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Original research

The effect of PVA coated film containing silver nanoparticles synthesized from aqueous *Satureja rechingeri* extract on shelf life of rainbow trout (*oncorhynchus mykiss*) fillet

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A B S T R A C T

This study investigated the effect of rainbow trout fillet's packaging (*Oncorhynchus mykiss*) on polyvinyl alcohol films that activated by silver nanoparticles; even though, it synthesized from aqueous extraction (*Satureja rechingeri*) with ultrasound and photochemical methods, increased the microbial shelf life (Antimicrobial properties) and qualitative properties of 160 piece of salmon alongside 4 kinds of packaging in 14 days at two refrigerator temperatures (4 and 8°C). The main compounds in *Satureja rechingeri* extract were carvacrol (83.2%), paracimene (3.11%), thymol (2.13%) and γ-terpene (1.63%). The evaluation of UV- VIS and TEM showed that diameter of S-AgNP was lower than 55 nm. According to this issue, the microbial load decreased in the sample of silver's nano covers that had ultrasound method ($p > 0.05$) and samples of nanosilver coating that had photo-chemical method; however, it had reduced effect on spoilage, also this process measured till fourteenth days of investigation. The results of this study showed we could use nanosilver's packaging of *Satureja rechingeri* aqueous for preserving raw and perishable foods such as fish. It is used to increase the shelf life and decrease the amount of spoil.

Keywords: Green biosynthesis; *Satureja rechingeri*; Nanocomposite; Polyvinyl alcohol; Fish; Shelf life

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1. Introduction

Seafood was so perishable and had a short shelf life. Because of biological compounds, the fresh fish was too sensitive. Fish was more perishable than meat, also after death, Because of several biochemical reactions, the freshness had lost (like changes in protein and lipid content, formation of biogenic amines) and microbial spoilage, also it was the reason of losing the quality and nutritional value of fish. How to preserve fishes to prevent loss of quality and nutritional properties was very noticeable (Ramezani et al., 2015; Matak et al., 2015). The cause of fish muscle's spoilage was an alternation of biological reactions like enzymatic activities, lipid and protein oxidation and microorganism's metabolic activity. Safety and shelf life related to food's spoilage and pathogenic microorganisms or pathogens (Matak et al., 2015; Dehghani et al., 2018). The biodegradable packaging and coatings could improve the fresh and frozen productions with delay the microbial growth, reducing lipid's oxidation and moisture as well as the functions of them as a food additive and had contained antimicrobials and antioxidants (Matak et al., 2015; Dehghani et al., 2018). The usage of various additives in coatings had some prons like slower release of these compounds from films. This additive included bacteriocins, protein and chitosan which have different effects on lipid oxidation, proteolytic and microbial corruption (Dehghani et al., 2018). In recent years, we could see a lot of investigation about plant essence for protecting quality and increasing shelf life of fishes (Alizadeh Amiri et al., 2017). In recent years, plant extract was the cause of increasing consumer's demand for natural products were increased, as well as they use instead of chemical's additives in the food industry. Demands were grown by consuming kind of packaging food, food compounds with nanoemulsions and antibacterial properties which could help increase the shelf life of packaged food (Salvia-Trujillo et al., 2013). The direct use of plant extract in preserving was limited,

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because of intensity cost, odor and inactivation of active compounds. The adequate solution for decreasing the dose of consumable essential oils was kept the effect of them. Also, in addition, this issue had an effect on natural compounds with biodegradable films. Edible films were like the matrices of proteins, polysaccharides or lipids and it prepared for alternate the food's surface properties (Alizadeh Amiri et al, 2017). *Satureja rechingeri* (*Jamzed rechingeri*) was one of the Iranian endemic species. This plant had yellow flowers, dense white fluffy and cover of dotted glands in both rows of leaves (Hadian, 2008). This plant was full of phenolic compounds with antioxidant properties, such as carvacrol and rosmarinic acid (Jamzad, 2008). Now, the aerial organs of this plant were mixture of Khuzestan *Satureja rechingeri* which was collected from natural habitats and pharmaceutical industry. Thus, cultivation and domestication were considerable. The cause of alternation in medicinal plant's growth, amounts and quality were alkaloids, glycosides, steroids, essential oils (Omid baigi, 2005). We could use nanotechnology in several food science fields like food processing, packaging and enrichment (Huang & Zhoui, 2015). New functions of nanotechnology in food productions contained the growth and improvement of foods, nanoparticles and additives were used as bioactive systems and innovation for packaging (Singh et al., 2017). The experts used nanoparticles (1- 100 nm) to make other structures of nano. Also, nanoparticles were fundamental to nanotechnology. The usage of nanotechnology in food packaging was functional method to have relation with increasing shelf life of foods. The reason for this technology event was a high amount of surface-to-volume in nanoparticles and this ratio had direct relation with nanoparticle's radius. According to decreasing dimension of nanoparticles, not only the power of surface's activity increased but also, the reaction of material and environment increased (Chaudhry et al., 2008; Damm et al., 2006). Active packaging had more properties than inhibitory features and we could achieve them by add active compounds and components to packaging system. This kind of packaging, illustrated the reaction of packaging properties change against internal and external environment change as well as it was important to protect the freshness of foods (Suppakul et al., 2003). Metal nanoparticles are used in different fields of science and industry (Rai et al., 2008). Silver nanoparticles (AgNPs) were considerable because of good conductivity, Chemical stability, Catalytic, photonic and optoelectronic properties (Cho et al., 2005), we could use silver as a disinfectant; However, the bactericidal properties was low. Also, the usage and development of antibiotics and antibacterial chemicals were widely limited. The development of producing AgNPs and reusing silver was like bactericidal process (Jiang et al., 2004). There were several chemical methods for nanoparticle's synthesis such as microemulsion (McLeod et al., 2007), Chemical's reduction methods (Brust et al., 1994), Electrochemical reduction (Zhang et al., 2002), radiation reduction (Hornebecq *et al*., 2003), photocatalytic reduction (Shchukin *et al*., 2003) and ultrasonic wave reduction (Zhang et al., 2003). Polyvinyl alcohol (PVOH) was the biggest polar synthetic polymer that product in all around the world and the main property was biodegradability (Majdzadeh & Nazari, 2010). It was the most abundant of water-soluble polymer's synthesis. The suitable mechanical's features and high thermal stability of polymer are used widely in industrial's fields (Huang & Yang, 2010). In this study, we investigate the influence of polyvinyl alcohol coated film which contained silver nanoparticles synthesized from the aqueous extract (*Satureja rechingeri*) with ultrasound and photochemical methods also increased the microbial shelf life of salmon fillet (*Oncorhynchus mykiss*).

2. Material and Methods

2.1. Materials

Satureja rechingeri was purchased from Khorram of Lorestan. Nanosilver powder (purity of 99%) was obtained from Sigma-Aldrich company which was located in the USA and the polyvinyl alcohol's granules (Food Grade) were obtained from Bandar Imam Petrochemical's complex. We were bought rainbow trout (medium - Sized) on farm which was located in Tehran (Shahrivar).

2.2. Preparation of Satureja rechingeri extract

The aerial organ (i.e. without roots because tuber's root is too small for extraction) in the flowering stage (The highest amount of extraction is in this stage and amount is in leaves) was prepared and dried in shadow. Materials, species and identification of *Satureja rechingeri* confirmation prepared and investigated the compounds with GC- MS and the results were delivered to the National Center for Medicinal plants. After that, we could see ground and powder forms of it. Thus extraction performs according to Iranian National Standard No. 2836. Experts mixed 20 g of plants powder and 80 mL of distilled water and put them in the laboratory for 24 h for preparing aqueous extraction. After that, they put it in a water bath (80°C) for 45 min. Firstly, they filtered with Buchner funnel then with Whatman (No. 2) filter paper and stored it at 4°C.

2.2.1. Gas Chromatography- Mass spectrometry (GC, MS)

The compounds of *Satureja rechingeri* extract were analyzed and identified by gas chromatography-mass spectrometry (GC-MS). Then the extraction was injected into chromatography system and obtained the compound's mass spectra. In this study, the experts used perking- Elmer, which is a kind of chromatography system and clarus 500 with capillary column 30 m long inner diameter 0.25 mm and inner layer thickness 0.25 mm HP-5M with column temperature program initially at 60°C with a stop for 2 min in this temperature, then increased the temperature to 280°C at a rate of 3°C and increased the column's temperature to 300°C for 2 min. The injection chamber temperature was 230°C and helium gas was used as a gas carrier with a rate of mL/min. The mass spectrometer used Hewlett-Packard 5973-6890 with 70V ionization energy, El detector and ionization temperature (220°C) (Al saraf et al., 2020).

2.2.2. Determination of MIC and MBC of Satureja rechingeri extract

The experts used microdilution method for the minimum amount of MIC and MBC of *Satureja rechingeri* extract. They prepared fresh cultivate of two kinds of bacteria like *S. aureus* and *E. coli* in tryptose broth medium (TSB; Quelab, Montreal, Canada) turbidity of 0.5 McFarland and dilute 2-4 to give turbidity of 1×10^6. The absorbance value of the McFarland solution at 625 nm was 0.12.

The test sterilized extract was prepared by needle filter with 0.45 diameter and different dilutions (dilutions serial) in broth

medium, Then they pour in 96 plates of polystyrene with various dilution's extraction which contain 100 µl of bacterial suspension (Tsai et al., 2008) as well as they used wells with 200 µL broth medium negative control and wells with bacterial and culture medium to positive controls. Wells considers as a control turbidity control (100 µl of Broth and 100 µl of per dilution). For each bacteria repeat three times, then cover the plates and incubate in an incubator with an anaerobic jar at 5°C for 5 hours. After 24 hours, the turbidity (1 nm) was read by ELISA reader: (Atat fax 2100). MIC consider as the lowest amount concentration of material that was the cause of turbidity reduction (90%). To determine the MBC, 20 µl took from each well with a sampler and didn't culture separately on Muller-Hinton agar medium as well as the plates incubated for 24 hours. When they didn't see any bacteria in density, they had used MBC. The current tests results were confirmed by repeating 3 times for each sample.

2.3. Synthesis of silver nanoparticles (AgNPs Synthesis)

Silver nitrate (0.001 m) (Merck, Germany) combined with *S. Rechingeri's* extract of two below methods (Narchin et al., 2018). In the photochemical method, nitrate is combined with *S. Rechingeri* (in a ratio at 1:4), then put in direct sunlight for 5 min. Color alternation (yellow to dark brown) illustrated the performance of reaction and nano-particle synthesis. This process had pH = 7 and stayed at room temperature for 24 h. In synthesis of nanoparticles with the ultrasound method, the extraction of the photochemical method was obtained by homogenizer ultrasonic with irradiation and ultrasonic waves at 40 Hz, also stayed in dark for 30 min at 40°C and pH = 7. Color alternation (light yellow to brown) showed the synthesis of nanoparticles. For separation of nanoparticles from solutions, the optical and ultrasound of nano-extraction centrifuge separately. This process was performed three times at 45°C for 15 min. After each step, the (supernatant) was discarded and filled with double ionized water. In the micro centrifugation step, the solution obtained from the microcentrifuge was stirred and poured into the microtubule. Also, reperform the previous process. The experts shook the microcentrifuge that was on side of falcon tubes poured. After that, they put Eppendorf under the hood without shaking and the supernatant opened with a syringe till the time that dried under the hood. After 72 h, nanoparticles dried and shared in microtubes. Overall they sent them to the laboratory for analysis.

2.3.1. Transmission electron microscopy

The quality and size of the dispersion of S- AgNps were evaluated by Transient electron microscope (TEM) (CM 120 is the model of Philips and Netherlands) (Emamifar et al., 2010).

2.3.2. X-ray diffraction test

The crystal structure of the AgNps was analyzed by X-ray diffraction (XRD) (PANalytical, XPERT- PRO). XRD patterns were recorded at the speed of 40/min (Jaiswal et al., 2020).

2.3.3. Ultraviolet spectroscopy test

The reaction of AgNPs was determined by (UV) spectroscopy. Plant's extraction of $AgNo_3$ (0.001 m) was homogenized, the reduction of $AgNO_3$ to AgNps measured and the adsorption recorded in the range of 300 – 800 (Alaraidh et al., 2014).

2.4. Production of S-AgNP/PVA films

S-AgNPS/PVA films, 5% PVA powder (by weight) (Merk, Germany) distilled in distilled water at 80°C for 1 h (Devi & Umadevi, 2014). The final Solutions cooled at room temperature and the bubbles disappeared. S-AgNPs which were produced with two previous methods poured in a flask and were with 0.75% glycerol as a plasticizer. PVA solution at 65°C for 25 min mined slowly and appeared S-AgNPs/PVA's colloidal which was viscous. The final films were made by casting method on a Teflon plate (Nippon Fusso, Japan).

2.5. Cover of Fillet's samples

Live *O. mykiss* fish with an average weight of 43.20 ± 220 g bought from fish market, transported to laboratory (less than 1 h), washed with cold water and cut with sterile instruments. The experts divided the 160 pieces of *O. mykisses* fillet with an average weight of 50 ± 2.3g into 4 groups (nano extract of *Satureja rechingeri*, silver with photo-chemical method, nano- extract's attendance of *Satureja rechingeri*, silver with ultrasound, cover's attendance which contained *Satureja rechingeri* extract and usual cover with attendance as a control group. They were wrapped with film and labeled for two refrigeration temperatures of 4 and 8°C (Table 1). Then transferred to special refrigerators with a thermometer and investigated them on days of 1, 3, 7 and 14).

Table 1. Introduction of treatments used in this research.

Treatment	Description
G_1	Without *S.rechingeri* extract (control), 4°C
G_2	Without *S.rechingeri* extract (control), 8°C
G_3	Without coating with PVA film containing *S.rechingeri* extract, 4°C
G_4	Coated with PVA film containing *S.rechingeri* extract, 8°C
G_5	Coated with PVA film containing nano extract of *S.rechingeri* /silver by ultrasonic, 4°C
G_6	Coated with PVA film containing *S.rechingeri* extract/silver by ultrasound method, 8°C
G_7	Coated with PVA film containing *S.rechingeri* extract/silver by photo-chemical method, 4°C
G_8	Coated with PVA film containing *S.rechingeri* extract/ silver plant by photo-chemical method, 8°C

2.5.1. Microbial analysis

The microbial parameters investigated the total enumeration of microorganisms and bacteria like *E. coli*, *Staphylococcus aureus*, *Salmonella* and psychrophilic bacteria and qualities properties 4 and 8°C on days of 1, 3, 7 and 14. The samples of fillet (25 g) were weighted aseptically and homogenized with 225 ml of sterile peptone water (0.1 %) (Merck, Germany) in a stomacher (lab blender 400, Italy) for 30 s at 260 rpm and room temperature. Decimal serial dilution was prepared for each sample in 0.1% peptone solution and 1 ml or 0.1 mL of samples, in two replications, for the total bacterial enumeration test (according to national standard 5272). The count of psychrophilic bacteria was determined in plate count agar (PCA, Merck). The plates were

incubated at 7°C for 10 days to count psychrophilic bacteria. Enumeration of salmonella according to Iranian standard No. 1810, *S. aureus* with bird parker agar medium and Iranian standard NO. 1- 8606 as well as *E. coli* with MPN and Iranian standard NO. 2946. Enumeration of *Salmonella*, *S. aureus*, *coliforms*, *E. coli* mold and yeast performed a burger control's samples to determine the health status. Thus, the hamburger's microbial properties must accordance with Iranian National No, 2304.

2.5.2. Sensory analysis of samples

The experts used Quality Index Method (QIM) to evaluate the sensory properties. Thus, 7 trained evaluators rated the factors (according to the standard's table) such as skin appearance, color, texture and odor of fillet from 0 till 20. (Excellent quality from 0 to 1.5, good quality from 1.5 to 3, average quality from 3 to 5 and up to 5, it is unacceptable (Nawaz, 2019).

Table 2. The most compounds in *Satureja rechingeri* extract.

Compounds	(%)	(RI) Retention time
α-Togen	0.12	926
α-pinene	0.11	937
Camphene	0.05	950
β- pinene	0.05	977
Mercin	0.20	988
α- Flandren	0.15	1001
α- terpinene	0.41	1016
paracimene	3.11	1021
γ-terpene	1.63	1061
Thymol	2.13	1288
Carvacrol	83.2	1298
Eugenol	0.21	1354
Caryophyllene oxide	0.27	1574
Total	91.64	

3. Results and Discussion

3.1. The results of most compounds in Satureja rechingeri extract's evaluation

In this study, the experts identified the most compounds of *Satureja rechingeri*, for instance: Carvacrol (83.2%), paracimene (3.11%), thymol (2.13%), γ-terpene (1.63%), α- terpinene (0.41%), Caryophyllene oxide (0.27%), eugenol (0.21%), mercin (0.20%), α-Flandren (0.15%), α-Togen (0.12%), α-pinene (0.011%), Camphene (0.05%) and β- pinene (0.05%). Paracimene was one of the most important monoterpenes knew in herbal medicines. It had antioxidant, antimicrobial and anticancer properties as well as cytokine modulation (Marchese et al., 2017). Cell- membranes of both gram-negative and gram-positive bacteria had been disordered by γ- terpine, α- terpinen and engenol (Oyedemi et al., 2009). Carvacrol was the main compound of savary extraction (Table 2) and could act as a chelating agent as well as led to reducing the metal ions (karmous et al., 2019). Eventually, nano-particles were made by green biosynthesis. Thus, the nano-composite of *Satureja rechingeri* extract/silver nanoparticles performed as a secure antibacterial. The results of Izadi et al. (2020) showed the most important compounds of Summer *Satureja rechingeri* essential oil such as carvacrol (42.40%), thymol (19.74%) and para -cement (19.8%). This plant's essential oils of IC50 were 0.15 ± 10.63 µg/ml, also this plant had an antibacterial and antifungal effect is stronger than the antibacterial effect. The minimal bactericidal concentration and minimum inhibitory concentration of summery *Satureja rechingeri* essence oils for all microorganisms was 0.15- 16 µg/ml. In this study, the bacterial effect of summery *Satureja rechingeri* on yeast, fungi, gram-positive bacteria (*Staphylococcus aureus* and *S. epidermidis*) and gram-negative bacteria (*Shigella flexneri*, *Serratia marcescens and Klebsiella pneumonia*) is more than amphotericin B antibiotics, vancomycin and gentamicin antibiotics.

Table 3.The results of MIC and MBC of *Satureja rechingeri* extract against the *S. aureus* and *E.coli*.

Bacteria	MIC* (µg/mL)	MBC* (µg/mL)
S. aureus	25.00 ± 0.04^{a}	50.00 ± 0.05
E. coli	12.50 ± 0.03^{b}	-

Different letters indicate a significant difference in the column ($p \leq 0.05$).
*MIC: Minimum inhibitory concentration; MBC: Minimum lethal concentration.

3.2. The results of MIC and MBC of Satureja rechingeri extract against the S. aureus and E. coli

The comparison of *Satureja rechingeri between* MBC and MIC which were against the *S. aureus* and *E. coli* (Table 3) showed the minimum inhibitory concentration against the *S. aureus* was more than *E. coli* ($p \leq 0.05$) and it didn't show the minimum lethal concentration against *E. coli*. The minimum lethal concentration (MBC) was the lowest concentration of antimicrobial which ultimate microorganism's death, as well as the minimum inhibitory concentration (MIC), was the lowest concentration of antimicrobial agent which had an inhibitory effect on a special microorganism's growth (Eloff, 1998). Plant extracts are widely used in the food industry and had antimicrobial properties on a wide range of microorganisms (Burt, 2004). Overall, it was difficult to compare the reported results on the different extracts of antimicrobial properties. The results of this issue were the different method of investigating these properties, their sources, plant's culture situations, different microbial strains and also different concentrations of bacteria used as an inoculant. Shahnazi et al. (2007) identified 34 compounds of *Satureja rechingeri* essence oils in their study. The main compounds were thymol, paracimene, γ-terpene, carvacrol and myrcene. Antibacterial effects of this investigation's results illustrated the essential oils of this plant used as an inhibiting growth on tested bacteria. In this study, carvacrol had the highest value (83.2%). Ultee et al. (2000) showed the dissolution of carvacrol after a maximum exposure time of 40 min in a phospholipid bilayer of *S. aureus* cells and it was alignment between the fatty acid chains. All cells of each strain had already been damaged after treatment with carvacrol. La Storia et al. (2011) showed the reduction in cell size, length and diameter for all strains in response to carvacrol treatment. This could reasonably be attributed to leakage of cytosolic fluids outside the cells. Muridi Kia et al. (2016) investigated the antimicrobial effect of *Satureja rechingeri* and ZnO nanoparticle on *S. aureus*, *also it* showed the amount of *Satureja rechingeri* hydroalcoholic extract of MIC for *S. aureus* was 3000 µg/ml as well as MIC of ZnO nanoparticle for *S. aureus* was 40 µg/ml. Teymouri et al. (2016) showed the antimicrobial effect of *Satureja rechinger was the* lowest inhibitory

concentration in the formation of biofilm in some important human bacterial pathogens (12.5-50 ppm). The highest inhibitory of *S. aureus* and the lowest inhibitory concentration of *Satureja rechingeri* extract were 12, 5 ppm.

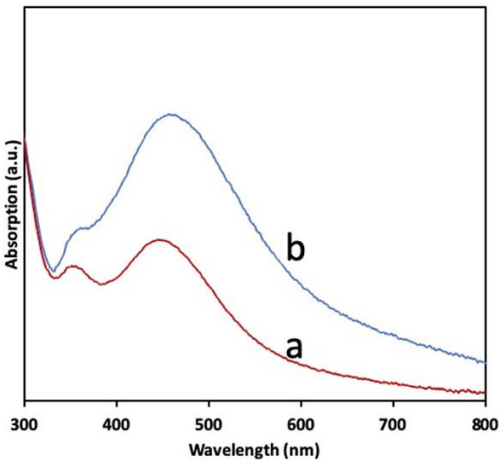

Fig. 1. Comparison of the absorption spectrum of *Satureja rechingeri*/silver nanoextract by (a) photo-chemical methods and (b) Ultrasound.

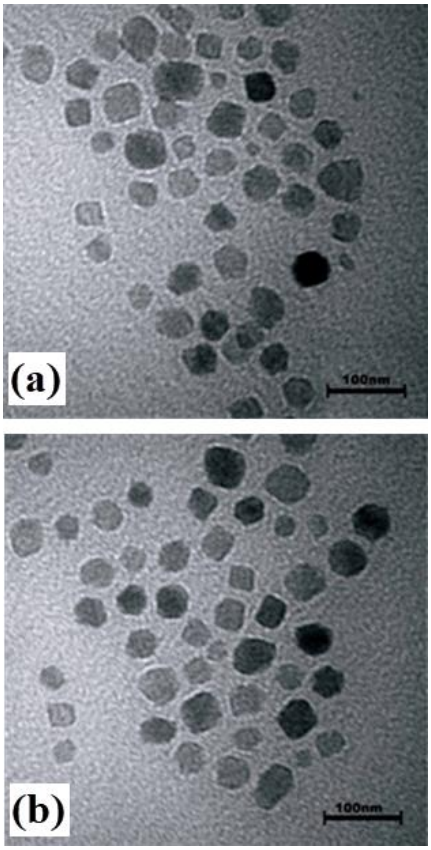

Fig. 2. Imaging obtained from a transmission electron microscope of *Satureja rechingeri*/silver Nano extract by (a) photo-chemical and (b) ultrasound.

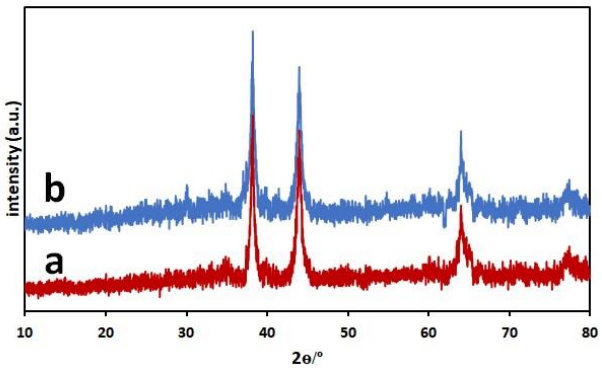

Fig. 3. X-ray diffraction diagram at 2θ angle by (a) photo-chemical and (b) ultrasonic.

3.3. *UV-Vis spectral evaluation*

The spectrophotometer was a kind of technique used to investigate the quantity of nano-particle. The peak's enhancement was an indicator of nano-particle formation in incubation period (Rajasekharreddy et al., 2010). Also, UV-Visible spectroscopy could use as a simple method for supervision of the nano-particles solution. The peak of UV- Vis AgNPs had influenced AgNP formation and it was in the range of 400- 500 nm (Goma, 2017). UV-visible spectrum prepared to prove the presence of silver nano-particles in samples. One of the most attractive metal nano-particles was optical properties that changed according to size and shape of nanoparticles in metal nanoparticles, plasmon resonance was responsible for optical properties which change with nanoparticles shape, size, distance from each other and refractive index. S. AgNP was produced by ultrasound and photochemical methods (Fig. 1). Also, it is recognized in the range of 430 and 475 nm. According to Ashraf et al. (2016) investigation, the range of AgNPs was 455 nm. The peaks demonstrated that AgNPs had polydispersity, comparison between both peak's wavelengths confirmed the TEM results.

3.4. *Evaluation of transient electron microscope (TEM)*

Microscopic techniques such as scanning electron microscopy and transmission electron microscopy were used for defining the size, shape and morphology of synthesized nano-particles material at the nanometer scale that are important because of unique chemical and physical properties and had different functions in various filled (Prasad et al., 2012; Jegadeeswaran et al., 2012). Nano-particles have unique properties depending on size and shape (Vanaja, 2011; Cai et al., 2013). TEM photos were prepared for comparing size, morphology and uniform distribution of the nanoparticles. TEM showed the size and dispersion of synthesized AgNPs. Also, these particles had different shapes like spherical and hexagonal (Fig. 2). Thus they spread irregularly. More than half of the synthesized S-AgNPs/pho had a diameter in the range of 35-55 nm but mostly the measurement of them was 75% which was lower than 30 nm according to a theory. The released energy of more than one wave as well as it showed the smaller nano-particles in an aqueous solution (Narchin et al., 2018).

Table 4. Changes in mesophilic bacteria population of trout samples during storage (Log CFU/g).

	Storage time (day)			
Treatment	1	3	7	14
G1	3.02 ± 0.2^{aA}	3.70 ± 0.4^{bB}	5.83 ± 0.5^{cB}	8.93 ± 0.2^{dB}
G2	3.16 ± 0.2^{aA}	4.52 ± 0.4^{aA}	6.41 ± 0.5^{aA}	10.93 ± 0.2^{dA}
G3	2.22 ± 0.2^{aB}	4.55 ± 0.4^{aA}	4.72 ± 0.5^{bC}	8.57 ± 0.2^{cB}
G4	2.22 ± 0.2^{aB}	4.51 ± 0.4^{aA}	5.52 ± 0.5^{cB}	10.82 ± 0.2^{dA}
G5	1.65 ± 0.2^{aC}	2.68 ± 0.4^{bC}	3.86 ± 0.5^{cD}	7.75 ± 0.2^{dC}
G6	1.65 ± 0.2^{aC}	3.52 ± 0.4^{bB}	4.63 ± 0.5^{cC}	8.19 ± 0.2^{dB}
G7	1.42 ± 0.2^{aC}	2.94 ± 0.4^{bC}	3.98 ± 0.5^{cD}	7.92 ± 0.2^{dC}
G8	1.42 ± 0.2^{aC}	3.72 ± 0.4^{bB}	4.80 ± 0.5^{cC}	8.65 ± 0.2^{dB}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

Table 5. Changes in psychrophilic bacteria population of trout samples during storage (Log CFU/g).

	Storage time (day)			
Treatment	1	3	7	14
G1	3.86 ± 0.2^{aA}	4.82 ± 0.4^{bA}	5.83 ± 0.5^{cB}	8.93 ± 0.2^{dB}
G2	-	-	-	-
G3	3.79 ± 0.2^{aA}	4.75 ± 0.4^{bA}	6.18 ± 0.5^{cA}	10.83 ± 0.2^{dA}
G4	-	-	-	-
G5	2.85 ± 0.2^{aB}	3.84 ± 0.4^{bB}	4.70 ± 0.5^{cC}	8.44 ± 0.2^{dB}
G6	-	-	-	-
G7	3.04 ± 0.2^{aB}	3.96 ± 0.4^{aB}	4.95 ± 0.5^{bC}	8.50 ± 0.2^{cB}
G8	-	-	-	-

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

Table 6. Changes in *Staphylococcus aureus* bacteria population of trout samples during storage (Log CFU/g).

	Storage time (day)			
Treatment	1	3	7	14
G1	0.077 ± 0.00^{aA}	0.77 ± 0.27^{aB}	1.76 ± 0.27^{bB}	3.80 ± 0.8^{cB}
G2	0.77 ± 0.00^{aA}	1.76 ± 0.00^{bA}	2.16 ± 0.27^{bA}	4.25 ± 0.2^{cA}
G3	0.77 ± 0.00^{aA}	0.77 ± 0.00^{aB}	1.76 ± 0.27^{aC}	3.00 ± 0.8^{bB}
G4	0.77 ± 0.00^{aA}	1.76 ± 0.00^{bA}	1.91 ± 0.27^{aC}	3.70 ± 0.8^{bB}
G5	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.27^{aC}	0.00 ± 0.8^{aC}
G6	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.27^{aC}	0.00 ± 0.8^{aC}
G7	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.27^{aC}	0.00 ± 0.8^{aC}
G8	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.27^{aC}	0.00 ± 0.8^{aC}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

Table 7. Changes in *E. coli* population of trout samples during storage (MPN: most probable number).

	Storage time (day)			
Treatment	1	3	7	14
G1	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	36 ± 0.03^{bA}	$<1100 \pm 0.03^{cA}$
G2	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	44 ± 0.03^{bA}	$<1100 \pm 0.03^{cA}$
G3	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	23.5 ± 0.03^{bA}	$<1100 \pm 0.03^{cA}$
G4	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	27.5 ± 0.03^{bA}	$<1100 \pm 0.03^{cA}$
G5	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	9 ± 0.03^{bB}	250 ± 0.03^{cB}
G6	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	12 ± 0.03^{bB}	460 ± 0.03^{cB}
G7	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	14 ± 0.03^{bB}	375 ± 0.03^{cB}
G8	$<3 \pm 0.03^{aA}$	$<3 \pm 0.03^{aA}$	19.5 ± 0.03^{bB}	1100 ± 0.03^{cB}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

3.5. Evaluation of X-ray diffraction results

X-ray or XRD is used as fuzzy analysis to investigate the size of particles and nano-particles. This issue was possible through the process and analysis of return X-ray. X-ray test used to recognize coating and crystal structure, also, identification of elements which used in the test sample was one of the most important functions of X-ray test (Fig. 3). Because of similar crystal structures, they had similar peaks as well as according to this issue, the X-ray pattern of AgNPs showed crystal arrangement. All of AgNp samples had been shown the peaks in 3182.38, 4975.44, 6119.64 and 5385.77 could relate with crystal structure of AgNP 111, 200. 200 and 311 peak densities of biosynthesized S- AgNPs illustrated the high amount of AgNP crystallization; even though, broad peaks showed low crystallization. In addition to the observed peaks, several non-routine peaks could show the existence of the phytochemical in solution (Firoozi *et al*., 2016). XRD pattern of S- AgNP peaks was similar to other expert's investigations (Gomaa, 2017) and showed the suitable green synthesis of silver nanoparticles. The use of ultrasound led to reducing the particles size of AgNPs; even though, this method led to stronger antimicrobial activities. Not only did nano-particles with the foregoing properties have a high contact surface and release a lot of Ag^{+}, but also, had more efficiency (Sotiriou & Pratsinis, 2010).

3.6. The results of rainbow trout's fillet's microbial tests during storage

3.6.1. Total count of living mesophilic bacteria

The results of the present study (Table 4) showed that control (without *Satureja rechingeri* extract and nanoparticles) and coated samples with alcohol polyvinyl film which contained an aqueous extract of *Satureja rechingeri* at both temperatures, had the highest mesophilic population and there wasn't statistically difference between the mentioned treatments ($p \leq 0.05$). The lowest mesophilic population was in the coated sample with polyvinyl alcohol film which contained *Satureja rechingeri*/silver nano-extract as well as it considered with ultrasound and phytochemical methods ($p \leq 0.05$). In all samples, the different temperatures didn't lead to different statistical. Over time (from day 1 till 14 days), the number of living mesophilic increased; However, the increase of mesophilic population was more pronounced from the third till the fourteenth day increased from the seventh to the fourteenth day.

3.6.2. Population of psychrophilic bacteria

The results of psychrophilic bacteria population (Table 5) showed that control and coated samples with polyvinyl film which contained aqueous *Satureja rechingeri* at 4°C had the highest population of psychrophilic bacteria. However, the coated with a film which contained aqueous sail flower's extract and stored at 8°C led to reducing the population of psychrophilic bacteria. Also, coated samples with polyvinyl film which *contained Satureja rechingeri*/silver nano-extract with ultrasound in both temperatures, had investigated. Eventually, the population of psychrophilic was lower than coated samples with polyvinyl which contained silver/ *Satureja rechingeri* nano- extract with photochemical methods. Overtime (from day 7 till day fourteenth), the population of living psychrophilic bacteria increased, ($p \leq 0.05$).

3.6.3. Population of Staphylococcus aureus

The results of the present study (Table 6) showed that *S. aureus was* only found in control and coated sample with polyvinyl Film which contained *Satureja rechingeri* of both temperature and trout fillet's coated with, silver/*Satureja rechingeri* nano- extract film (product with ultrasound and photo-chemical methods) led to protect the quality of samples till day 14. Overtime (third day till the fourteenth day) in sample G1 at 4°C, G2, G3 and G4 at 8°C. Also, the number of staphylococcus bacteria increased ($p \leq 0.05$) AgNPs antimicrobial activities on gram-negative bacteria were more than germ-positive. This issue was attributed to germ-negative bacteria that had a thick wall. Haji et al. (2016) showed that *S. aureus* was more sensitive (in chitosan nanocomposite/PVA) than *E. coli*. Monoterpenes are the main components of *Satureja rechingeri* (more than 90%) which inhibited the growth of *S. aureus* (Trom et al., 2005). Other researchers' investigations were the same as the present study which showed *S. aureus* was more sensitive than *E. coli* (Alboofetileh et al., 2014; Alizadeh, 2015; Paredes et al., 2014; Pirtarighat et al., 2019). The effect of the photo-chemical method and silver/*Satureja rechingeri* extract was more than the ultrasound method (same effect on both bacteria) (Narchin et al., 2018). The comparison which we assigned, depended on some factors for instance: Size of AgNP, sort of nanoparticles (Meral et al., 2019), bacterial resistance, growth stage, main extract compounds and test method (Alizadeh, 2015; Paredes et al., 2014). Salem et al. (2015) said that the trout fillet was placed in chitosan films which contained different concentrations of pomegranate peel extract; also, the amount of *S. aureus* on the sixth day at 4°C was 2 CFU log/ g. We could attribute the comparison of this study with the present study, as a kind of extract, because the volume of NP required inhibiting the growth of *S. aureus* which is affected with Ag and ZnO nanocomposites, was usually the same.

3.6.4. Population of E. coli

The results of the present study (Table 7) showed that in the first and third days of investigating, there wasn't statistically comparison in *E. coli* population and on other days, the highest number of the population belonged to control samples in both temperatures. There wasn't statistically comparison between the mention and coated sample in polyvinyl film which contained aqueous *Satureja rechingeri* ($p \leq 0.05$). The lowest number of *E. coli* was assigned in the coated sample that alongside polyvinyl film which contained silver/*Satureja rechingeri* nano- extract (Ultrasound method) at 4°C. There wasn't statistically comparison between the mention Sample and coated sample that alongside polyvinyl film which contained silver/*Satureja rechingeri* nano extract with photochemical method (in both temperatures) and coated sample with polyvinyl film which contained silver/*Satureja rechingeri* nano extract with ultrasound to 8°C. Overall, from the first till the fourteenth day, the population of *E. coli* increased ($p \leq 0.05$); however, this increase was more obvious on the third day till the fourteenth day.

3.6.5. The results of Salmonella search

The results showed that salmonella didn't see in samples.

Table 8. Changes in the appearance score of trout fillet specimens during the storage.

	Storage time (day)			
Treatment	1	3	7	14
G1	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	1.00 ± 0.03^{bA}	1.71 ± 0.03^{cA}
G2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	1.00 ± 0.03^{bA}	1.71 ± 0.03^{cA}
G3	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.85 ± 0.03^{bB}	1.00 ± 0.03^{cC}
G4	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.85 ± 0.03^{bB}	1.14 ± 0.03^{cB}
G5	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.71 ± 0.03^{bC}	0.85 ± 0.03^{cD}
G6	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.85 ± 0.03^{bB}	0.85 ± 0.03^{bD}
G7	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.85 ± 0.03^{bB}	1.00 ± 0.03^{cC}
G8	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.85 ± 0.03^{bB}	1.14 ± 0.03^{cB}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

Table 9. Changes in color score of trout fillet specimens during storage.

	Storage time (day)			
Treatment	1	3	7	14
G1	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	1.28 ± 0.03^{cA}	1.71 ± 0.03^{dA}
G2	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	1.28 ± 0.03^{cA}	1.71 ± 0.03^{dA}
G3	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	1.00 ± 0.03^{cB}	1.00 ± 0.03^{cD}
G4	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	1.00 ± 0.03^{cB}	1.57 ± 0.03^{dB}
G5	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	0.71 ± 0.03^{cD}	1.00 ± 0.03^{dD}
G6	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	0.85 ± 0.03^{cC}	1.28 ± 0.03^{dC}
G7	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	0.85 ± 0.03^{cC}	1.28 ± 0.03^{dC}
G8	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bA}	0.85 ± 0.03^{cC}	1.28 ± 0.03^{dC}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

Table 10. Changes in texture score of trout fillet specimens during storage.

	Storage time (day)			
Treatment	1	3	7	14
G1	0.00 ± 0.00^{aA}	1.00 ± 0.05^{bB}	1.85 ± 0.03^{cC}	2.42 ± 0.03^{dB}
G2	0.00 ± 0.00^{aA}	1.85 ± 0.05^{bA}	2.28 ± 0.03^{cA}	2.85 ± 0.03^{cA}
G3	0.00 ± 0.00^{aA}	0.85 ± 0.05^{bC}	2.00 ± 0.03^{cB}	2.42 ± 0.03^{dB}
G4	0.00 ± 0.00^{aA}	1.00 ± 0.05^{bB}	2.00 ± 0.03^{cB}	2.14 ± 0.03^{dC}
G5	0.00 ± 0.00^{aA}	0.14 ± 0.05^{bE}	1.57 ± 0.03^{cD}	2.00 ± 0.03^{dD}
G6	0.00 ± 0.00^{aA}	0.14 ± 0.05^{bE}	1.85 ± 0.03^{cC}	2.14 ± 0.03^{dC}
G7	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bD}	1.85 ± 0.03^{cC}	1.85 ± 0.03^{cE}
G8	0.00 ± 0.00^{aA}	0.28 ± 0.05^{bD}	1.85 ± 0.03^{cC}	2.14 ± 0.03^{dC}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

Table 11. Changes in Overall acceptability of trout fillet specimens during storage.

	Storage time (day)			
Treatment	1	3	7	14
G1	0.00 ± 0.00^{aA}	2.00 ± 0.05^{bB}	5.28 ± 0.03^{cC}	7.57 ± 0.03^{dB}
G2	0.00 ± 0.00^{aA}	2.28 ± 0.05^{bA}	6.28 ± 0.03^{cA}	8.43 ± 0.03^{dA}
G3	0.00 ± 0.00^{aA}	1.86 ± 0.05^{bC}	4.85 ± 0.03^{cD}	4.85 ± 0.03^{cE}
G4	0.00 ± 0.00^{aA}	1.86 ± 0.05^{bC}	5.43 ± 0.03^{cB}	6.30 ± 0.03^{dC}
G5	0.00 ± 0.00^{aA}	1.86 ± 0.05^{bC}	3.25 ± 0.03^{cE}	4.75 ± 0.03^{dE}
G6	0.00 ± 0.00^{aA}	1.86 ± 0.05^{bC}	3.31 ± 0.03^{cE}	5.00 ± 0.03^{dD}
G7	0.00 ± 0.00^{aA}	1.86 ± 0.05^{bC}	3.00 ± 0.03^{cF}	4.80 ± 0.03^{dE}
G8	0.00 ± 0.00^{aA}	1.86 ± 0.05^{bC}	3.33 ± 0.03^{cE}	5.00 ± 0.03^{dD}

Different uppercase letters indicate a significant difference in the column and different lowercase letters indicate a significant difference in the row ($p \leq 0.05$).

3.6.6. Evaluation of sensory test's results

3.6.6.1. Appearance score

The results of the present study (Table 8) illustrated that on the first and third days there wasn't statistically difference in samples appearance score; even though, on other days, control and coated samples with polyvinyl film which contained *Satureja rechingeri* (in both temperatures), had the highest appearance score ($p \leq 0.05$). Over time, from the first day till the fourteenth day, the appearance score decreased ($p \leq 0.05$); however, the appearance score increased on the third to the fourteenth day.

3.6.6.2. Color Score

The results of the present study (Table 9) showed that on the first and the third days, there wasn't statistically comparison in a color score of sample; although, control and coated samples alongside polyvinyl film which contained *Satureja rechingeri* in both temperatures, had the highest color score ($p \leq 0.05$). Over time, from the first to the fourteenth day, the color score decreased ($p \leq 0.05$). However, this score increased from the third till the fourteenth day.

3.6.6.3. Texture score

The results of the present study (Table 10) showed that on the first and third day, there wasn't statistically difference in tissue score of samples; Even though, on other days control sample and coated sample with polyvinyl film which contained *Satureja rechingeri* (in both temperature) had the highest tissue score ($p \leq 0.05$). Over time, from the first to the fourteenth day, the tissue score of samples decreased; however, the tissue score increased from the third till the fourteenth day.

3.6.6.4. Overall acceptability

The results of the present study (Table 11) showed that there wasn't statistically difference in total acceptance. A score of samples on first and third days and on other days Control samples and coated samples alongside polyvinyl which contained *Satureja rechingeri* (in both temperatures) had the highest appearance score ($p \leq 0.05$). Over time (from the first day till the fourteenth day) the total acceptance score decreased ($P \leq 0.05$); however, this score was more obvious on 3 till 14 days. In the present study, the score of appearance, odor, color, tissue and total acceptance of fish samples had accepted when the score was 4. The sensory score increased during 14 days of refrigeration ($p \leq 0.05$). In control samples and trout fish packed in film that contained *Satureja rechingeri*, whereas the low coated Performance, Fish spoilage and other high microbial activity during storage, weren't acceptable (without considering the temperature, from third days). Khodanazary et al. (2019) said that the shelf life of spotted fish fillets (*scomberomorus commerson*) coated with pomegranate peel extract with gelatin (G)-poly caprolactone was 6 days (khodnazri, 2019). Arfat et al. (2015) said that the shelf life of bacon fillets with zno/nano-particles of basil leaves essence in LDPE increased. Khoshbouy lahidjani et al. (2020) said that curcumin nanoemulsion knew as an antimicrobial compound and couldn't delay the salmon fillets' spoilage more than 5 days while it stored in the refrigerator.

4. Conclusion

To conclude, the present study seeks to address the green synthesis of AgNPs/*S. rechingeri*. The physical targeted criteria of AgNPs fabricated through ultrasound, and photochemical methods resulted in preferred properties, features, and desired nano-scale. The importance of these techniques was related to their antimicrobial efficiency and the environmentally friendly AgNPs-*S. rechingeri*/PVA synthesis. AgNP/Ult.PVA and S-AgNP/Pho.PVA films with size mostly smaller than 35-50 nm diameter were effectively postponed the mesophilic and psychrophilic bacteria growth until the 7th day of the cold storage period, whereas fish samples enclosed in *S. rechingeri*/PVA and control films could be edible up to 3rd day of cold storage due to their counts exceeded 6 log CFU/g. The bacteriological analyses were confirmed with the sensory evaluation so that *O. mykiss* samples enfolded with AgNP/Ult.PVA and S-AgNP/Pho. PVA films had desirable sensory criteria which made them be edible up to the 7th day even more. These two types of films were also capable of completely eliminating *S. aureus* from *O. mykiss* samples up to two weeks. The AgNP/Ult of *E. coli* was less sensitive to AgNP/Ult, PVA and S-AgNP/Pho.PVA films compared to the other afore-mentioned bacteria through the cold storage. It suggested that AgNP/Ult.PVA and S-AgNP/Pho.PVA films could be used as coating films for *O. mykiss* at cold preservation to prolong the shelf life up to the 7th day in fish trails.

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Not applicable.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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