepared, biodegradable, eco-friendly and increase the solubility and bioactivity of some compounds compared to other solvents [21]. This solvent is an alternative to water in nature, and they have shown the solubility of flavonoids that are insoluble in water, but still having water in its supramolecular structure due to the hydrogen bonds formed between hydroxyl groups [22].

In the present work, the extraction of plant chemical components is performed by using eco-friendly solvents, such as water and NADESs [23]. There has been no investigation of the synthesis of MNPs with plant extracts obtained by a green process. The synthesis of the MNPs will be conducted through thermic processes in absence of light, as well as, assisted by LED light [24].

1.2. Specialized terminology

- Polyphenols: Are considered the most active compounds of many vegetables and plants, also they are nutraceuticals that could act as neuroprotective agents due to their antioxidant ability [25].
- Deep eutectic solvents (DES): A mixture of organic or inorganic solid compounds that liquifies and forms stable eutectics at optimum conditions [26]. The difference of these solvents from the IL (Ionic liquids) is that the DES are easy to prepare in a pure state and exhibit high solubilities.
- Natural deep eutectic solvents (NADES): Are considered natural because of the constituents of the eutectic mixture, that are primary metabolites like sugars and amino acids [27].
- Plant secondary metabolites: Bioactive compounds of the plant that enable them to survive under stress conditions; some of them are: terpenoids, alkaloids, phenolic compounds [10], [26].
- Photon induced method: Method that makes controlled reducing action and the creation of uniform AgNPs, without the need of additional absorption promoters [24].
- Characterization: The evaluation of the functional aspects of the synthesized nanoparticles [28].
- Green synthesis: A way of synthesis that solves the devastating effects of physical and chemical techniques. It is a bottom-up approach, where the NPs are produced through oxidation/reduction process of metallic ions [1]. The synthesis of NPs using plant extracts is a single-step method, with lots of metabolites [1].
- Green extraction: A type of extraction that the industry prefers due to their non-toxic nature where the organic solvents are green, such as DESs and NADESs [11].
- Nanoparticles (NPs): Are particles at a size range between 1 and 100 nm [3].

- Antibacterial activity: Resistance to a wide range of bacterial pathogens [29]. The plants have an array of defense mechanisms like the secondary metabolites that combat pathogens before they could make a serious damage [19].

2. METHODOLOGY

The collection of the agro wastes was directly from the market or from agro-industries that produce orange juice, banana pure, guacamole and chocolates.

2.1. Raw material preparation

This process was standardized in the GICAS laboratory [30], however, the protocol was slightly modified to the needs of this investigation.

The avocado seeds were chopped and triturated to obtain particle size between 2nm and 500 µm and refrigerated. Clean cacao pod husks and orange peels were dried in the oven at 40°C for 1 night and then crushed and kept in a dried place.

2.2. NADES preparation

The Liquid/Solid ratio (solvent/raw material) was 10:1, solvents used were water, ethanol 70%, methanol 80% and a sodium acetate/glycerol (3:1) NADES. Alcohols were stirred at 300 rpm for 5 hours and then filtered [30], whereas the NADES, was macerated at ambient temperature in the dark for 72h without stirring, and finally, filtered.

2.3. Soxhlet extraction

The process of extraction for all the agro-industrial wastes was the same [30]. 9,00 g of the raw material was placed in a cellulose thimble and the placed in the Soxhlet apparatus, and in the round bottom flask were placed 150 mL of water, ethanol 70% and methanol 80% (triplicated), for 5 hours. Then, all the samples cooled until reached ambient temperature for approximately 1 hour). The resulting extracts were concentrated in the rotary evaporator and then lyophilized.

2.4. Synthesis of AgNPs assisted by led light (blue, green, red, dark)

2.4.1. Preparation of extract solution.

The lyophilized extracts were prepared with a 1:100 extract/water solution ratio. In this case, it was used 0,5g of extract and 50 mL of water.

2.4.2. Preparation of silver salt solution.

A 2.5 mM solution of $AgNO_3$ was prepared in a 25mL volumetric flask. This solution was fresh prepared the day of experiment to reduce error in the results.

2.4.3. Synthesis of AgNPs.

A mixture of extract/silver salt solution at a 2:1 ratio was prepared in a dark ambient and transferred to two UV-Vis cuvettes, one to be placed in front of a LED-light panel and the other one in a place with no light entry.

Before turning on the LED-light, an aliquot of 300 µL was taken and mixed with 2000 µL of distilled water and characterized in the UV-Vis at time 0 (in absence of light); after the aliquot was taken, the led-light was turned on, and the aliquots at 5, 10, 20, 40, 60 minutes, and then each hour were taken, for a period of 6 hours, approximately. This process was repeated with different LED color light used.

2.4.4. Analytical methods.

The absorption spectra of Ag colloids were recorded using a UV-Vis spectrometer (CECIL) with a wavelength range from 300 to 1000 nm and a pathlength of 10mm. The blank solutions were prepared substituting the silver nitrate solution with distilled water. The FTIR spectra were recorded from 4000 to 650 cm^{-1} regimes using an Agilent Cary 630 ATR-FTIR analyzer. For the study of the size and morphology of the AgNPs, a FEI Spirit Twin with LaB6 filament transmission electron microscope (TEM) was used operating at a voltage of 80 kV.

3. RESULTS AND DISCUSSION

The results obtained from the plant extracts, the AgNPs synthesized using those extracts and its corresponding characterization are summarized in this section of this study. Table 1 shows the agro-residues, and solvents and the extracts obtained from Soxhlet extraction and maceration extraction. * The same procedure as the others was followed, but the extract was not useful for the formation of AgNPs.In the following section, the extracts obtained, and their use for NPs synthesis is presented. UV-Vis, TEM and FTIR characterization of synthesized materials is also shown for analysis and discussion.

Table 1: Extracts and solvents used in this study.

EXTRACT	AGRO-INDUSTRIAL RESIDUE	SOLVENT
1	Avocado seeds	Water
2	Avocado seeds	Sodium acetate:glycerol (1:3)
3	Cacao pod husks	Water
4	Cacao pod husks	Sodium acetate:glycerol (1:3)
5	Orange peels	Water *
6	Orange peel	Sodium acetate:glycerol (1:3)

The extractions were successfully performed. The Soxhlet extraction shown in Figure 2: was carried out in accordance with the analytical technique. The aqueous extracts were made by using the two most common plant secondary metabolites extraction methods: maceration and Soxhlet extraction. The NADES extracts were prepared by maceration extraction, the easiest and low cost/energy method of extraction. During the extraction process, the coloration of the avocado seed is bright dark orange, as it is shown in Figure 2, which leads to the conclusion that the extraction of almost all biomolecules has been performed. In fact, each sample was subjected to extraction for three times, in the second and third time, the color was orange and pale yellow, respectively.

Figure 2: Soxhlet extraction of avocado seeds metabolites.

Once the extracts were obtained, MNPs synthesis with and without LED-light irradiation was conducted. **Error! Reference source not found.** shows the formation of the AgNPs using avocado seeds extract irradiated with LED blue light (a picture of the system can be found in the ANNEX 7.3). Along the irradiation time, the appearance and increment of the surface plasmon resonance was observed, which is characteristic for the formation of AgNPs. A peak maximum around 435 nm was observed, related to the formation of quasi-spherical silver nanoparticles.

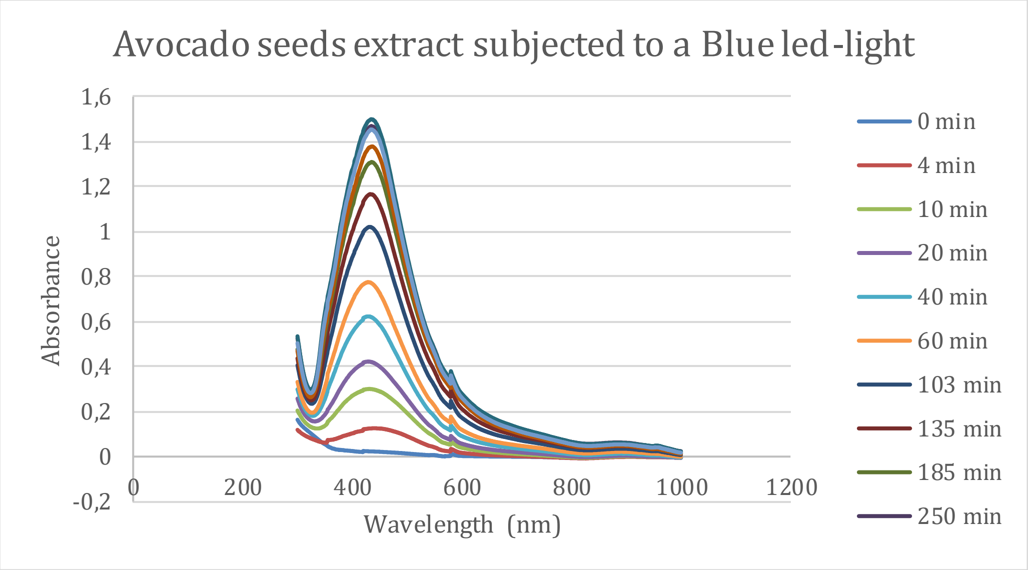

Figure 3: UV-Vis characterization and comparison of absorbance vs. wavelength of the AgNPs synthesized from avocado seeds extract in water.

For a better understanding of the AgNPs formation using orange peel aqueous extract, peak maximum vs. time irradiation curves graph is depicted in Figure 5. It is noted that after 6 hours of light irradiation, almost all silver salt was consumed for NPs formation. In Figure 4, AgNPs formation is shown using different color LED lights and without light irradiation. Green LED light is considerable slower for AgNPs formation, and after 6 hours, the synthesis did not end. For irradiation with red LED light, the AgNPs formation was negligible. For samples mixed without light irradiation, no NPs formation was observed during the reaction time [24]. Similar behavior was observed using the rest of plant extracts shown in Table 1.

The formation of AgNPs could be seen as a color change phenomenon, solutions went from transparent pale orange/yellow to brown within 10 minutes, and as time passed the lighter brown color changed to a solid dark brown, as shown in the ANNEX 7.3. For almost all extracts the change of the coloration was a qualitative parameter to confirm the start of the AgNPs synthesis. However, for extracts from orange peels aqueous extract even when therein water, NPs production was color change. not observable in the UV/Vis spectra, as shown in Figure 4.

According to the literature, some of the flavonoids that are the target in orange peel extract are the phenolic antioxidants (gallic acid, ferulic acid, para-coumaric acid). Also, they mention a step of pectin remotion from orange waste [32] and relaying on the laboratory experience, that step would help in the maceration extraction using water. The visual observation of color change is one of the most mentioned ways to predict the formation of the MNPs, followed by UV-Vis and TEM for characterizing them. The experiment was conducted in triplicate and each time the result was the same. Finally, the reaction occurring with the silver salt and the orange peel extract is difficult to control because of the high number of biomolecules in the solution that can interfere in the synthesis [32].

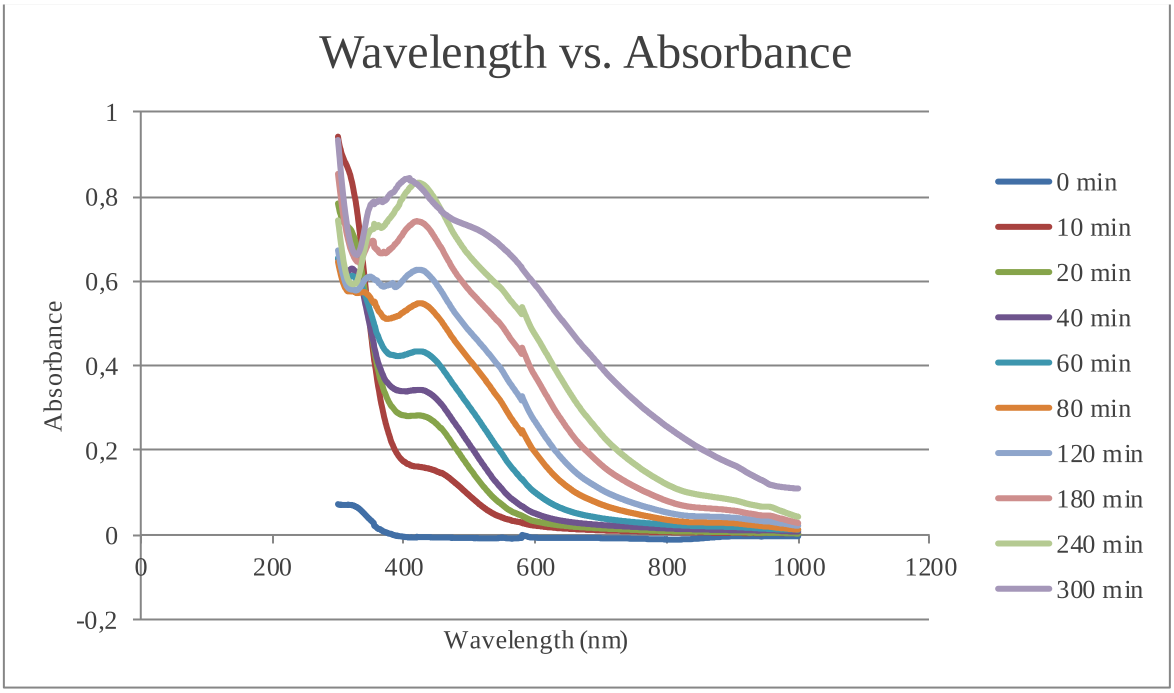

Figure 4: UV-Vis absorbance vs. wavelength of the AgNPs synthesized from orange peel aqueous extract.

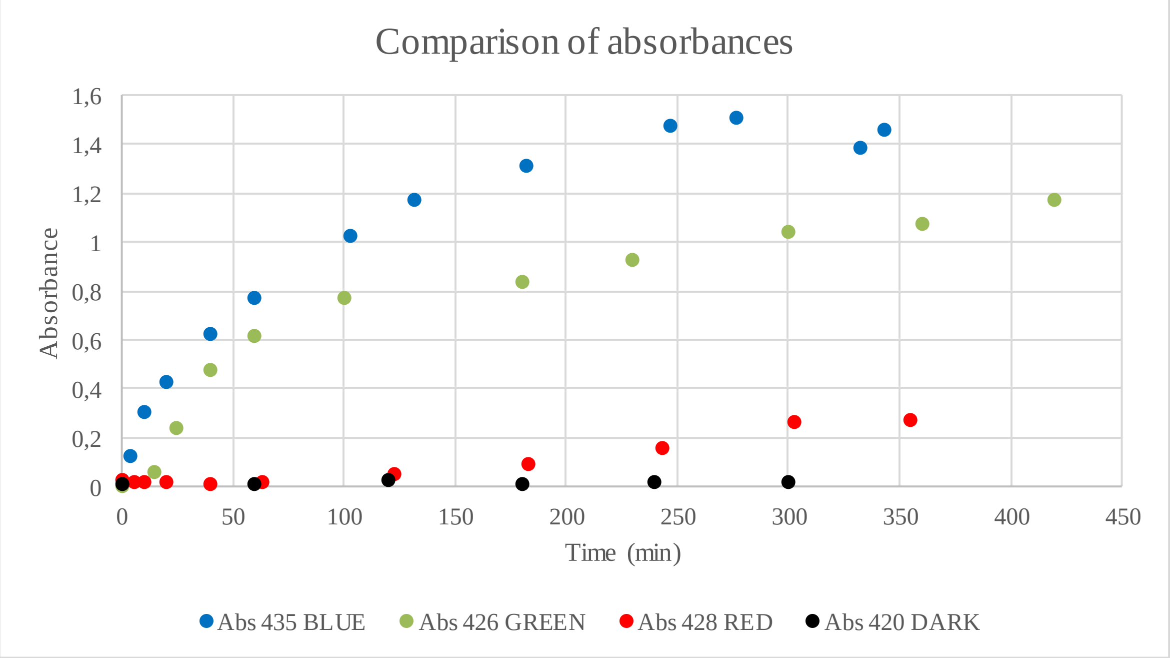

Figure 5: Curves of comparison of the absorbance vs. time of formation of the AgNPs synthesized from avocado seeds extract in water with different led lights.

The curve of Figure 7 shows a comparison for NPs formation between cacao and avocado extracts in water irradiated with blue LED light. As shown, NPs formation using cacao extract seems to be faster compared to the avocado one. Faster synthesis is normally associated with more homogenous particle distribution.

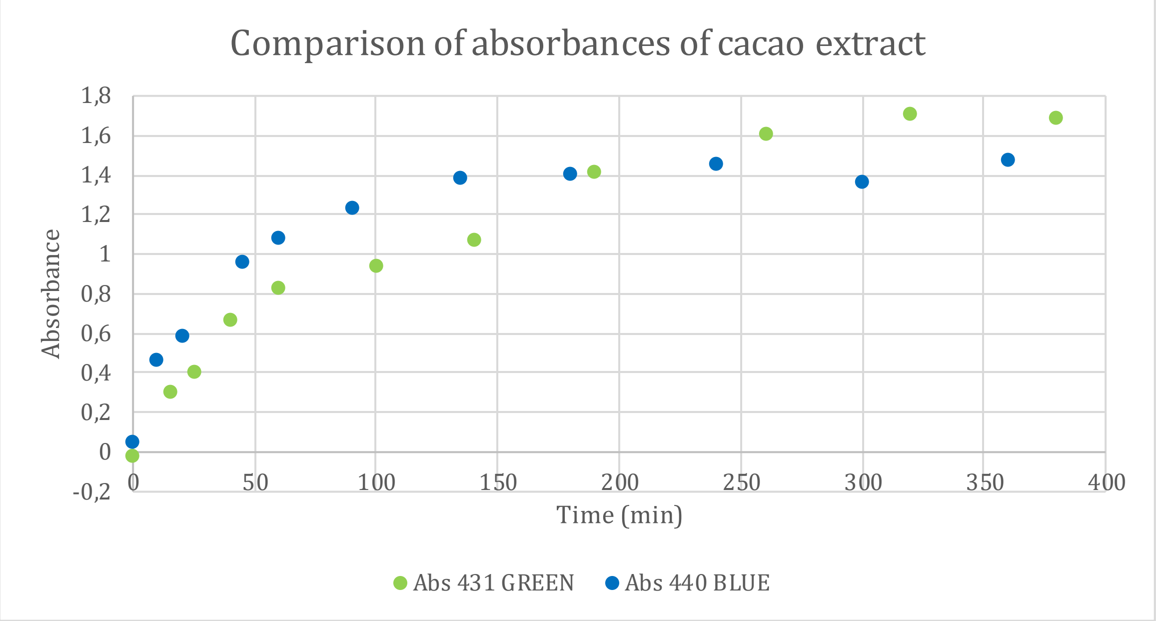

Figure 6: Curves of comparison of the absorbance vs. time of formation of the AgNPs synthesized from cacao pod husks extract in water with the most relevant LED lights.

3.1. AgNPs synthesized with cacao pod husks and avocado seeds extracts

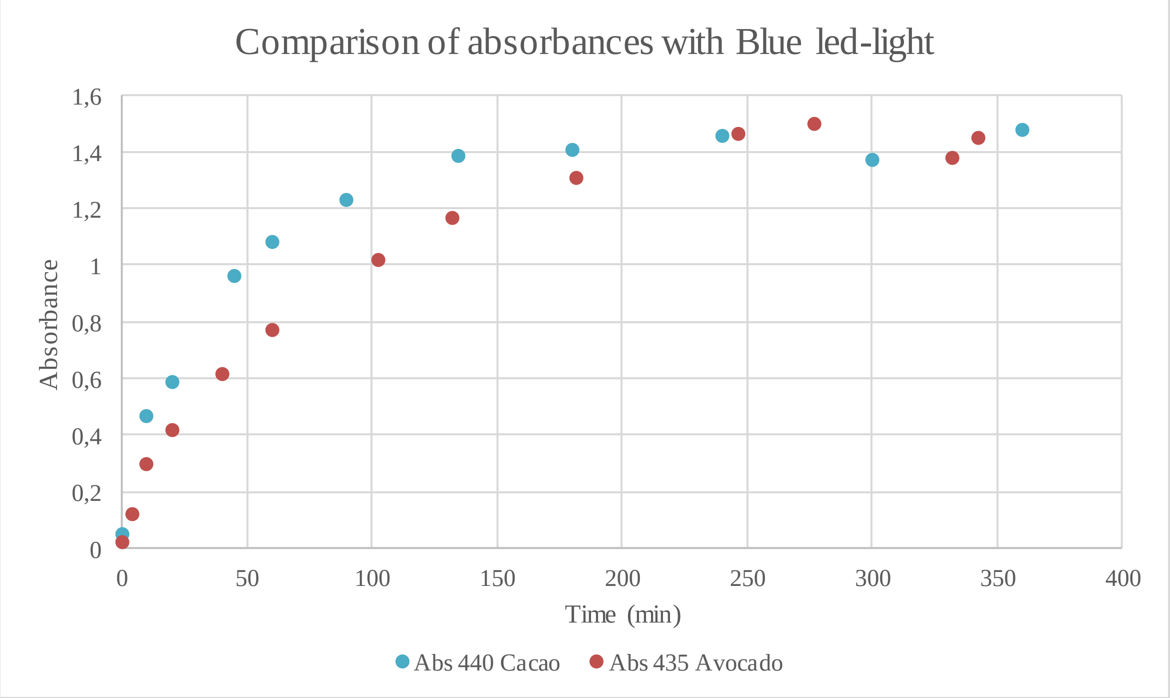

Figure 7: Curves of comparison of the absorbance vs. time of formation of the AgNPs synthesized from avocado seeds and cacao aqueous extracts with blue led light.

FTIR is a powerful tool to study the functional groups of the biomolecules, such as terpenoids and flavonoids, their role is to act as reducing and capping agents for the synthesis of AgNPs [9]. FTIR spectra for extracts and synthesized NPs are presented in Figure 8. The peaks show that there are the AgNPs formed. The -OH groups donate electrons, that oxidize the hydroxyl groups, and this reduce the Ag+ ions to form the AgNPs [13].

Something to notice is that the existent metabolites in the cacao pod husk and in the avocado seed are the same, as they were explained in table 2, but also have a reduction in the amines peak. The reduction of the peak intensity could be due to redox reaction between the biomolecules and the Ag+ ions; biomolecules such as flavonoids and other secondary metabolites [33]. This means the biomolecules blended with the AgNPs and the extracts are working as reducing and capping agents [34].

3.2. FTIR spectra of avocado seed and cacao peel extracts and AgNPs.

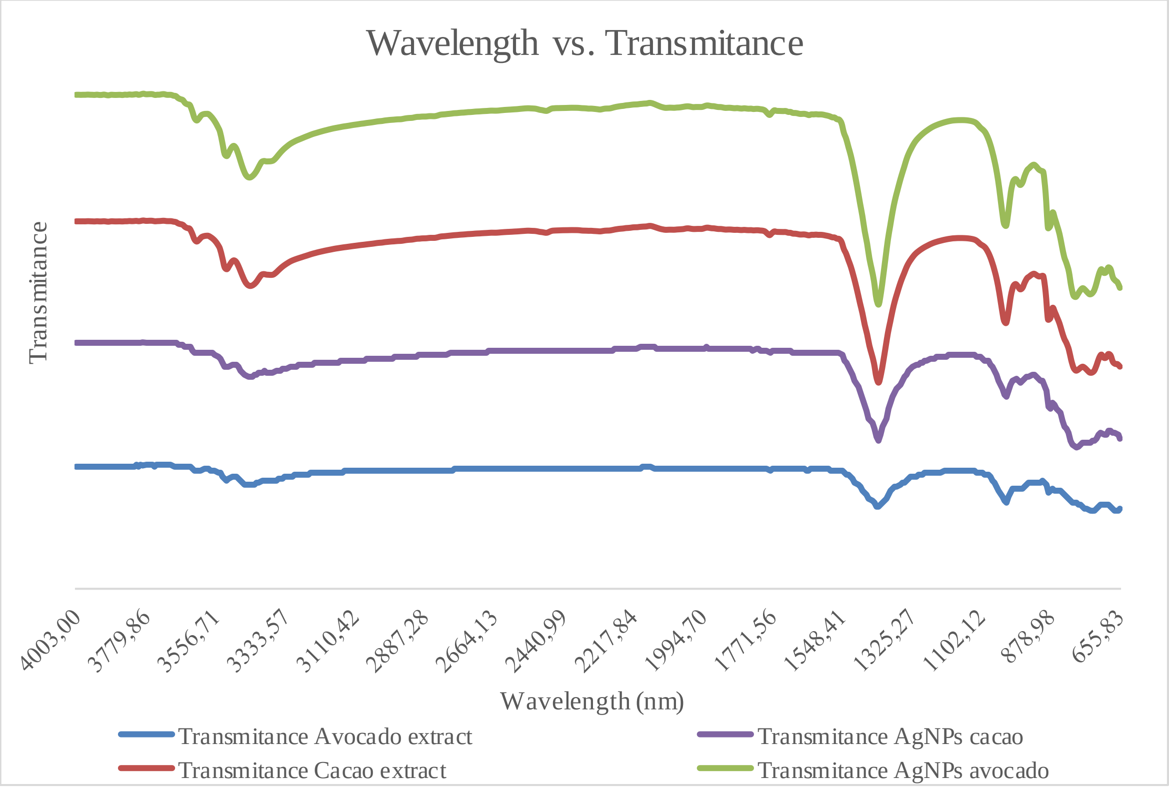

Figure 8: FTIR characterization and comparison of transmittance vs. wavelength of the AgNPs synthesized from avocado seeds and cacao pod husk aqueous extracts.

Table 2: Identification of the vibrations of the plant extracts and the synthesized AgNPs.

# OF PEAK	**Extract (CM^{-1})**	**AgNPs (CM^{-1})**	**Vibration MODE**
1	3500	3500	-OH
2	3400	3400	Amides
3	1400	1400	C=C C-H
4	1028	1028	C-O-C
5	1000	1000	C-N
6	870	870	-C=C
7	700	700	Amines

Description. The vibrational mode showing the fingerprint of the bond at certain wavelength where the transmittance peaks appear.

The peak in 3500 cm^{-1} is the -OH stretching vibration associated to alcohols/phenols. The peak at 3400 cm^{-1} showed the N-H bond for the existing amide groups in the sample. In approximately 1400 cm^{-1}, the peak can be attributed to bending vibrations of -C-H and C=C, that infer the presence of aromatic compounds; the C=C shows the bond between the extract and the nanoparticles formed. Peak at a wavelength of 1028 cm^{-1} showed the stretching vibration of the ester groups. In peak at 1000 cm^{-1}, the bond fingerprint seen is of C-N. Peak at 870 cm^{-1} are attributed to the polyol group. Finally, peak at 700 cm^{-1} is the vibration of amine groups. As it is noticeable, the last one just exists in the extract samples, and the samples with the NPs, have a variation in the absorbance. Most of the species found correspond to terpenoids [9].

3.3. TEM AgNPs with avocado extract

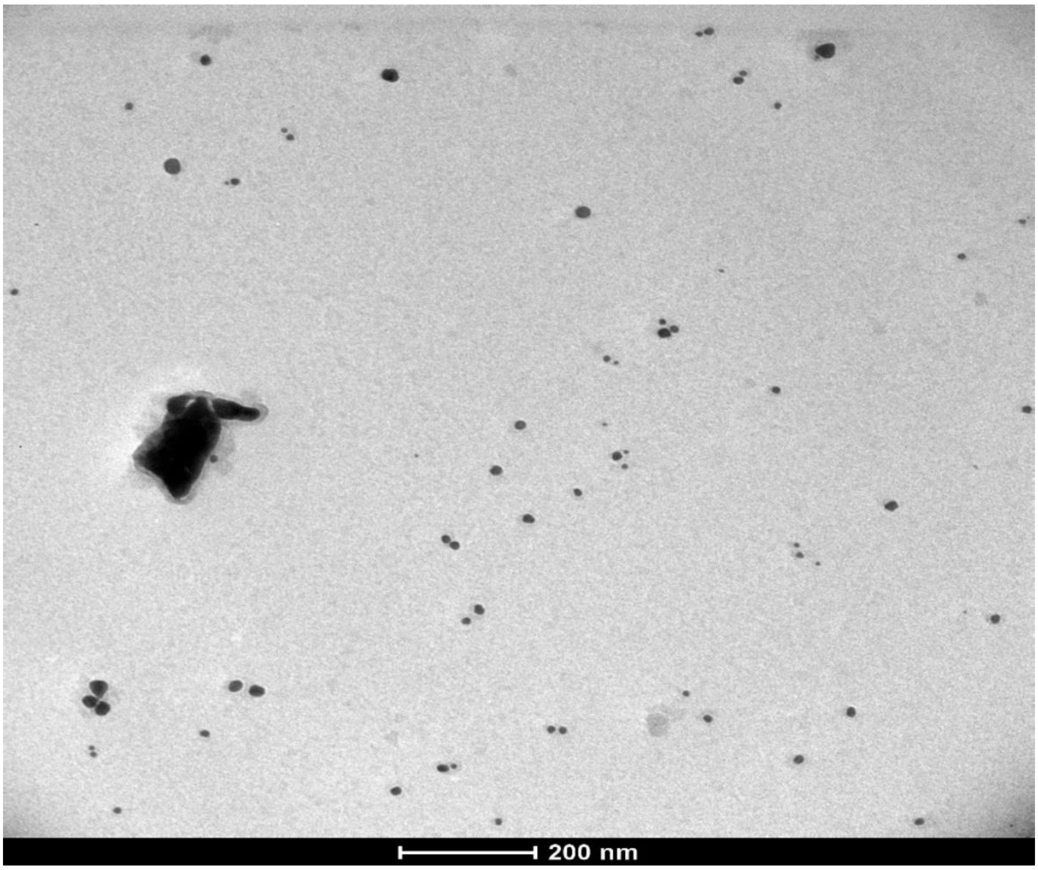

Figure 9: TEM characterization of avocado seeds extract obtained with water as solvent.

For having a proof of NPs formation, TEM imaging was developed. This type of characterization gives information of particle size, morphology of the nanoparticles and size distribution of them. The particles individually are measured in this characterization method, and it shows if there is agglomeration or if there is a good dispersion [35]. It is noted that the metallic nanoparticles have a coating around them, and as it can be seen, the particles shape is predominantly spherical. However, some agglomeration is seen, but in general it is loosely agglomerated. The meaning of an absorbance of 435 is seen in the TEM image, the nanoparticles have quasi-spherical shapes. This shape depends on the concentration of the extract used.

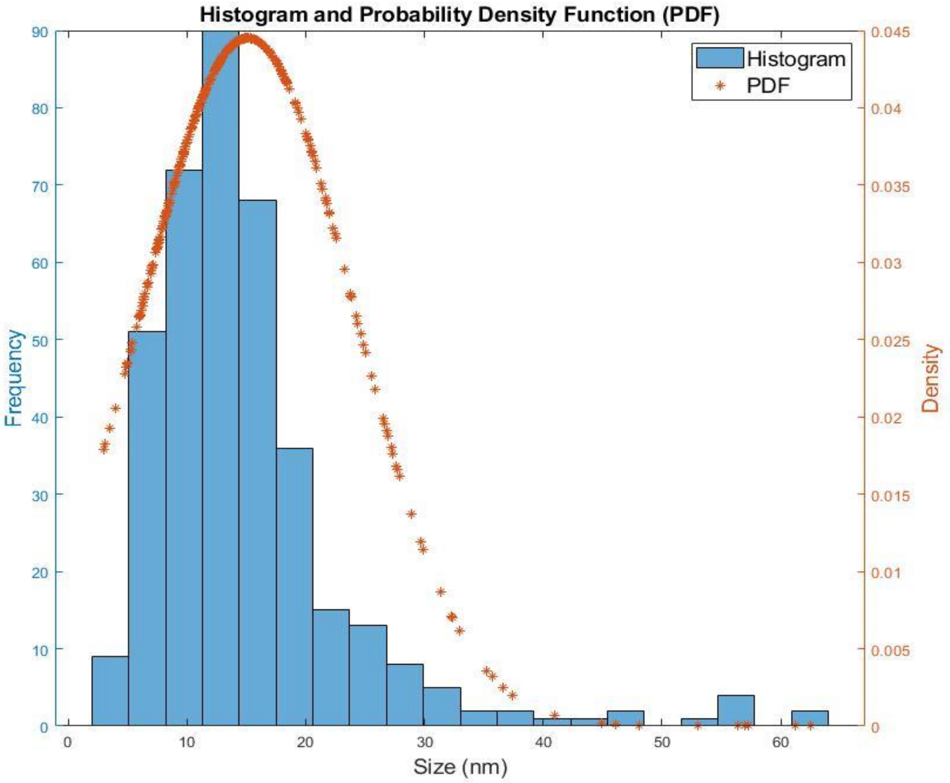

Figure 10: Number frequency histogram of the distribution of AgNPs in avocado seed extract with water as solvent.

The graphic (Fig 8) illustrates the frequency of occurrence vs. the size range. Histograms deal with quantitative data that is continuous, so the curve drawn through the histogram is the curve of the size-frequency of the particles. The TEM images indicate that is a Gaussian distribution with skewed to the right, which means the values are positive, and most of the particles have a size around 15 nm, with a standard deviation of 8,96% that indicate homogeneous AgNPs [36].

In the right side of the graph, there is density, it provides information of the area of each bin that represents the percentage of NPs in the bin. So, at a height of 0,045, times the width of each bin, around 3, the multiplication 0,045*3=0,135 so, 13,5% of all the NPs characterized, have a size of ≈ 15 nm.

Table 3: Average particle size TEM

EXTRACTS (WATER)	**AVOCADO SEED**	**CACAO POD HUSK**	**ORANGE PEEL (NADES)**

PARTICLE SIZE MEDIA	15,12	14,81	37,90
STANDARD DEVIATION	8,96	8,28	14,05

Description. This table shows the particle size and the standard deviation of each sample obtained from TEM characterization.

The TEM characterization selects the particles to be measured in accordance with the visual selection of the sample from the operator. The size of the AgNPs is good and in Figure 7, the distribution of the AgNPs showed that the avocado seed extract was correctly made, and the methods extracted the metabolites that covered the NPs to reduce agglomeration without the need of an external stabilizer.

Table 4: Average particle size from DLS

EXTRACTS (WATER)	**AVOCADO SEED**	**CACAO POD HUSK**	**ORANGE PEEL (NADES)**
PARTICLE SIZE MEDIA	74,5	61,7	3993,5
STANDARD DEVIATION	50,0	36,5	1482,6

Description. This table shows the particle size and the standard deviation of each sample obtained from Dynamic Light Scattering (DLS) characterization.

This type of characterization is the most used for characterizing nanoparticles. It measures the scattered laser light that passes through the colloids, to obtain their particle size and size distribution in an aqueous solution. DLS considers the total sample, including agglomerates and big particles. So, this characterization shows the total size of the agglomeration. The Orange Peel sample has a bigger media and standard deviation compared with the TEM obtained data, this results from the existence of agglomerated particles, therefore the DLS values show the total size of the agglomerated [37].

NADES Cacao

NADES Avocado

NADES Orange

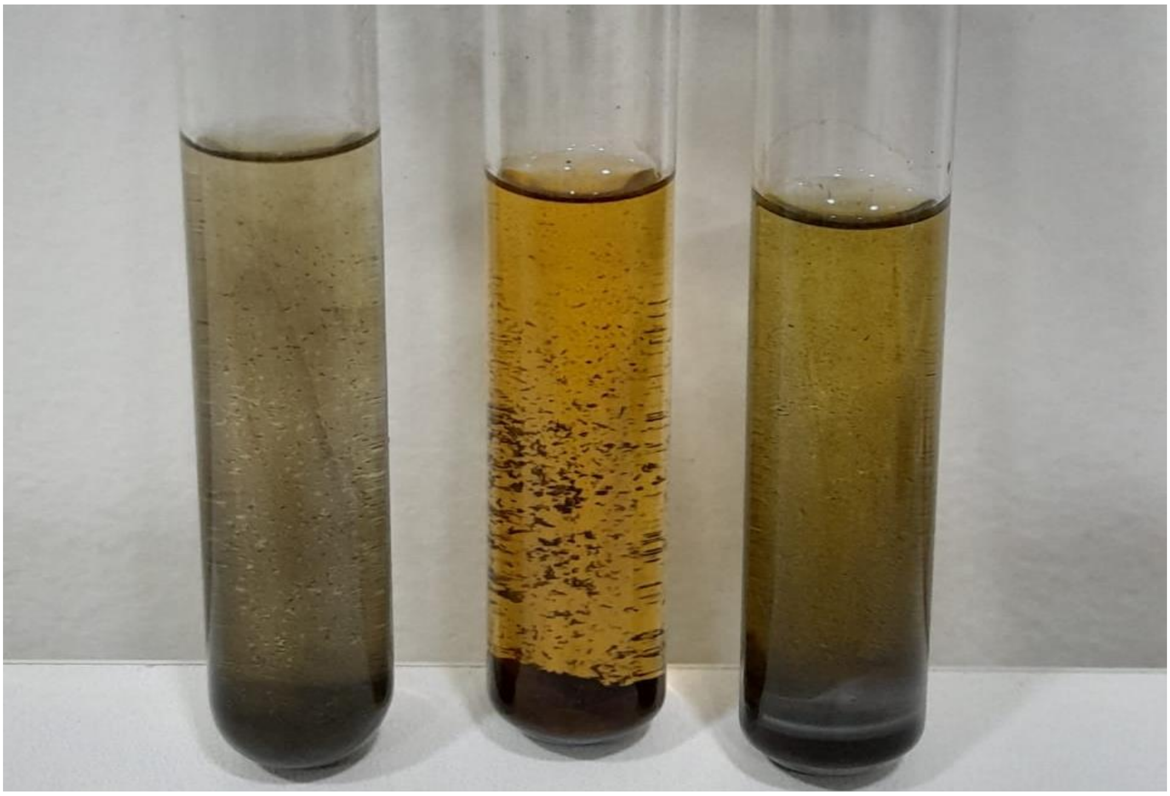

Figure 11: AgNPs obtained with extract of cacao, avocado and orange peels using NADES.

On the contrary to what was obtained using extract using water as solvent, agglomeration of AgNPs formed was observed when using NADES, as shown in Figure 9. That is why there are precipitates at the bottom of the three solutions. The agglomeration is due to attracting forces such as Van der Waals and due to the nature of the solvent itself. Also, less stability shows the tendency to agglomerate and form large particles, different situation showed with the other solvents, that due to the presence of polyphenols that form a negative ambient around the particles, so it creates a force that beat the Van der Waals forces, which prevents agglomeration of the particles [18].

The physical properties of NADES like solubilizing capacity, with a variation of water content in it can help to have higher solubility of plant secondary metabolites, but high concentrations of the extract usually lead to agglomeration and large NPs formation, this is due to the excess of reducing agents which causes a secondary reduction process that occurs on the surface of the NPs already formed [38]. Other property of this solvent is the polarity that can vary with the addition of water since it affects its solubilizing capacity. This could happen because of the rupture of hydrogen bonding between the glycerol and sodium acetate because the structure of the NADES changes with the addition of water to it [22]. Another possible reason of the formation of agglomerated and unstable AgNPs is the pH [34].

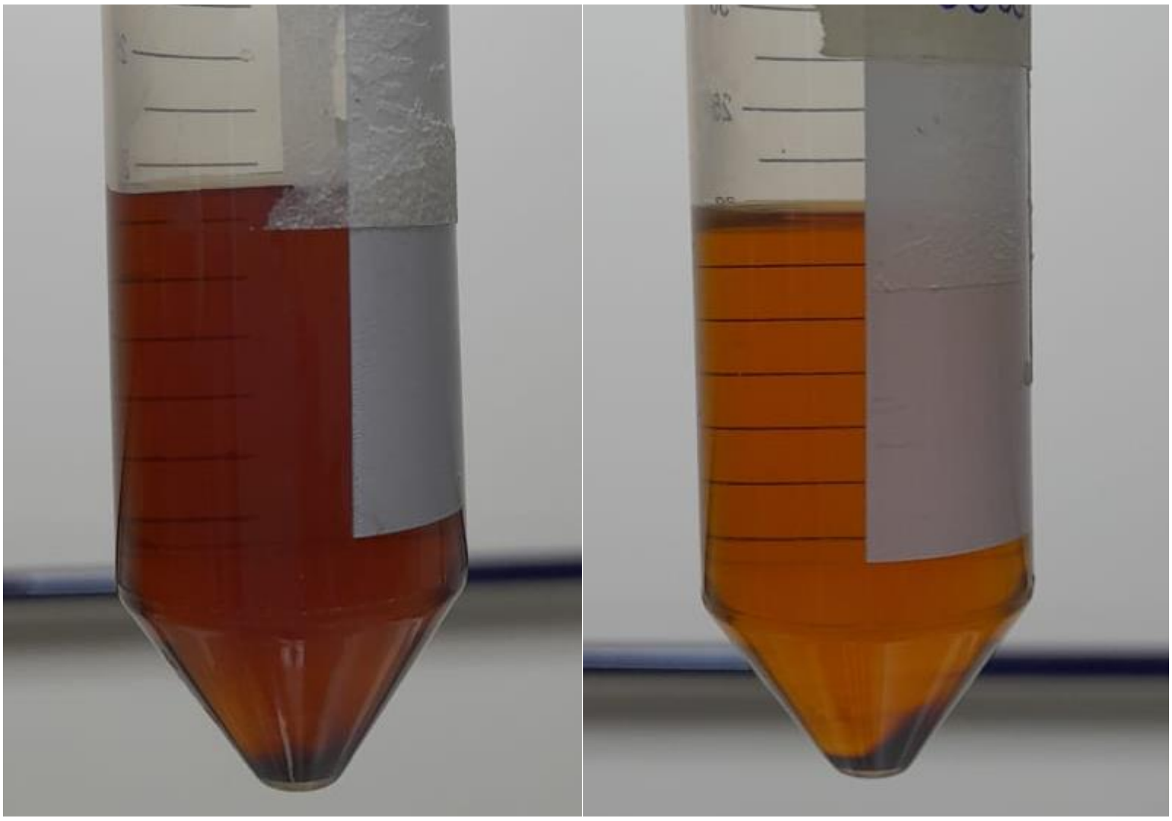

Figure 12: Centrifugation for the separation of NPs from solution.

Finally, for obtaining separated NPs form the solvent solution. AgNPs were centrifuged at 4000rpm three times for 1 hour each, washed with distilled water to redissolve the nanoparticles and to remove biomolecules not fixed to the AgNPs. As it is seen, the color of the mixture starts to pale when a precipitate starts to be visible; the precipitate are the nanoparticles separated from the solution. In future work, these particles will be studied for antimicrobial properties.

4. GENERAL DISCUSSION AND CONCLUSION

It was proven that the AgNPs can be synthesized with the use of green solvents and that maceration can extract the same plant metabolites just like the Soxhlet extraction does, but with a greener process due to shortage of drinking water in the world; except for the orange peel maceration with water because of the content of pectin in the albedo of the orange, which formed a viscous liquid with a texture like soaked oatmeal; so, in this case, the Soxhlet extraction was better.

The use of plant extracts reduced the limitations of the physicochemical synthesis of AgNPs due to the phytochemicals presented in these extracts, which are the responsible of the reduction of Ag ion and the production of the NPs. The reduction of Ag occurs in two steps, nucleation and then growth (process in which there is a nuclei formed over a crystal and where the growth of other crystal layer will happen [39]).

The UV-Vis gave information about the wavelength of the sample that provide the certainty of the formation of AgNPs, and the velocity of the synthesis with each plant extract used. The stability of the NPs is excellent when they reach the total

According to literature, the extract of orange peel should be the best capping and reducing agent for the synthesis of the AgNPs, but in this investigation that did not occur. The conclusions that can be made are from a failure in the extraction, could be light exposure of the lyophilized orange extract that could have affected the plant metabolites because of its photo sensibility, changing the properties of them and resulting in the no reducing and capping action for the synthesis of the AgNPs.

The FTIR characterization method showed the stability of the AgNPs with avocado seeds and cacao pod husks extracts obtained through Soxhlet extraction method, showing the presence of almost the same primary metabolites in each sample. Because of lack of time, the FTIR of the extracts obtained from NADES and maceration process could not be made. But according to literature, the NADES in fact, are better at extracting the plant primary metabolites than the Soxhlet extraction. Hence, it is expected to see peaks with a larger transmittance than the ones seen of the extracts in Figure 8 and probably more peaks in other wavelengths. Therefore, it is a cost effective and simple technique to determine the molecules that have reducing action [28].

One probable reason of the agglomeration of the AgNPs synthesized in the NADES is the ratio of the NADES dissolved, with the salt. It was experimented with a 2:1 and 3:1 ratio but in all the cases, agglomeration occurred. According to literature, using a 90% diluted NADES help the use of the extract because of the reduction of the viscosity, but also due to the concentration of the extract as mentioned before, in Figure 9. In this case, it was not performed that way because of the objective of comparing with the same parameters, in this case, knowing the concentration of the extract. Apparently, that should have not been the parameter used because of the differences in the physical and chemical properties of the solvents (water and NADES). Literature says NADES increases the solubility of primary metabolites that cannot be dissolved in water [22] so that creates a gap between the relation it was tried to be made.

The nanoparticle size media of DLS shown in table 3, and TEM, shown in table 2, are different, usually the size obtained from DLS is larger than TEM. This occurs due to the influence of Brownian motion, when large particles collide with thousands smaller ones that are at different velocities and directions making the large particles move randomly. So due to the image of the TEM the morphology of the particle is quasi-spherical and has a uniform distribution, that with a variation of the concentration of the extract, could start being spherical.

5. RECOMMENDATIONS

- Extracts with NADES, in fact, are capable of NPs formation, however more studied is needed for controlling the NP agglomeration during synthesis.
- A more complete study, principally using the FTIR technique, could give insights for a better control of NPs formation with NADES as solvents.
- A different approach for methodologies comparison parameters, according to the properties of each solvent used could give a better understanding of them.
- Varying the concentration of the extract and silver salt would be interesting for forming more homogeneous colloids.
- Making again the extraction of plant metabolites from orange peels and comparing the raw material with pectin and without it, to know it that was the cause of the non-formation of the AgNPs.

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7. ANNEXED 1: PREPARATION OF THE EXTRACTS AND AGNPS

7.1. Soxhlet extraction

1/4

Figure 13: Soxhlet extraction from avocado seed in water done in triplicate (Start of extraction).

2/4

Figure 14: Soxhlet extraction from avocado seed in water done in triplicate (Hour number 2 of extraction).

3/4

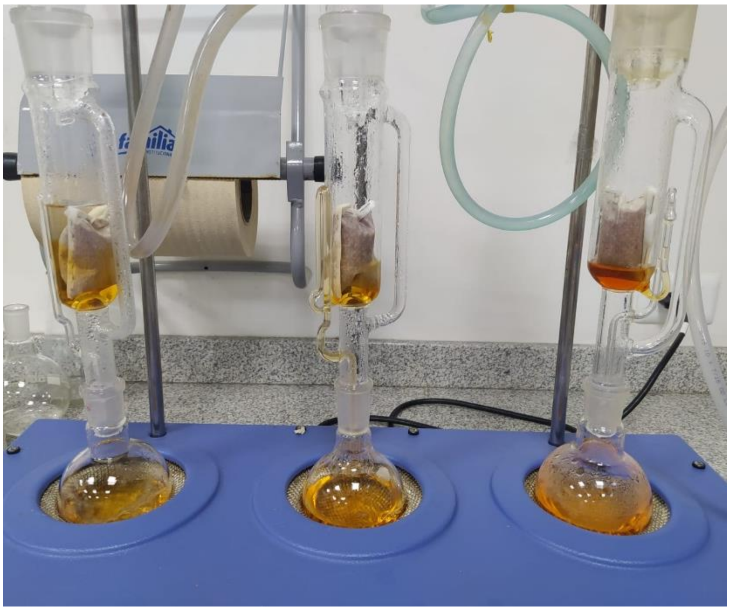

Figure 15: Soxhlet extraction from avocado seed in water done in triplicate (Hour number 4 of extraction).

4/4

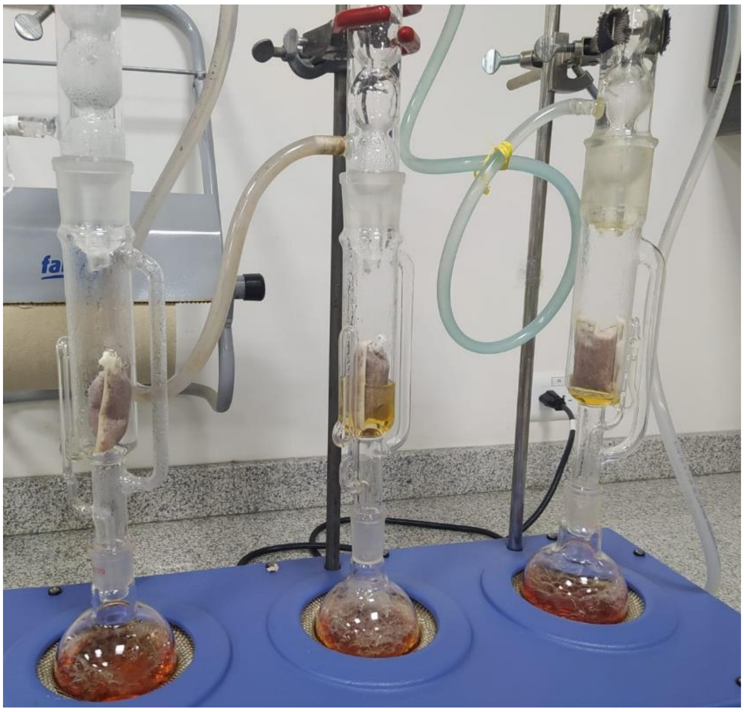

Figure 16: Soxhlet extraction from avocado seed in water done in triplicate (Hour number 5 of extraction).

7.2. Maceration extraction

Figure 17: Orange in aqueous maceration (filtration step).

Figure 18: Cacao pod husk in aqueous maceration.

7.3. AgNPs synthesis

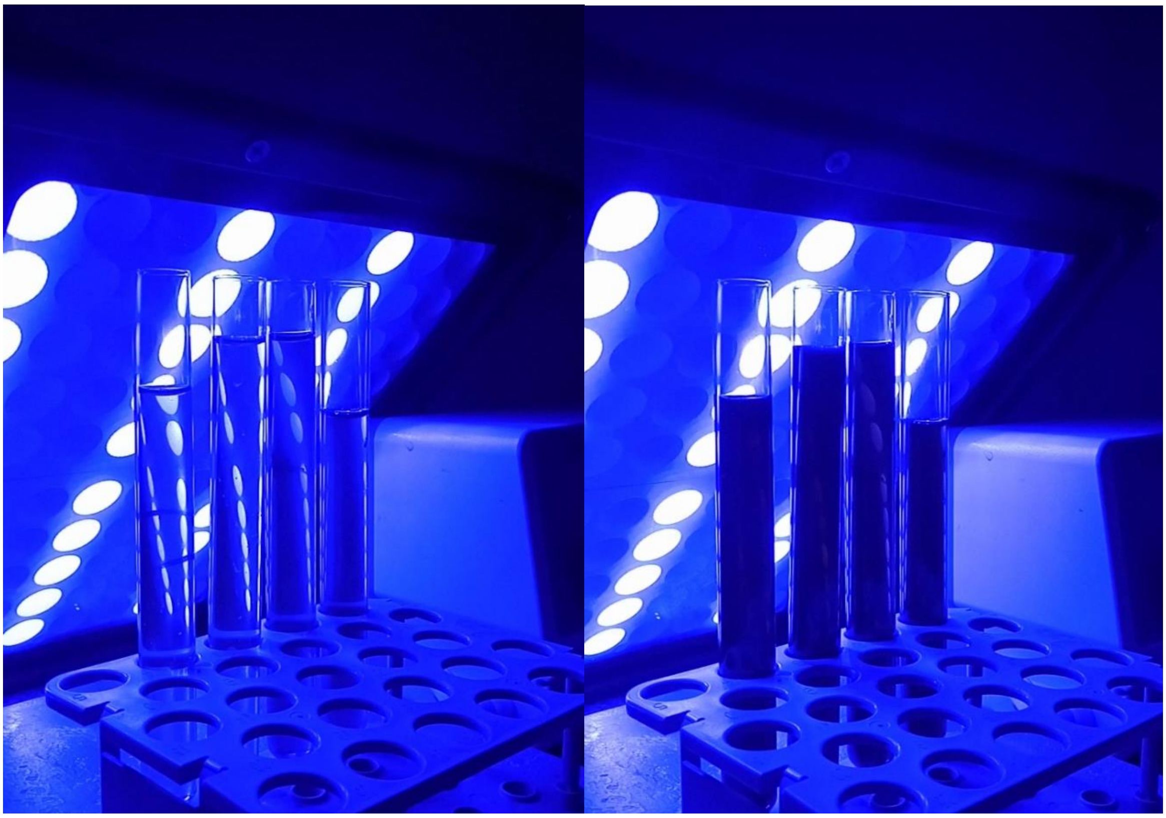

Figure 19: AgNPs synthesis with aqueous avocado seed extract assisted by blue LED light.