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Synthesis of Silver Nanoparticle and Formulation of Nanogel Using Pomegranate Peels Extract

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Abstract: *Nanoparticles are the ultrafine particles that has a size range of 1-100nm. These particles are widely used since the rapid growth of Nanotechnology has taken over the formulation aspects. A combination of a nanotechnology with the herbal formulations has been always shown greater therapeutic effects and minimal side effects. Silver nanoparticles were used formulation. The phytoconstituent namely anthocyanins found in pomegranate peels has shown a good antioxidant effect. The use of peels instead of the whole fruit made the formulation cost effective and a productive formulation out of the biowaste. The anthocyanins were standardized using various standardization techniques like TLC, UV, HPLC. The Green synthesis method was used to synthesize the silver nanoparticles which was an environment friendly method which required minimal chemicals The synthesized silver nanoparticles were evaluated by the analysis techniques like SEM and TEM. The nanogel is a semisolid dosage form that, due it its smaller particle size and increased surface area on application has a greater advantage which provides great penetration through the skin. The formulated nanogels were evaluated as per their evaluation parameters. The formulation was tested for the DPPH radical scavenging activity to check the antioxidant potential of the formulation. The formulation was then tested for the drug release by using the Franz Diffusion apparatus. The nanogel was compared to the standard parameter and was found out to be comparable to the standards.*

Keywords: Nanoparticles (NPs) ,Silver Nanoparticles (AgNPs), Green Synthesis, Pomegranate Anthocyanins, Pelargonidin, Topical Delivery, Transdermal, Drug Deliver, Nanoparticles, UV Spectroscopy, Nanogel

I. INTRODUCTION

According to the definition from NNI (National Nanotechnology Initiative), nanoparticles are structures of sizes ranging from 1 to 100 nm in at least one dimension. However, the prefix “nano” is commonly used for particles that are up to several hundred nanometers in size [1]. NPs have the potential to improve the stability and solubility of encapsulated cargos, promote transport across membranes and prolong circulation times to increase safety and efficacy [2][3].

For these reasons, NP research has been widespread, generating promising results in vitro and in small animal models. These NPs can therefore be utilized as more complex systems including in nanocarrier-mediated combination therapies to alter multiple pathways, maximize the therapeutic efficacy against specific macromolecules, target particular phases of the cell cycle or overcome mechanisms of drug resistance. However, despite this extensive research motivated by the NNI, the number of nanomedicines available to patients is drastically below projections for the field, partially because of a translational gap between animal and human studies [4][5].

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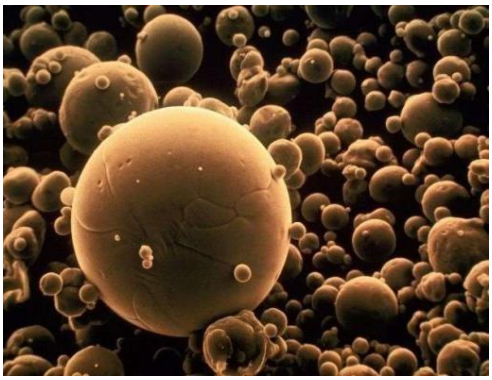

Fig. 1 Nanoparticles

TYPES OF NANOPARTICLES:

1. Inorganic Nanoparticles:

Inorganic materials such as gold, iron and silica have been used to synthesize nanostructured materials for various drug delivery and imag ing applications [6].

2. Polymeric Nanoparticles:

Polymeric NPs can be synthesized from natural or synthetic materials, as well as monomers or preformed polymers allowing for a wide variety of possible structures and characteristics. The most common forms of polymeric NPs are nanocapsules (cavities surrounded by a polymeric membrane or shell) and nanospheres (solid matrix systems) [7].

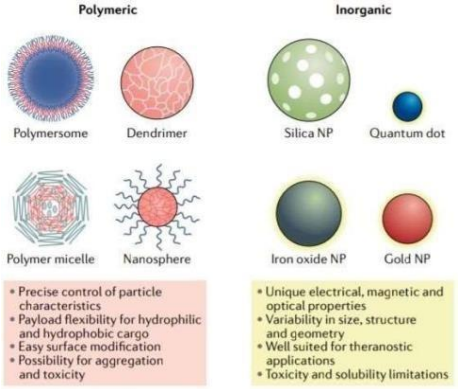

Fig. 2 Types of nanoparticles

SILVER NANOPARTICLES:

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. .Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well- defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs shows much promise[8]

SYNTHESIS OF SILVER NANOPARTICLES:

Generally, the synthesis of nanoparticles has been carried out using three different approaches, including physical synthesis, chemical synthesis, and biological methods (Green Synthesis).

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Physical Synthesis: In physical methods, nanoparticles are prepared by evaporation- condensation using a tube furnace at atmospheric pressure. Conventional physical methods including spark discharging and pyrolysis were used for the synthesis of AgNPs. The advantages of physical methods are speed, radiation used as reducing agents, and no hazardous chemicals involved, but the downsides are low yield and high energy consumption, solvent contamination, and lack of uniform distribution.

Chemical Synthesis: Chemical methods use water or organic solvents to prepare the silver nanoparticles. The above-mentioned methods are extremely expensive. Additionally, the materials used for AgNPs synthesis, such as citrate, borohydride, thio-glycerol, and 2- mercaptoethanol are toxic and hazardous. Apart from these disadvantages, the manufactured particles are not of expected purity, as their surfaces were found to be sedimented with chemicals.[9]

TOPICAL DELIVERY OF NANOPARTICLES:

Nanoparticles as drug carriers can potentially enhance drug's specificity, bioavailability, and therapeutic efficacy while improving patient compliance during therapy. Skin entry of nanoparticles through the trans-appendageal route, which includes the hair follicles, sweat glands, the sebaceous and the pilosebaceous glands has been reported. This enables nanoparticles to penetrate the superficial layers of the stratum corneum i.e. the outermost protective layer of the skin. Percutaneous penetration studies determine how much of a chemical penetrates the skin, and thereby absorbed into the systemic circulation. Dermal penetration is considered to occur by passive diffusion; however, biotransformation of the test substance within the viable regions of skin (metabolism) prior to systemic absorption can occur [10].

ANTHOCYANINS:

Anthocyanins are the key colour molecules of pomegranate present in various parts of the pomegranate trees, including leaves, flowers, and fruits.. It seems, however, that the white flowers and anthocyanin- less fruits are more susceptible to browning and radiation damages (personal communication). The accumulation of anthocyanin in young pomegranate leaves also suggests that it acts to protect the tissues from abiotic and biotic stresses during leaf development [11]

II. MATERIALS AND METHODS

PLANT PROFILE: Pomegranate Fruit

Common Name: Pomegranate, Delima.

Botanical Name: *Punica granatum L.*

Family: Punicaceae [12].

Description: Punica granatum, commonly known as the pomegranate, is a deciduous fruit- bearing shrub or small tree known for its distinctive, leathery-skinned fruit. Pomegranates have been cultivated for their fruit, juice, and medicinal properties for thousands of years. Punica granatum (Pomegranate) is a small tree which measures between 5-8 meters tall. It has ability to adapt adverse ecological conditions. The Pomegranate can be also divided into several anatomical compartments including seed, juice, peel, leaf, flower, bark, and root with each possessing
interesting pharmacological and toxicological activities. The edible fruit is a berry which is about 5-12 cm in diameter with a rounded hexagonal shape, thick reddish skin and around 600 seeds, each surrounded by a water-laden pulp (aril) ranging in colour from white to deep red or purple, the aril is the edible part of the fruit. The seeds are embedded in a white, spongy, astringent pulp.

Chemical Constituents: Gallic acid, Ellagic acid, Punicalin, Punicalagin, Caffeic acid, Ellagitannins, Pelletierine alkaloids, Luteolin, kaempferol, Quercetin.

Uses:

Reduces hyperpigmentation without any side effect

Packed with antioxidants this extract combats acne breakouts and makes the skin blemish free and radiant

Also shields the skin against ultraviolet damage and slow down signs of aging

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Pomegranate peel extract when combines with seed oil enhances procollagen synthesis, which combats the enzymes that break down collagen and promote skill cell growth [13].

Fig.3 Pomegranate fruit

CHEMICAL PROFILE:

Pelargonidin

Synonym: 2-(4-hydroxyphenyl) chromenylium-3,5,7-triol.

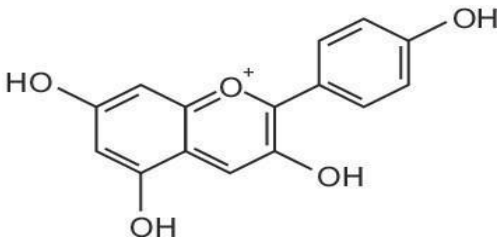
Molecular Formula: $C_{15}H_{11}O_5$.

Molecular Weight: 271.24 gm/mol

Melting Point: >349.85 ^{0}C

Description: Pelargonidin is an anthocyanidin cation that is flavylium substituted by a hydroxy group at positions 3, 5, 7 and 4'. It has a role as a plant metabolite. It is a conjugate acid of a pelargonidin [14]

Structure:



Pelargonidin

III. METHODOLOGY

A. EXTRACTION OF ANTHOCYANINS:

Before extraction, pomegranate peel powder was prepared by purchasing fresh pomegranate fruit which were peeled to obtain fresh peels.

These peels were washed thoroughly with water and were shade dried to retain the phytoconstituents.

The shade dried peels were grinded in a mixer grinder to obtain coarse powder.

This dried powder was extracted by maceration using solution of methanol and concentrated HCl for 24 hours.

After 24 hours above solution was filtered to get alcoholic extract of anthocyanins.

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Fig.4Dried powder of pomegranate peels

Fig.5 Pomegranate peels extract

B. FORMULATION:

Synthesis of Silver Nanoparticles:

Preparation of 1 mM Silver Nitrate Solution ($AgNO_3$):

1 mM of Silver Nitrate solution was prepared by adding 0.1699 g of silver nitrate to 1 Litre distilled water [16].

Green Synthesis of Silver Nanoparticles (AgNPs):

1 mM of silver nitrate ($AgNO_3$) solution was added drop-wise to 50 ml of the extract solution and the reaction mixture was stirred at 200 rpm at room temperature on a magnetic stirrer.

The dark brown coloured resulting reaction mixture indicated the formation of AgNPs.

Finally, the coloured mixture centrifuged at 1000 rpm for 20 minutes.

Synthesized PG-AgNPs are stored until further studies [17].

Fig.6 Magnetic Stirrer

Fig.7 Centrifuge Machine

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C. Formulation Of Nanogel:

Nanogel is prepared by following steps:

- Accurately weighed quantity of synthesized silver nanoparticles obtained by green synthesis (drug), and Tween 40 as stabilizer are dissolved in glycerol while stirring.
- The aqueous phase is prepared by soaking Carbopol- 934 in distilled water for 24 hours.
- The drug containing phase is then agitated and added drop by drop into the aqueous phase during homogenization to form emulsion.
- The emulsion converted into nanodroplets by homogenizer which formed O/W emulsion.
- Homogenization was continued for one hour. Triethanolamine added to form the gel with continuous stirring to nanogel [18].

Formula table:

Sr. No.	Ingredients	Quantity	Purpose
1	Drug Extract	20gm	Active Ingredient
2	Carbopol-934	5gm	Gelling Agent
3	Tween-40	0.005ml	Stabilizer
4	Glycerol	0.25ml	Hydrating Agent
5	Triethanolamine	0.5ml	Surfactant
6	Methyl Paraben	0.125gm	Preservative
7	Propyl Paraben	0.125gm	Preservative
8	Distilled Water	Qs	Vehicle/ Diluent

Table 1 Formulation Table [18].

D. EVALUATION FOR NANOGELS:

Following methods are used for evaluating nanogels;

Organoleptic Evaluation:

- Appearance: It is done by visual examination.
- Colour: The colour of the nanogels was determined by the visual inspection.
- Odour: Odour of the nanogels was determined by taking the small amount of nanogel in a petri plate and slightly sniffing above it.
- Texture: The texture was determined by rubbing it in between index finger and thumb.

Viscosity:

The viscosity of the nanogel was determined by using Brookfield viscometer.

Steps:

- Fit the spindle number 64 to the motor of Brookfield viscometer.
- Adjust the height of viscometer in such a way that the spindle is dipped in the sample up till the marking on the spindle.
- Turn on the viscometer and set the required rpm and turn on the motor.
- Note down the reading when the values on viscometer screen becomes constant or stable

Spreadability:

It is used to determine the spreading ability of the topical formulation.

Steps:

- Mount a slide on a solid surface.
- Place a small amount of formulation on this slide.

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- Take another slide and tie a weight of 20gm to it.
- Place this slide on the formulation and allow the weight to freely suspend over the edge.
- Note down the time in seconds required for the slide to move along the sample or the formulation.
- Note down the readings in the set of 3 and calculate mean [19]. Formula used:

pH Measurement:

It is measured by using Digital pH Meter.

Steps:

- Take 100ml distilled water in a beaker and dip the electrode in it.
- Turn on the pH meter and check if the pH of the distilled water is 7 or not.
- If it is not 7 then make it 7 by using acid or base.
- Once the pH of distilled water is 7 turn off the pH meter.
- Now, dissolved 1gm of sample or formulation into the distilled water.
- Dip the electrode in the beaker and note down the reading [20].

Antioxidant Activity:

The free radical scavenging activity of given test compounds were determined by DPPH scavenging method.

Procedure:

- In this method, 0.1mM DPPH solution was prepared in methanol by adding
- 39.4 mg of DPPH in 1000 ml of methanol, and to 0.5 mL of this solution,
- 1.5 mL of test compounds given test compounds dissolved in DMSO were
- added at concentration (1000 µg/mL).
- The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes.
- Then the absorbance was measured at 517 nm using non-coated 96 well plate on microplate reader.
- Vitamin C was used as standard compound. Reduction in absorbance by test compounds indicates radical scavenging activity.
- The scavenging activity by the DPPH radical was determined by; DPPH scavenging effect (% inhibition) = {(A0 -A1)/A0) ×100}
- Where, A0 is the absorbance of the control reaction, and A1 is the absorbance test compound or vitamin C [21]

IV. RESULTS AND DISCUSSION

PRELIMINARY TESTS:

Preliminary chemical tests were performed where anthocyanins were detected and presence of Pelargonidin was observed [24].

Test for Anthocyanins and Polyphenols:

Test	Observation	Inference
Extract+ Heat with 2M HCl for 5 min at 100^0C.	Colour stable	Anthocyanins are present.
Extract + Acetic acid	Red colour	Polyphenols are present.

Table 2 Test for anthocyanins and polyphenols

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Test for Flavonoids and Tannins:

Test	Observation	Inference
i. Shinoda test Extract + Mg wires + HCl + 95% ethanol (5ml)	Pink colour observed	Flavonoids are present.
ii. Extract + 5% $FeCl_3$ solution	Deep blue-black colour observed	Tannins are present.

Table 3 Test for flavonoids and tannins.

SCANNING ELECTRON MICROSCOPY:

The presence and the morphology of the silver nanoparticles was observed in the SEM analysis by changing the magnification power viz. 1.00 KX, 5.00 KX, 10.00 KX, 25.00 KX.

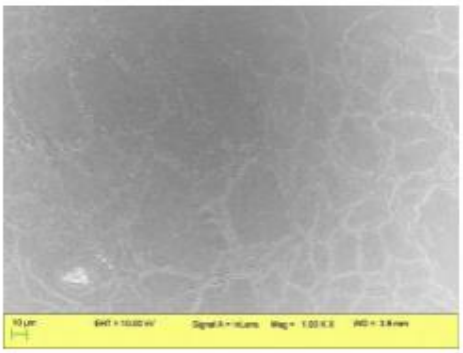

Fig.8 SEM Images AgNPs at 1.00 KX

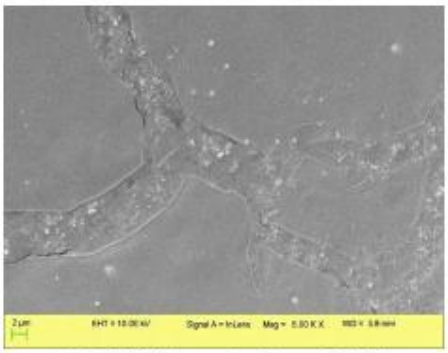

Fig.9 SEM Images AgNPs at 5.00 KX

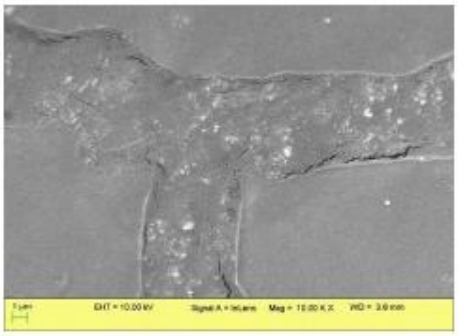

Fig.10 SEM Images AgNPs at 10.00 KX

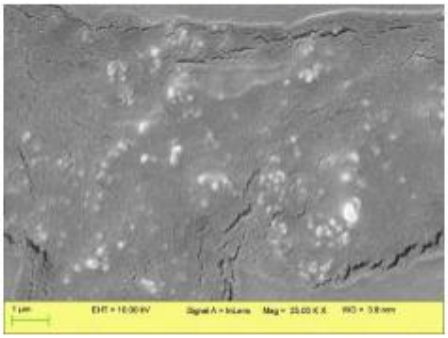

Fig. 11 SEM Images AgNPs at 25.00 KX

TRANSMISSION ELECTRON MICROSCOPY:

The size, shape, and crystallinity of the AgNPs were observed in the TEM analysis. The size of the nanoparticles was found to be ranging from 1-100 nm

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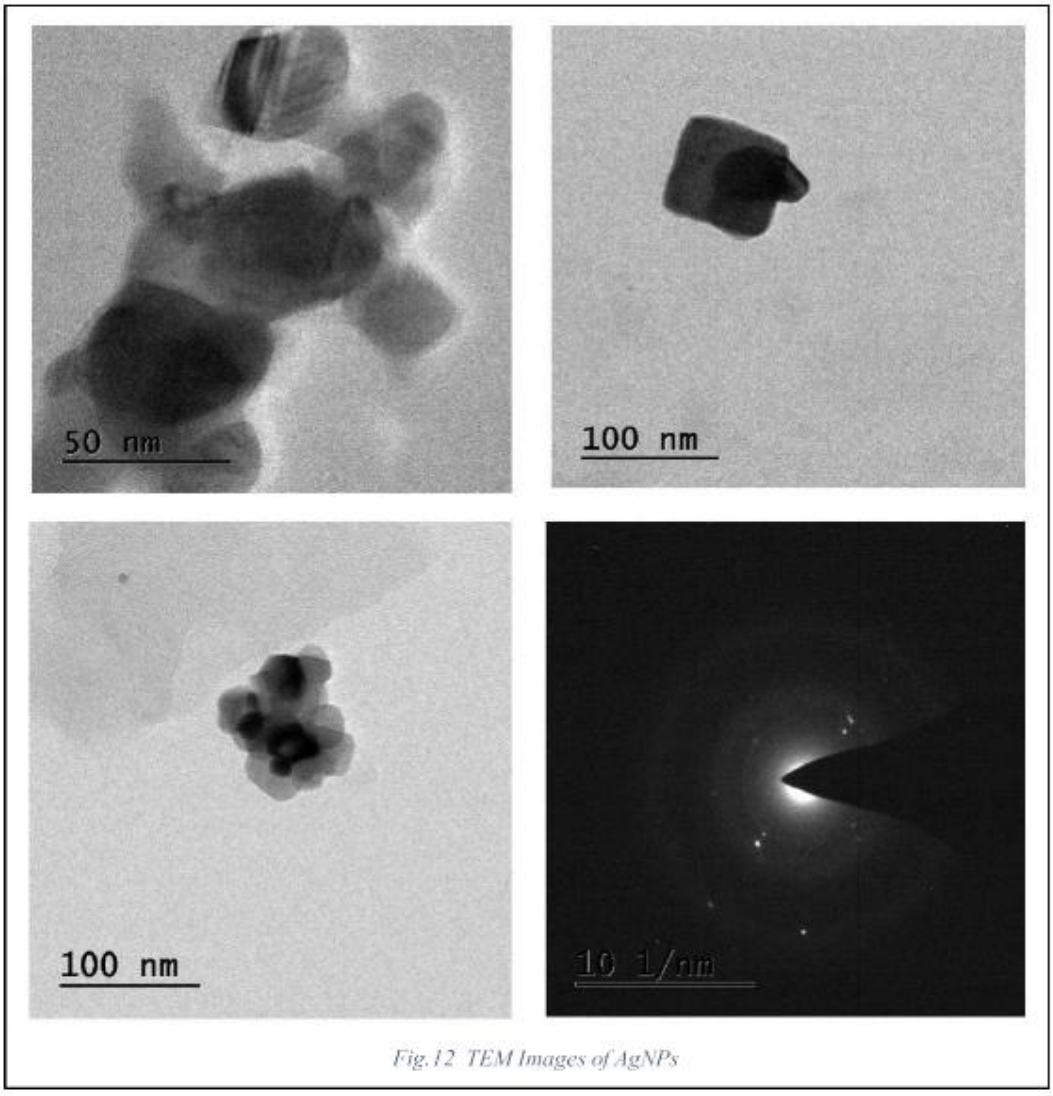

Fig. 12 TEM Images of AgNPs

PHYSICAL PARAMETERS OF NANOGEL:

Organoleptic Evaluation:

Appearance: Translucent and jelly-like.

Colour: Orange

Odour: Fruity odour

Texture: Smooth.

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Viscosity: The viscosity of the nanogel was checked by using the Brookfield viscometer where the achieved results were: 4690 cP and the percent torque was found to be 39.1%.

pH Measurement:

The pH of the formulation was found to be 5.06.

Spreadability:

The spreadability of the formulation was found to be 24.90 gm/cm^2.

Fig. 14 Determination of Spreadability

ANTIOXIDANT ACTIVITY:

Test compounds	Concentration (µg/mL)	Absorbance	Scavenging activity (%) DPPH
Pomegranate Peels Extract	Blank	0.756	0
	STD 1	0.06	92.06
	STD 2	0.072	90.48
	STD 3	0.249	67.06
	STD 4	0.304	59.79
	STD 5	0.333	55.95
	STD 6	0.364	51.85
	SAMPLE 1	0.214	71.69
	SAMPLE 2	0.263	65.21
	SAMPLE 3	0.298	60.58
	SAMPLE 4	0.36	52.38
	SAMPLE 5	0.433	42.72

Table 4 Effect of Test Compound on DPPH Radical Scavenging Activity

By the analysis of the DPPH radical scavenging activity, the percentage of antioxidant activity of the standard was found to be 92.06% and that of the test compound was found to be 71.69%. By this we can say that, though the antioxidant activity of the sample did not reach the standard result, but it is comparable to the standard.

Concentration (µg/mL)	Absorbance
Sample 1	0.214
Sample 2	0.263
Sample 3	0.298
Sample 4	0.36
Sample 5	0.433

Table 6 Effect of Sample on DPPH

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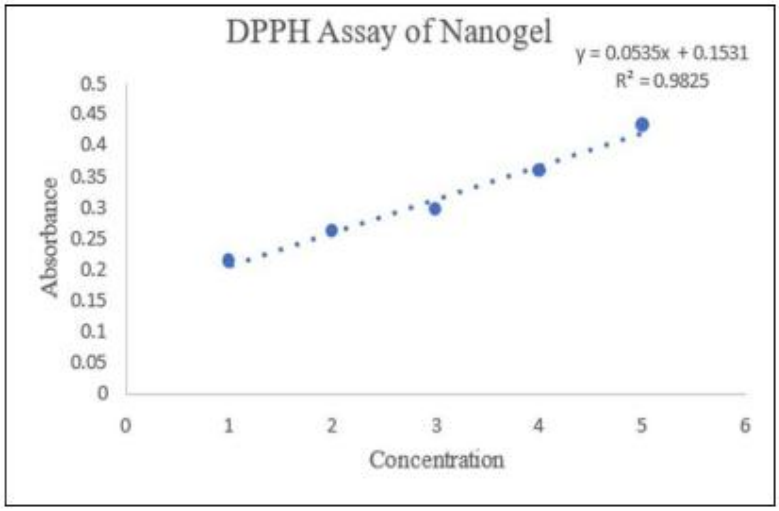

Fig.18 Antioxidant Activity of The Formulation

IV. CONCLUSION

Pomegranate is widely used due to its various therapeutic properties. In this study we will prepare an extract of pomegranate peels which will be further used for synthesis of nanoparticles. Nanoparticles are tiny materials which have many advantages as topical drug delivery which includes higher drug deposition in target region, better physicochemical stability, etc. This investigation suggests Green Synthesis to remain the suitable method for synthesizing the silver nanoparticles. The green synthesis of AgNPs turns out to be a cost- effective method which can synthesize the nanoparticles for Nano gel formulation. A Nano gel containing herbal extract as prime active ingredient makes it an enhanced formulation and can be used for various topical effects. The use of the biowaste for a formulation is productive and also cost efficient. The formulation containing the antioxidant property helps in the treatment of several skin problems that are caused due to the presence of free radicals. These antioxidants donate an electron to the free radicals which in turn stabilizes the unpaired electron in the free radicals. This effect can treat the skin problems. Thus, the use of herbal extracts in a formulation can make the formulation with minimal or no side effects. As the increase in the use of nanotechnology, it gives an upper hand to combine nanotechnology with herbal formulations for better and safer effects.

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