



ANALYSING THE PIGMENTS OF *Tagetes erecta* INCORPORATED WITH SILVER NANOPARTICLES AND INVESTIGATING THEIR APPLICATIONS

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ABSTRACT:

Nanoparticles, with dimensions ranging from 10⁻⁹ meters, have gained attention due to their unique Physico-chemical properties. Silver nanoparticles (AgNPs) are particularly attractive due to their optical, electrical, and antimicrobial properties, making them suitable for various industries such as biosensors, composite fibers, superconducting materials, medical imaging, pharmaceuticals, and cosmetics. AgNPs' antibacterial action is the most studied, offering new pharmacotherapeutic perspectives in the context of rising resistance to pathogenic bacteria. Green synthesis is more beneficial than traditional chemical synthesis due to its cost-effectiveness, reduced pollution, and improved environmental and human health safety. *Tagetes erecta*, also known as "marigold," is a popular plant known for its decorative qualities and carotenoids extraction. It has a rich history of use in traditional medicine, particularly in Mexico and South America, for curing various diseases. Microorganisms attack fiber only when the fiber is damp, and synthetic fibers repel microorganisms due to their high aquaphobicity. However, textiles contain cotton fiber, which can cause microbial growth, causing irritation and smell problems. To protect fabrics from bacterial growth, eco-friendly methods are needed, including medical garments, sanitary napkins, socks, underwear, disposable wipes, and carpets.

KEYWORDS: Silver nanoparticles, *Tagetes erecta*, pigment, antimicrobial and colour fastness

1. INTRODUCTION

Nanoparticles are those that have dimensions in the range of 10⁻⁹ meters and possess unique Physico-chemical properties. The special characteristics of nanoparticles have gained increased attention in recent years, leading to advancements in research and the development of nanotechnology as an interdisciplinary field. Nanotechnology has a wide range of applications across various industries, including medicine, pharmaceuticals, cosmetics, energy, agriculture, environment, and food. (Bianca Ivanescu *et al.*, 2021). Conventional physical and chemical techniques are becoming out-of-date due to their use of toxic substances, high energy consumption, extravagant processing expenses, and production of nanoparticles with low stability due to their tendency to aggregate. Green synthesis is more beneficial than the traditional chemical synthesis because it costs less, decreases pollution, and improves environmental and human healthy safety. (H. Padalia *et al.*, 2014)

Tagetes erecta (commonly known as “marigold”), a member of the *Asteraceae* family, is a popular plant known for its decorative qualities and the extraction of carotenoids. It is a small shrub, which grows up to 1-2 m and it is used widely in our traditional system of medicine for curing various diseases. The problems related to microorganism's growth in textiles are with respect to hygiene and fabric deterioration. It results in damage of fabric by causing stain and discolouration or by deteriorating the material itself.

Therefore, there is a requirement to inhibit microbiological growth on fabric both in industrial and apparel use. oday, within the era of eco-friendly operation, it became vital usage to provide hygiene and freshness. But the main constraint is the growth of microorganisms, which are responsible for the decay, staining and odour of textiles. Other than this effect, even microbes can affect human being by spreading diseases and infections. (Suneeta *et al.*, 2021)

Nature has gifted many antimicrobial property carrying florals. One such kind is *Tagetes erecta* universally recognized as Marigold. Here an exploration was done to deliberate the role of the concentrate obtained from marigold petals as an antimicrobial dyeing and considered its dyeing consequences on cotton fabrics. Pigments are present in leaves, fruits, vegetables, and flowers; they are also found in skin, eyes, and other animal structures and in bacteria and fungi. Pigments have been a well-known pharmacological activity. (Siddharthan *et al.*, 2020)

In our exploration of biological methods for nanoparticle synthesis, we opted to utilize *Tagetes erecta*, an ornamental plant that is prevalent in various geographical regions. Our selection of this plant was based on its widespread availability and the extensive research conducted on its antibacterial activity. This study investigates the synthesis of silver nanoparticles using green chemistry and their subsequent characterization as well as to assess their application in fabric dyeing which emphasis on their antibacterial property.

2. MATERIALS AND METHOD

2.1 Collection of samples

Tagetes erecta (Marigold) was sourced from the local farmer in Udumalpet, Tiruppur district, Tamil Nadu and was authenticated by Tamil Nadu Agricultural University (TNAU) with authentication number: BSI/SRC/5/23/2024/TECH – 254, dated on 08th April,2024, Coimbatore.



Fig 1 - Marigold flowers



Fig 2 - Shadow dried petals

2.2 Extraction of pigments

Fresh petals were collected and thoroughly washed with tap water, followed by distilled water. The washed petals were then shadow dried and were crushed to obtain a powdered form. To create these extracts, 20 grams of pulverized plant material was combined with 200 millilitres of distilled water. The mixtures were thoroughly blended using a magnetic stirrer for a duration of 30 minutes. Subsequently, the extracts were filtered using a Whatman filter no. 1 and topped up to the desired level with the same solvent. The resulting liquid extracts were then stored in a refrigerator at 4°C for future use. (H. Padalia *et al.*, 2014)



Fig 3 – The pigment extracted from *Tagetes erecta*

2.3 Green Synthesis of AgNPs

The green synthesis method was followed for the synthesis of silver nanoparticles. For the reduction of Ag^+ ions, 1 mL *T. erecta* extract was added dropwise into 100 mL of 1 mM aqueous solution of AgNO_3 and heated at 60–80°C for 1 hour. The color change was seen from deep brown to reddish brown, indicating the formation of silver nanoparticles. After establishing the reaction conditions, the colloidal solutions were centrifuged at 5000 rpm for 30 min. For the purification of nanoparticles, the supernatant was removed and the AgNPs were redispersed in distilled water, centrifuged, and finally separated. This operation was repeated twice, and the obtained AgNPs were dried in an oven at a temperature of 40°C, until a constant mass. The synthesized samples were kept at 4°C.



Fig 4 - Titration of AgNO_3 solution and extract



Fig 5 - Synthesized AgNPs

2.4 Characterization of the Synthesized AgNPs

The formation of AgNPs was observed through the color change of *T. erecta* extracts after being mixed with AgNO₃. The synthesized AgNPs were characterized using UV-visible spectroscopy, with the UV-Vis spectra being recorded in the 250–600 nm range to distinguish the maximum surface plasmon resonance (SPR).

To investigate the different functional groups of the extract and those involved in the AgNP synthesis, the FTIR spectra of both extracts and AgNPs were recorded in scan intervals of 400 – 4000 cm⁻¹. Additionally, the morphology and dimensions of nanoparticles were investigated by SEM analysis. SEM analysis enables the high-resolution of single nanoparticles (NPs) with sizes well below 10nm. It gives the ability to detect nanoparticles and investigate their properties. (H. Padalia et al., 2014)

2.5 Fabric Dyeing

2.5.1 Source

Decent quality marigold flowers and leaves were purchased from the farmer. The dyeing of the cotton fabric was passed through three phases; extraction of dyeing elements from the plant sources, antimicrobial dyeing, and mordanting.

2.5.2 Fabric

Plain woven cotton fabric available in the local marketplace was bought.

2.5.3 Extraction of antimicrobial dyeing element

The decent quality flowers of marigold were cleaned followed by drying under shadow. These dried petals were crushed and boiled with water for 2 hours at 50°C - 95°C. The extract was then sieved to get a clear solution. (D. B. Patil *et al.*, 2016)

2.5.4 Scouring of cotton followed by dyeing

The natural colour dyeing procedure is followed to convey the antimicrobial finish to a material. The bath was prepared by using 3% NaOH, 2% soda, and 1% surface-active agent to clean the cotton fabric. The cotton fabric was dipped in the bath and boiled for 2-3 hours for the scouring process. After scouring the fabric was soaked in clean water for half-hour before dyeing or mordanting. This fabric was coloured with dye extract by maintaining M:L ratio as 1:40. A Standard dyeing method was employed for the same and the dyed samples were undergone mordanting. (Suneeta *et al.*, 2021)

2.5.5 Mordanting

A mordant is an organic reagent, which supports forming a complex between dye and fabric. In textiles, mordants were used to fix the colour in dyeing exclusively for cotton fabrics. Alum (Potassium aluminum sulphate) was used to treat dyed textiles. (Suneeta *et al.*, 2021). The mordant solutions with 2% and 4% concentrations were prepared depending on the weight of the material. The fabric to liquor ratio was maintained as 1:50. The dyed fabric samples were soaked into the mordant bath initially at 60°C for half-hour. Mordant samples were then dried under shadow.



Fig 6 – Plain woven cotton fabric



Fig 7 – Cotton fabric dyed with marigold pigment

2.6 Colour fastness tests

Colour fastness tests signify the standard of the apparel colour. Every fabric after dyeing undergoes colour fastness tests with respect to washing, rubbing, perspiration and light fastness.

2.7 Assessment of antibacterial activity

The Agar well diffusion method was adopted, which is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile tip, and a desired volume of the antimicrobial agent or extract solution at desired concentration is introduced into the well and the plates were incubated for obtaining the desired results. (Suneeta et al., 2021). The microorganisms, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae* were used.

3. RESULTS AND DISCUSSION

3.1 Characterization of AgNPs

3.1.1 UV – Visible Spectroscopy analysis

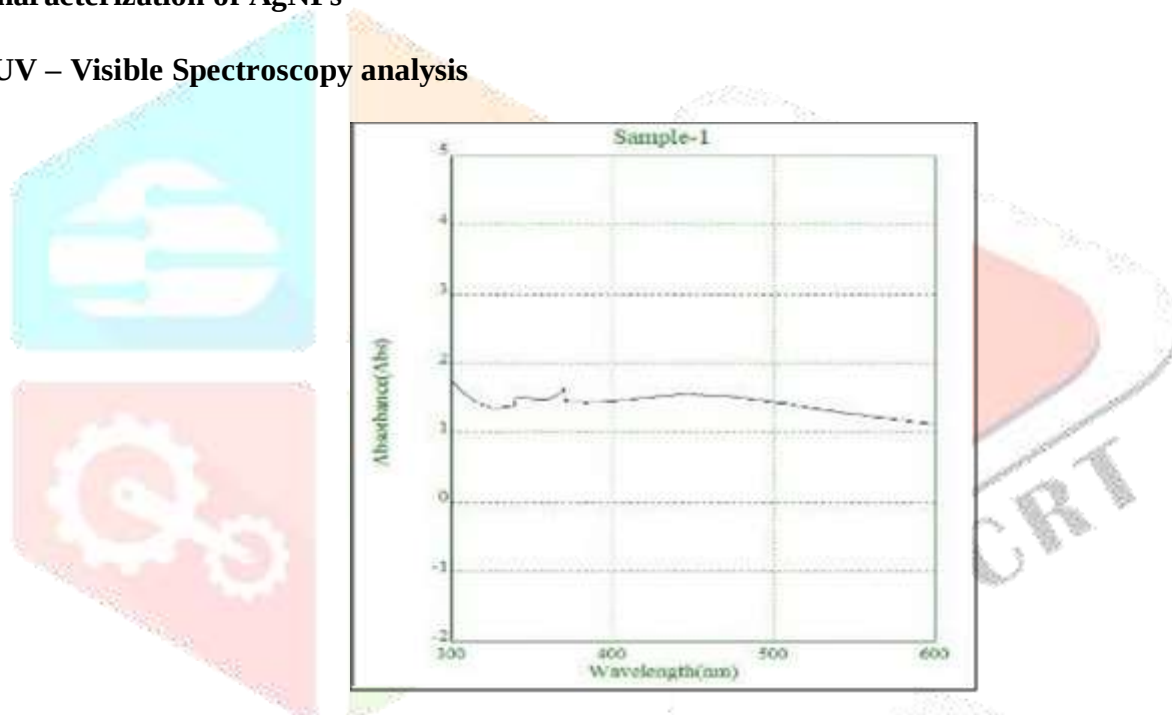


Fig 8 - UV Visible Spectroscopy analysis of the AgNPs synthesized using the extract of *Tagetes erecta*

To confirm the formation and proper stability of silver nanoparticles, their absorption spectrum was read after 24 hours in the range of 400–450 nm using a spectrophotometer. Results of UV - Visible Spectroscopy analysis showed that the extract of AgNPs showed highest absorbance at 446 nm with 1.96 au (Absorbance unit) which are similar to the results of Suneeta *et al.*, (2021) which showed the higher absorbance for the AgNPs at 430nm with the value of 1.5 au and they confirmed the presence of silver nanoparticles in their sample.

3.1.2 FTIR Analysis:

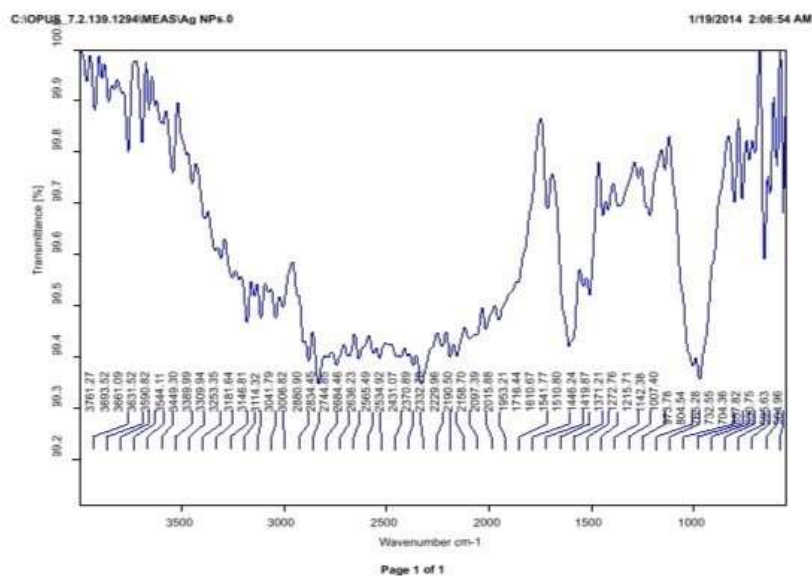
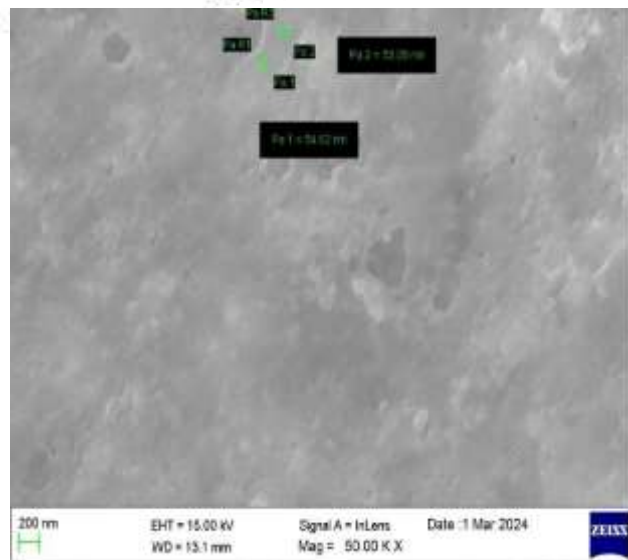
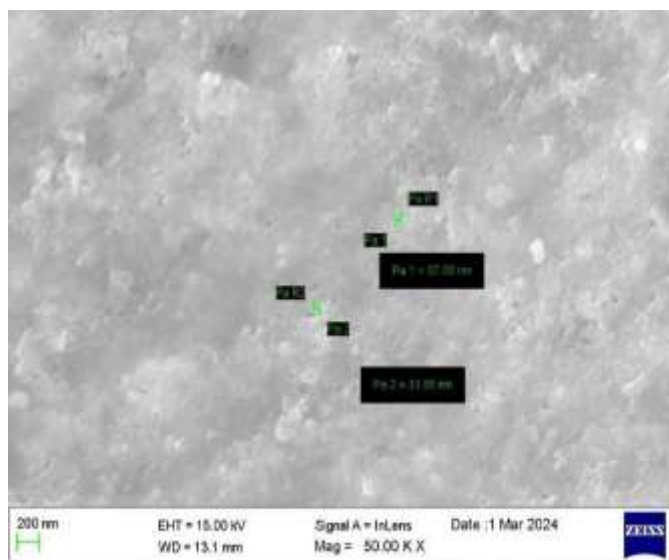


Fig 9 - FTIR analysis of the silver nanoparticles synthesized using the extract of *Tagetes erecta*

The FTIR (Fourier transform infra-red spectroscopy) was recorded in the range of 400–4000 cm^{-1} . Various modes of vibrations were identified and assigned to determine the different functional groups present in the *T. erecta* extract. The FTIR spectra of extracts and of their derived AgNPs are depicted in Figure 9.

The extracts of *T. erecta* showed strong absorption bands at 3524 cm^{-1} and 3449 cm^{-1} corresponding to the stretching vibration of O-H groups in alcohols and phenols. Bands at 2922 cm^{-1} and 2853 cm^{-1} for *T. erecta* extracts are assigned to N-H bonds in amides and C-H stretching vibrations, respectively. Common intense bands for both extracts present at 1610 cm^{-1} and 1007 cm^{-1} denote C=O and C-O-C stretching vibrations, respectively. Stretching vibrations of the aromatic ring and phenyl groups were also identified through the absorption bands at 804 cm^{-1} and 600 cm^{-1} which are similar to the results depicted by H. Padalia *et al.*, (2014).

3.1.3 SEM Analysis:



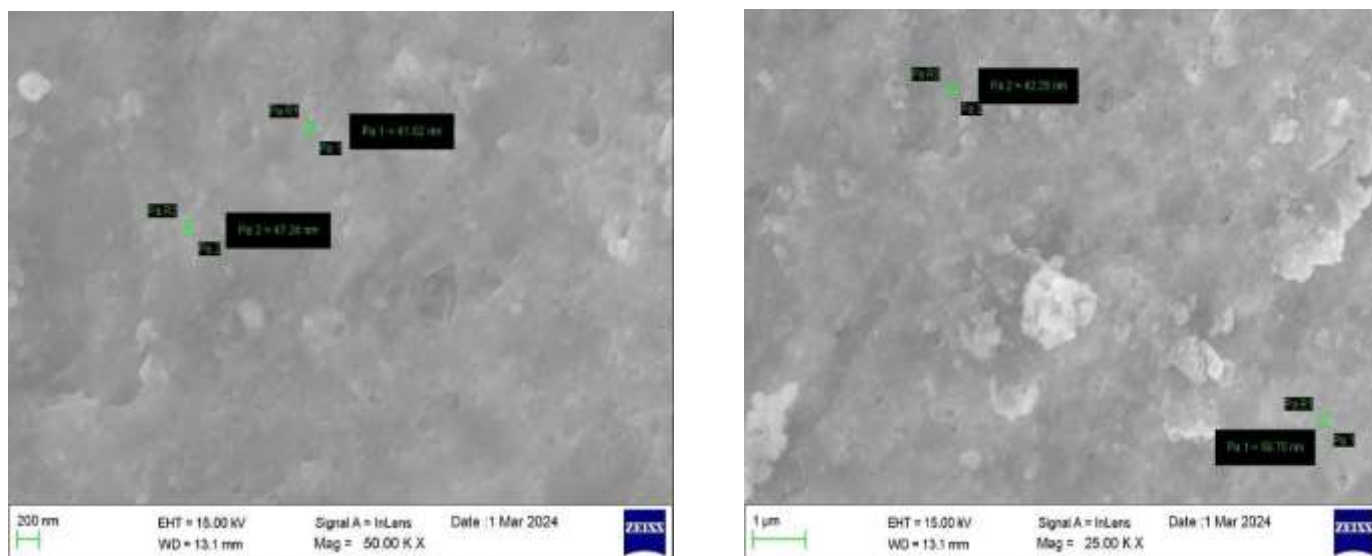


Fig-10 comprising of A, B, C and D are the SEM images which shows the various sizes of the nanoparticles present in the sample

The particle size of the prepared silver nanoparticles using *Tagetes erecta* flower extract were measured using a scanning electron microscope. The appearance of bright spherical particles is noticed, which confirms the formation of silver nanoparticles and that the size measured using a scanning electron microscope were (Fig.10A, 10B) between 31.56 nm to 59.75 nm, which indicates the presence of silver nanoparticles.

These results are close to the ones reported by Kuppusamy *et al.*, (2015) reported silver nanoparticles produced from extract of broccoli. The results showed average size of silver nanoparticles thus formed to be around 35–50 nm and spherical in shape.

The nanoparticles present in Fig 10A were of sizes 31.55 and 37.08nm, whereas in Fig 10B it was of sizes 41.02 and 47.24nm. Then nanoparticles present in Fig 10C were of sizes 42.25 and 59.75nm, whereas in Fig 10D it was of sizes 53.06 and 54.02nm.

3.2 Colour Fastness Analysis:

Table 1: Colour Fastness of mordant samples

S.no	Mordant	Mordant %	Colour Fastness to Washing Grade	Light Fastness Grade	Colour Fastness in Precipitation		Colour Fastness to Rubbing Grade	
					Acid	Alkaline	Dry	Wet
1	Alum	2	4	4	4	4	4-5	4
2	Alum	4	5	4	4-5	4	4-5	4

The colour fastness to washing, light, rubbing and perspiration on dyed cotton sample with alum as a mordant is presented in Table 1. The dyed samples showed good wash fastness of 4 for both mordant percentage concentration. There is no such change in washing fastness with the increase in the percentage of mordants. (Suneeta *et al.*, 2021)

The dyed samples are also subject to light fastness test. The samples mordant with 2% and 4% concentration of alum shows good fastness towards light. The good to excellent light fastness properties of the fabric indicates that close by are no such superficial unsettled natural dyes left over on the fibre apparent after soaping and washing.

The perspiration fastness value for 2% and 4% of alum shown good fastness in presence of alkaline medium. The fastness value remains constant as 4 even with increase in percentage of mordants in presence of alkaline medium. It indicated that there is no effect of high percentage of mordents in alkaline medium. Same results were observed in the presence of acid medium too.

Colour alteration to dry rubbing for the canned example is found with superb resistance in the series of 4-5. It is observed that during dry rubbing there is no colour discoloration ranged between no staining and negligible staining (4-5).

3.3 Antibacterial Susceptibility Analysis

Table 2: Antibacterial activity of the marigold pigment and AgNP against gram negative and gram positive organisms

Marigold pigment	Zone of Inhibition in mm			
	<i>E coli</i>	<i>Klebsiella pneumoniae</i>	<i>S aureus</i>	<i>Bacillus cereus</i>
Without AgNP	6	6	7	7
With AgNP	9	10	13	11

These are the mean values of the triplicates performed

The results of antibacterial susceptibility test of the marigold pigments clearly showed that the pigments of *Tagetes erecta* and AgNPs synthesized from the extracts having the antibacterial activity against the gram-positive and gram-negative organisms. The pigments of *Tagetes erecta* shown maximum zone of inhibition for *S aureus* and *Bacillus cereus* with 7 mm and the minimum zone of inhibition for *E coli* and *Klebsiella pneumoniae* with 6 mm and the AgNPs synthesized from the extracts of *Tagetes erecta* showed the maximum zone of inhibition for *Staphylococcus aureus* with 13 mm and the minimum zone of inhibition for *Bacillus cereus* with 11 mm. These results correlate with the results performed by H. Padalia *et al.*, (2014). Their results for the antibacterial activity of the AgNPs showed the maximum zone of inhibition for *Staphylococcus aureus* and the minimum zone of inhibition for *Bacillus cereus*.

Table 3: Antibacterial activity of the marigold pigment dyed fabric against gram-negative and gram-positive organisms

Cotton Fabric	Zone of Inhibition (mm)			
	<i>E coli</i>	<i>Klebsiella pneumoniae</i>	<i>S aureus</i>	<i>Bacillus cereus</i>
Without dye	< 2	< 2	< 2	< 2
With dye	6	2	13	9

The results of antibacterial susceptibility test clearly shown that the marigold pigment dyed fabric incorporated with AgNP having the antibacterial activity against the gram-positive and gram-negative organisms. The dyed samples showed maximum zone of inhibition for *Staphylococcus aureus* with 13 mm and the minimum zone of inhibition for *Klebsiella pneumoniae* with 2 mm. These results correlate with the results performed by Suneeta *et al.*, (2021). Their results showed maximum zone of inhibition for *Staphylococcus aureus* with 11 mm and the minimum zone of inhibition for *Klebsiella pneumoniae* with 6 mm.

4. SUMMARY AND CONCLUSION

Tagetes erecta which are characterized as eco-friendly, economical, and more effective approach than physical and chemical approach. The plant extract not only functions as a reducing agent but also coats the produced nanoparticles, providing them with stability. *Tagetes erecta* has been considered a good reducing agent for the preparation of stable colloidal silver nanoparticles. The silver nanoparticles prepared using marigold extract have intense absorption peak in the visible region with the peak at 446 nm in the UV Visible Spectroscopy. The optimal condition for the synthesis of silver nanoparticles were at 35 - 37°C for 24 h. These silver nanoparticles were stable in the in the fridge at 4°C for a maximum period of 15 days.

On the other hand, the antibacterial tests showed that these nanoparticles have antibacterial activity against the *E coli*, *S aureus*, *Bacillus cereus* and *Klebsiella pneumoniae*. Common colour can be effectively extracted from blossoms of marigold. With the extraction process the antimicrobials are extracted. The coloured samples show great fastness towards light, wash and sweat. Fabric with medium to excellent fastness properties indicated no such unpredictable natural dye port on cloth apparent even later shampooing and clothes wash. It indicates the natural dye were possibly fixed well. The colour fastness test authenticated the procedures followed for dyeing especially with the natural resources are precise. It was found that the fabric dyed with marigold extract showed resistance for few organisms. At the same time such treated fabrics show gradual decrease in bacterial activity to wash in each cycle.

From my study I conclude that the pigments of marigold had a better performance for production of desirable silver nanoparticles with antibacterial activity. The outcome demonstrates that the dyeing capability of marigold blossoms acts as a phenomenal hotspot for material colouring against microorganisms. These prevent microbial invasion and can be used for medical garments, sanitary napkins, disposable wipes, etc.

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